PROXIMAL TUBULAR RENAL DYSFUNCTION AMONG HIV PATIENTS ON TENOFOVIR VERSUS TENOFOVIR SPARING REGIMEN IN WESTERN KENYA.

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A RESEARCH PROPOSAL SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, MOI UNIVERSITY.

DECLARATION

Student's declaration

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I dedicate this work to Mathew, Mia and Myles Koech who inspire me to keep moving forward.

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ABSTRACT

Background: Tenofovir Disoproxil Fumarate (TDF) is the most widely used Anti-Retroviral Therapy (ART) drug due to its potency, safety profile and WHO recommendation. TDF causes proximal tubular renal dysfunction (PTRD) leading to Fanconi syndrome, acute kidney injury and chronic kidney damage. Modest rates of about 2-4% of TDF related toxicity based on estimated Glomerular Filtration Rate (GFR) have been described; while TDF induced PTRD has been reported to be 22%. Beta 2-microglobulin (B2M) in urine has been validated as a highly sensitive/specific marker for tubular proteinuria. TDF toxicity is more likely among African patients, it is reversible and TDF may be renal dosed in patients with dysfunction.

Objectives: To assess proximal tubular renal dysfunction, global renal function and their determinants among patients on TDF versus TDF sparing regimen.

Methods: This was a cross sectional study among HIV infected patients attending Academic Model Providing Access to Healthcare (AMPATH) program. The primary outcome of interest in this study was PTRD while the secondary outcome of interest was estimated GFR. PTRD was defined as any two of beta 2 microglobulin in urine, metabolic acidosis, normoglycemic glucosuria and fractional excretion of phosphate. Student t test, chi square, likelihood ratio and their non-parametric equivalents were used to test for statistical significance. Univariate and Multivariate logistic regression analysis was carried out.

Results: A total of 516 participants were included in the final analysis, 261 on TDF while 255 were on non-TDF based regimens. The mean (SD) age of all participants was 41.5 (12.6) years with majority being female (60.3%). The proportion of PTRD was 10.0% versus 3.1% in the TDF compared to TDF-sparing group (P<0.001). Mean estimated GFR was 112.8 (21.5) vs 109.7 (21.9) ml/min/1.73mm^3 (P=0.20) for the TDF compared to TDF-sparing group. TDF users were more likely to have PTRD compared to those not using TDF, adjusted Odds Ratio (AOR) 3.0, 95% CI 1.12 to 7.75. Hypophosphatemia was found to have a specificity of 88.8% and a low sensitivity of 23.5% in detecting proximal tubulopathy.

Conclusion: There was significant tubulopathy in HIV patients on TDF compared to TDF-sparing group without significant difference in estimated GFR. The clinical significance of these findings may not be clear in the short term. Serum phosphate levels are not useful as a proxy for detecting tubular dysfunction.

LIST OF ABBREVIATIONS

AMPATH	Academic Model Providing Access to Healthcare
AUC	Area Under Curve
ART	Antiretroviral Therapy
B2M	Beta 2 Microglobulin
BMI	Body Metabolic Index
CD4	Cluster of Differentiation
CG	Cockcroft- Gault
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
dAMP	Deoxyadenosine Monophosphate
dATP	Deoxyadenosine triphosphate
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
FEphos	Fractional Excretion of Phosphate
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HIV	Human Immunodeficiency Virus
KDIGO	Kidney Disease Outcomes Quality Initiative
MDRD	Modification of Diet in renal disease
MHC	Major Histocompatibility Complex
MRP 2	Multidrug Resistant Proteins
MRS	Medical Records System
MTRH	Moi Teaching and Referral Hospital
NGAL	Neutrophil Gelatinase Associated Lipocalin
OAT	Organic Anion Transporters
PTRD	Proximal Tubular Renal Dysfunction
RBP	Retinol Binding Protein
ROC	Receiver Operating Characteristics
TDF	Tenofovir Disoproxil Fumarate
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Proximal tubular renal dysfunction

Proximal tubular renal dysfunction was defined as any 2 out of 4 parameters including normoglycemic glucosuria, metabolic acidosis, Beta 2 microglobulinuria, and fractional excretion of phosphate >20%.

Tubular proteinuria

Tubular proteinuria was defined as presence of excessive amounts of Beta 2 microglobulin in urine in excess of 0.3mg/mmol.

Phosphate wasting

Phosphate wasting was defined as a fractional excretion of phosphate (FEphos) of >20% among participants if normal serum phosphate levels (0.85 to 1.45 mmol/l) or >10% among participants with hypophosphatemia (serum phosphates of <0.85 mmol/l) (Ezinga *et al.*, 2014; Pitisci *et al.*, 2014).

$$FEphos = \frac{\text{urinary phosphate (Up)x plasma creatinine (Pcr)}}{\text{plasma phosphate (Pp)x urinary creatinine (Ucr)}} \text{ X } 100$$

Normoglycemic glucosuria

The definition of normoglycemic glucosuria in this study was defined by detectable glucose in urine by dipstick despite a random blood glucose of less than 11.1mmol/l.

Renal function/ Estimated GFR

The National Kidney Foundation's Practice Guidelines for Chronic Kidney Disease was used to establish a cut point, eGFR <90 mL/min/1.73 m^2, for decreased kidney function. Normal renal function was defined as estimated GFR ≥120 ml/min per 1.73 m^2 (K/DOQI, 2002).CKD-EPI creatinine equation was used to calculate the estimated GFR (Levey *et al.*, 2009).

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CHAPTER ONE: INTRODUCTION

1.1 Background

HIV (Human Immunodeficiency Virus) infected people have 4 times higher risk of renal disease compared to HIV uninfected people (Islam *et al.*, 2012). HIV related renal disease has multifactorial causes including HIV itself, co-infections, co-morbidities and their treatment (Röling *et al.*, 2006). Antiretroviral use has led to improvements in HIV and renal related outcomes (Lacey, 2014). Some ART drugs have however been noted to cause renal damage through mechanisms such as tubulointerstitial injury, and through drug interactions with other concomitant medications (Kalyesubula and Perazella, 2011; Szczech *et al.*, 2004). Since the advent of ART, HIV patients are living longer thus non-infectious co-morbidities and renal toxicities have become important areas of research and contributors to morbidity (F. A. Post, Wyatt, *et al.*, 2010)

Tenofovir Disoproxil Fumarate (TDF) was recommended by WHO 2013 guidelines as the first line of therapy in combination with other anti-retroviral drugs (WHO, 2013). It is an oral prodrug of Tenofovir, which is an acyclic nucleotide analogue of adenosine monophosphate (Kohler *et al.*, 2011). TDF is dephosphorylated to its active form tenofovir diphosphate, in this form tenofovir inhibits HIV-1 replication through inhibition of reverse transcriptase inhibitor (Grim and Romanelli, 2003).TDF at a daily dose of 300mg for adults has been shown to reduce HIV viral load effectively when combined with other antiretrovirals (Lyseng-Williamson *et al.*, 2005). Randomized trials of tenofovir have confirmed similar efficacy when compared to other first line regimens containing, zidovudine, stavudine or abacavir (J. E. Gallant *et al.*, 2004; Pozniak, 2008).

TDF use may lead to Fanconi syndrome, acute or chronic kidney injury by triggering kidney damage through proximal tubular damage (Fernandez-Fernandez *et al.*, 2011; Rodriguez-Nóvoa *et al.*, 2010). Fanconi syndrome, an infrequent disorder of proximal tubular function, may be hereditary or acquired in HIV-positive persons exposed to Tenofovir. Tenofovir induced Fanconi syndrome is defined by normoglycemic glucosuria, mild to moderate proteinuria, urinary phosphate wasting, hypokalemia, hypouricemia and metabolic acidosis with normal anion gap (Gupta *et al.*, 2014). Modest rates of TDF related renal dysfunction have been described in literature with 1-2% of renal dysfunction reported (Brennana *et al.*, 2011; Fux *et al.*, 2007). Most studies however report global kidney function using estimated glomerular filtration rate (GFR) yet early detection of TDF associated nephrotoxicity requires testing for proximal tubular renal dysfunction (PTRD)(Cooper *et al.*, 2010). Studies investigating proximal tubular dysfunction report a high prevalence of subclinical dysfunction, ranging from 15 - 22%, among HIV infected patients (Labarga *et al.*, 2009; F. Post, 2014).

Some of the factors associated with TDF-induced renal toxicity consist of prolonged duration of antiviral treatment, severe immunosuppression, underlying renal disease, older age, African ethnicity, female gender and concomitant administration of other drugs that are toxic to the kidney (Crum-Cianflone *et al.*, 2010). When combined with protease inhibitors such as ritonavir, TDF causes more kidney injury. Majority of cases (70%) of TDF induced renal toxicity occur in combination with ritonavir boosted protease inhibitors (Izzedine *et al.*, 2009; Zimmermann *et al.*, 2006). Much of the incident renal dysfunction in HIV patients using TDF has been attributed to pre-existing renal pathology (Brennana et al., 2011).

Detection of TDF associated toxicity while it is still early or mild requires specific investigations for proximal tubular injury (Cooper et al., 2010). Proximal tubular injury can be determined through urinalysis for glucose and protein, serum phosphate and bone fracture rate (Campbell *et al.*, 2012; Del Palacio *et al.*, 2012a). Proteinuria due to proximal tubular injury can be detected through finding of low molecular weight proteins in the urine. Low molecular weight proteins are biomarkers of tubular function and examples such retinol binding protein (RBP), beta 2 micro globulin (B2M) and neutrophil gelatinase associated lipocalin (NGAL) can be measured from urine (Del Palacio *et al.*, 2012b). B2M in urine has been validated as a highly sensitive marker for assessing proximal tubular proteinuria (Del Palacio et al., 2012a; Kinai and Hanabusa, 2005). WHO guidelines do not emphasize the need or frequency of monitoring renal function in persons on TDF, leaving this to the clinicians' discretion. Furthermore, subclinical toxicity is missed when serum creatinine used to assess the global renal function (WHO, 2013).

1.2 Problem statement

Given its proven antiviral potency, good safety profile and guideline recommendation, TDF has become the most prescribed antiretroviral drug. There is rapid scale up of ARV therapy in resource constrained settings with updated WHO guidelines recommending starting therapy in all patients with HIV infection regardless of their CD4 count (WHO, 2015). TDF forms a major component of 1st line regimen or 2nd line regimen if patients had no previous exposure. TDF is provided at no cost through donor and government funding and therefore easily accessible to HIV patients. TDF is also now prescribed as pre-exposure therapy for discordant couples (CDC, 2018).

Tubular dysfunction from TDF may be subclinical and progressive over time (Rodriguez-Nóvoa et al., 2010). Several cases of kidney abnormalities involving proximal tubular function have been described (Karras *et al.*, 2003; Malik *et al.*, 2005; Verhelst *et al.*, 2002). Tubular dysfunction may pass unrecognized given that glomerular function is well preserved in most cases. Early forms of renal dysfunction have previously been evaluated through laboratory monitoring of serum creatinine and estimated GFR calculated using either the Cockcroft Gault (CG) or the modification of diet in renal disease (MDRD) formula. This is estimation requires the use of serum creatinine which has the disadvantage of delaying detection of tubular dysfunction since it only gets deranged in advanced disease (Bygrave *et al.*, 2011). The modest effects of TDF on the kidneys as reported in previous studies do not prevent the use of TDF in resource constrained settings where monitoring of kidney function is not feasible. Therefore, TDF use continues despite these modest toxicities (Kyaw *et al.*, 2015).

1.3 Study Justification

WHO recommends further research into TDF use to improve understanding of the magnitude of TDF toxicity and associated factors for added renal dysfunction such as comorbidities, age, and use concomitant nephrotoxic drugs (Kyaw et al., 2015).

HIV infected Africans are known to have a higher risk for kidney disease, potential for rapid deterioration to end stage renal disease compared to Caucasians in developed countries (Lucas *et al.*, 2008). Chronic kidney disease is 3 times more frequent in Africa than in industrialized countries with nephrotoxicity in HIV patients considered an important complication (Naicker, 2009; Stohr *et al.*, 2011). This higher risk of kidney disease presents a serious public health issue given the high cost of keeping individuals with end stage renal disease alive by renal replacement modalities

as well as morbidity and mortality even among those with moderate forms of kidney disease (Pollak, 2008). The long-term consequences of proximal tubulopathy may also be related to accelerated bone mineral loss, osteomalacia and fractures or further renal damage (Grant and Cotter, 2016).

This study adds new knowledge on subclinical tubular injury among HIV infected persons and has the potential to improve guidelines on laboratory monitoring. With increase in uptake of TDF in resource limited settings, monitoring protocols for TDF toxicity need to be simplified. The utility of biomarkers in assessing renal function has been explored in this study to help in validation of simple markers to detect tubular injury. When detected early, TDF associated toxicity is reversible and can be renal dosed for patients who have renal dysfunction (Some *et al.*, 2017).

1.4 Research Questions

1. What is the proportion of proximal tubular renal dysfunction among HIV-infected patients on TDF regimen compared to patients on TDF sparing regimen?
2. What is the mean global renal function among HIV infected patients on a TDF regimen compared to patients on a TDF sparing regimen?
3. What are the determinants of the association between proximal tubular renal dysfunction and TDF use among HIV infected patients?
4. What is the sensitivity and specificity of hypophosphatemia in detecting proximal tubular renal dysfunction?

1.5 Research Objectives

1.5.1 Broad objective

To assess proximal tubular renal dysfunction among HIV infected patients on TDF regimen compared to those on TDF sparing regimen

1.5.2 Specific objectives

1. To compare the **proportion of proximal tubular renal dysfunction** among HIV infected patients on a TDF regimen versus patients on TDF-sparing regimen.
2. To compare the **mean GFR** among HIV infected patients on TDF regimen versus patients on TDF sparing regimen.
3. To assess **determinants of the association** between proximal tubular renal dysfunction and TDF use in HIV infected patients.
4. To **determine sensitivity and specificity of hypophosphatemia** in assessing proximal tubular renal dysfunction in HIV patients on ART.

CHAPTER TWO: LITERATURE REVIEW

2.1 Pharmacology of TDF

2.1.1 Introduction

TDF is the pro-drug for tenofovir diphosphate which is the only analogue nucleotide reverse transcriptase inhibitor approved by the Food and Drug Administration (FDA) (Markowitz *et al.*, 2014). TDF is used for the treatment of HIV-1, Hepatitis B and for pre-exposure prophylaxis against HIV infection. Randomized placebo controlled trials have shown that TDF at 300mg significantly reduce viral load compared to placebo within 6 months (Chapman *et al.*, 2003).

Tenofovir alafenamide fumarate (TAF) is a more potent prodrug that achieves higher intracellular tenofovir levels compared with TDF. Unlike TDF, TAF is initially stable in plasma before conversion to tenofovir intracellularly (Markowitz et al., 2014).

2.1.2 Pharmacokinetics of TDF

TDF is a soluble diester of tenofovir and has an oral bioavailability of 25% even while patients are fasted. After a 300mg oral dose of TDF to fasted patient, maximum serum levels (cmax) are attained within 0.6 to 1.4 hours (Kearney *et al.*, 2006). Tenofovir pharmacokinetics are dose proportional up to a dose of 600mg even with repeated doses or food intake. High fat meal has been reported to increase the area under the curve (AUC) of tenofovir by approximately 40% and Cmax by 14%. Tenofovir is minimally bound to plasma (7.2%) and has a volume distribution of 0.8 litres/kg. The half-life of Tenofovir is 13 hours and it is 70-80% excreted in urine (Joel E. Gallant and Deresinski, 2003). Dosing based on gender or weight is not necessary although a dose reduction may be required in the elderly and in those with

renal dysfunction. Cytochrome P450 enzymes do not participate in metabolism of TDF.

2.1.3 Pharmacodynamics of TDF

TDF is given orally and then goes through a first pass metabolism in the liver to form and an analogue of the endogenous deoxyadenosine monophosphate (dAMP) (Anderson *et al.*, 2011). Tenofovir, the major circulating form, is taken up by target cells where it is phosphorylated to its active moiety tenofovir diphosphate (Naesens *et al.*, 1996). Tenofovir diphosphate prevents DNA polymerization by competitive inhibition of dATP during reverse transcription leading to chain termination of the viral DNA synthesis process (von Kleist *et al.*, 2012). Tenofovir diphosphate therefore prevents the machinery of the virus that enables it to continue replication (Delaney *et al.*, 2006).

2.1.4 Pharmacotherapeutics

Although generally well tolerated, TDF causes minor adverse effects that may not be significant to result in drug discontinuation. These effects include dizziness and gastro-intestinal discomfort (Molina *et al.*, 2014; Zolopa *et al.*, 2013). The key adverse effect of TDF is renal tubular dysfunction, which may vary from minor alterations in plasma creatinine rise to major renal tubular dysfunction or Fanconi's syndrome (Arribas *et al.*, 2008; Cassetti *et al.*, 2007). TDF also causes bone mineral density loss due to wasting of phosphate in urine and renal related osteodystrophy (Ustianowski and Arends, 2015).

Tenofovir is cleared through active tubular secretion and partially by filtration in the glomerulus. Several transporter proteins participate in the active tubular secretion.

Tenofovir enters the tubular cells through organic anion transporters (OAT) 1 and in basolateral membrane. Once inside the tubular cells, Tenofovir is secreted by multidrug resistant proteins (MRP) 2 and 4 through an active process (Kohler et al., 2011). TDF in the tubular cell can lead to mitochondrial toxicity and interference with the normal function resulting in subsequent death of the cells (Rodriguez-Nóvoa et al., 2010). Prolonged use of TDF has also been shown to lead to chronic renal failure. The deterioration in renal function is reported to be about 13.0 ml/min per 1.73 m^2 after 12 months of treatment in TDF-treated groups (J. E. Gallant *et al.*, 2005).

2.2 Renal tubular function

2.2.1 Role of renal tubules

The kidney plays an essential role in preserving blood pressure, fluid and electrolytes balance mainly through the nephron tubules (Arthur and John, 2011). The glomerulus filters 25% of normal cardiac output while the tubules reabsorb electrolytes and water that have been filtered at the glomerulus. (Zhuo and Li, 2013).The proximal tubular part of the nephron is responsible for reabsorbing 65% of solutes which includes amino acids, low molecular weight proteins and electrolytes (Rector, 1983). Proximal tubules also regulate glucose metabolism and maintain body acid-base homeostasis by reabsorbing all the filtered glucose and 80% of bicarbonate (Bakris *et al.*, 2009; Boron, 2006).

2.2.2 Renal biomarkers for tubular function

Biomarkers of renal function indicate the GFR and tubular function of kidneys (Gowda *et al.*, 2010). These markers may be serum or urinary markers. Estimated GFR represents the overall functioning of the kidney and is most commonly measured using serum creatinine (Traynor *et al.*, 2006). Several formulae for determining the

GFR using serum creatinine exist and include; Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, Modification of diet in renal disease (MDRD) and Cockcroft-Gault (CG) formulae (Cockcroft and Gault, 1976).

Estimated GFR presents the overall function of the kidney and may not be sufficient to detect early tubular damage (Cooper et al., 2010; Levey et al., 2009). Low molecular weight proteins (LMWPs) are tiny molecules that are easily filtered through the glomerulus then reabsorbed and broken down by the proximal tubule. These low molecular proteins are present in the urine in negligible amounts if the tubular function is normal. Presence of excessive amounts of tubular proteins in the urine is indicative of tubular dysfunction (F. A. Post, Wyatt, et al., 2010). Examples of these LMWPs are Beta 2 microglobulin (B2M) Retinol binding protein (RBP), Cystatin C, and Neutrophil gelatinase associated lipocalin (NGAL)(Gowda et al., 2010).

B2M is a 12 kilo Dalton (kDa) protein present on all nucleated cells and a constituent of Major histocompatibility complex (MHC) class 1 molecules. RBP is a 21kDa LMWP is plasma bound to transthyretin with the 10% unbound fraction freely filtered by the glomerulus and reabsorbed by the proximal tubule. NGAL is a 25kDa protein produced in many tissues and highly induced during inflammation (Coca and Perazella, 2002). Cystatin C, an inhibitor of cysteine proteinases, is freely filtered by the glomerulus then reabsorbed at the tubules where is it partially catabolized and the remaining product excreted in urine (Conti *et al.*, 2006). Kidney injury molecule-1 (KIM-1) is a trans-membrane protein that is over expressed in proximal tubular cells when there is ischemia of the renal tubules (Ichimura *et al.*, 1998).

Measurement of urinary B2M is a highly sensitive biomarker for proximal tubule injury. Studies have shown an association between urinary B2M with results of immunohistochemical staining of KIM-1 in renal biopsy and consequently with proximal tubular injury (Han *et al.*, 2002).

2.2.3 Clinical manifestations of proximal tubular toxicity

Long term use of TDF may cause progressive proximal renal tubular dysfunction before overt drop in GFR (Rodriguez-Nóvoa et al., 2010). Proximal tubular dysfunction may be manifested as selective re-absorptive defects leading to hypokalemia, aminoaciduria, glucosuria, phosphaturia, uricosuria, or bicarbonaturia (proximal or type II renal tubular acidosis). These defects constitute Fanconi syndrome. Low molecular weight proteinuria also known as tubular proteinuria, is usually modest, rarely > 2 g/day. (Del Palacio et al., 2012a; Rodriguez-Novoa *et al.*, 2010)

Reported kidney complications in persons on TDF include Fanconi syndrome, diabetes insipidus, acute tubular necrosis, acute renal failure, chronic kidney disease, proximal renal tubular dysfunction and bone loss (Tourret *et al.*, 2013). Fanconi syndrome was first described in 2002, persons with non-gap metabolic acidosis, low serum electrolyte levels (phosphate, potassium, uric acid), tubular proteinuria, glucosuria with normoglycemia and aminoaciduria (Verhelst et al., 2002). Acute renal failure may result from tubular necrosis due to the effect of the tubular damage. Renal function has been noted to improve a few months after withdrawing TDF, but some renal derangement may persist (Tourret et al., 2013).

TDF associated defect of tubular function may also result in decreased 1-α hydroxylation of vitamin D and reduced tubular reabsorption of vitamin D–binding protein (Fernandez-Fernandez et al., 2011). Secondary hyperparathyroidism from TDF use has been described in vitamin D–deficient people (Rosenvinge *et al.*, 2010). Persons on TDF have also been reported to have hypophosphatemia, osteomalacia, bone pain, decreased bone mineralization, and bone fractures have also been described in (Haverkort *et al.*, 2011).

The only sign of TDF-specific toxicity on histologic examination is giant mitochondria and inclusions in the cytoplasm of proximal tubular epithelial cells Other features are loss of brush border, prominent eosinophilia with tubular ectasia, simplification of the cytoplasm and prominent nucleoli (Tourret et al., 2013).

2.3 Review of studies assessing Tenofovir nephrotoxicity

2.3.1 Studies assessing tubular function

Studies assessing PTRD as the main outcome use the presence of 2 or more markers of tubular damage to ascertain the presence of tubular damage. In a review of tubular toxicity among HIV infected patients on ART, Del palacio in 2012 defined clinically meaningful PTRD as the presence of 2 or more proximal tubular alterations. The definition of PTRD should include one Fanconi defining alteration such as normoglycemic glucosuria. Subclinical tubular toxicity can be determined from tubular proteinuria and phosphate wasting (Del Palacio et al., 2012a).

Dauchy et al in a cross-sectional analysis within a hospital-based cohort of 399 HIV-1 infected attending routine clinic in France found a prevalence of 6.5% (95% CI 4.2% - 9.5%) of PRTD. This study also found significant association between PTRD and TDF use OR 5.22, p =0.03(Dauchy *et al.*, 2011). PTRD was defined as the presence

of 2 or more of normoglycemic glucosuria, hypophosphatemia, metabolic acidosis, hypouricemia and B2M in urine. Using an almost similar definition for PTRD as the Dauchy et al study, Pitisci et al in 2014 found a prevalence of 10% in an Belgian cohort (Pitisci et al., 2014).

A cross sectional study in Madrid, Spain found a prevalence of 22% of PTRD amongst 284 HIV-infected TDF users. This study measured serum and 24-hour urinary markers of renal tubulopathy such as aminoaciduria, phosphaturia, uricosuria, B2M and glucosuria. The study also determined the significant predictors of the association for PTRD to be use of TDF (OR 21.6 $p< 0.001$) and older age (OR 1.1 $p=0.01$) (Labarga et al., 2009).

2.3.2 Studies assessing estimated GFR

Estimated GFR or creatinine clearances have also been used to determine TDF associated nephrotoxicity. Despite varying definitions, modest rates of nephrotoxicity have been reported by studies using GFR as the measure of TDF induced nephrotoxicity. These studies have led to the conclusion that there is no need for limiting TDF use even in resource limited settings where monitoring of renal function is not possible. Other studies of different populations taking TDF, including some in Africa, have failed to demonstrate significant decreases in GFR (L. B. Mulenga *et al.*, 2008; Reid *et al.*, 2008; Stohr et al., 2011).

Serum creatinine changes reflect advancement in renal dysfunction (Slocum *et al.*, 2012). Other markers such as phosphate wasting are more reflective of proximal tubular dysfunction associated with TDF. The frequency of tubulopathy may go unreported since previous studies do not report specific detailed analysis for diagnosis

of phosphate wasting (Atta and Fine, 2009; F. A. Post, Moyle, *et al.*, 2010; Tourret et al., 2013). Tenofovir-treated persons who experience a GFR drop by >25% from baseline and to a level <60 mL/minute/1.73 m^2, a different antiretroviral is recommended (Lucas *et al.*, 2014).

A prevalence of 4% of renal dysfunction was determined among 318 HIV patients on TDF attending a Naval Medical center in San Diego. Renal dysfunction was defined as GFR rate <60ml/min (Crum-Cianflone et al., 2010).

In 2011, a study among 890 South African patients initiating TDF found only 2.4% prevalence of nephrotoxicity. Nephrotoxicity was defined as any decline in kidney function over 48 weeks of study from baseline using Creatinine clearance calculated by Cockcroft Gault (CG) equation (Brennana et al., 2011).

A Singapore cohort followed for 24 weeks had comparable results to the south African study with 2.2% prevalence of nephrotoxicity reported among 226 Asian patients on TDF. Nephrotoxicity was defined in this latter study as reduction of creatinine clearance to less than 50ml/min (Chua *et al.*, 2012).

In a10-year cohort study, a 52% versus 40% cumulative incidence of reduced renal function among participants on TDF versus non-TDF was reported. This study was conducted on an open cohort of 2532 HIV infected patients in Montreal, Canada. Although this study found a decline in GFR due to TDF, it was a mild decline given the definition of reduced renal function used. Reduced renal function was defined as decline of GFR to <90ml/min over a 3-month period (Laprise *et al.*, 2013).

2.3.3 Determinants of renal function among patients on TDF

Determinants of renal function among patients on TDF have also been extensively studied. Combination of TDF and protease inhibitors such as ritonavir has been found to increase the occurrence of TDF renal toxicity. Studies approximate that 70% of TDF induced renal toxicity may follow combination of TDF with ritonavir boosted protease inhibitors (Izzedine et al., 2009; Zimmermann et al., 2006). Risk factors identified for TDF-induced renal toxicity include existing renal disease, prolonged duration of antiviral treatment, older age, female gender, African ethnicity, severe immunosuppression, and concomitant administration of other nephrotoxic drugs (Crum-Cianflone et al., 2010).

A study on a Swiss Cohort reported female gender (HR 1.62 95%CI 1.17 – 2.25), Diabetes Mellitus (HR 2.34, 95%CI 1.24 – 4.42) and combination with protease inhibitors (HR 1.71, 95%CI 1.30 – 2.24) as significant predictors for TDF related reduction in GFR in multivariate models (Fux et al., 2007).

Significantly higher renal dysfunction was associated with long duration of TDF use (OR 1.54, 95% CI 1.10 – 2.15) and older age (OR 1.99, 95% 1.22 – 3.24) in a San-Diego cohort of HIV infected TDF users (Crum-Cianflone et al., 2010).

Reduced renal function among patients on TDF with older age (HR 1.06 95% CI 1.05 – 1.07), lower baseline renal function (HR 0.93), alcohol use (HR 1.57 95% 1.07 – 2.28) and PI use (1.82 95%CI 1.61 – 2.05) was described in Canada (Laprise et al., 2013). A study among south Africans with HIV reported a relative risk of 3.2 (95%CI 1.3 to 7.8) for death among those with moderate renal dysfunction versus normal renal function over 48 weeks (Brennana et al., 2011).

2.3.4 Sensitivity of phosphate in detecting PTRD

Serum hypophosphatemia and urinary phosphate wasting have been determined as sensitive markers of TDF toxicity (Kinai and Hanabusa, 2005; Waheed *et al.*, 2015). Eleven out of 15 patients with phosphate wasting were discovered in an Egyptian study. A study in a swiss Cohort found 42 – 50% of TDF exposed patients with phosphate wasting while 4% of ART naive patients had phosphate wasting (Fux et al., 2007). An increase in urinary low molecular weight proteins has been used as a gold standard to determine TDF related PTRD (Kinai and Hanabusa, 2005).

CHAPTER THREE: METHODOLOGY

3.1 Study design

This was a cross-sectional study design comparing outcomes (proximal tubular renal dysfunction and global renal function) in TDF regimen use (exposed) versus TDF-sparing regimen (unexposed) groups at the same point in time.

3.2 Study site

Ambulatory HIV care at Moi Teaching and Referral Hospital (MTRH) is provided by the Academic Model Providing Access to Healthcare (AMPATH) program. AMPATH program is collaboration between Moi Teaching and Referral Hospital, Moi University College of Health Sciences, and a group of North American academic medical centers led by Indiana University. The program has enrolled over 160,000 HIV-positive patients in over 144 clinical sites in both urban and rural western Kenya over the last 15 years. MTRH clinic has 4 modules and a youth clinic where HIV patients receive care. Participants for this study were selected from Modules 1,2,3 and the youth clinic where adolescent patients are followed up.

3.3 Study population

3.3.1Target population

The target population comprised of HIV-infected persons attending AMPATH's MTRH clinics in western Kenya. The results of this study are generalizable to all HIV infected patients within the MTRH catchment area in western Kenya. Approximately 12,000 HIV infected patients on ART are enrolled in AMPATH’s urban MTRH clinic with about 3,000 seen monthly.

3.3.2 Sample size

A sample size was calculated for each objective of the study and the largest sample size selected.

Objective 1: To compare proportion of PTRD in exposed and unexposed participants:

A formula for comparison of proportions was used. A sample size of **106** for each group was needed to demonstrate a 16% difference in tubular dysfunction among the exposed and unexposed groups with 90% power and 0.05 significance level plus 10% to account for non-responders and missing data using the formula below (Kirkwood R Betty and Sterne A Jonathan, 2003). The proportion estimates used for the formula were from a Spanish study which found 22% proximal tubular dysfunction among those exposed to TDF and 6% dysfunction in those on TDF sparing regimen (Labarga et al., 2009).

$$n = \frac{\left\{u\sqrt{[\Pi_0(1-\Pi_0)+\Pi_1(1-\Pi_1)]}+\ v\sqrt{[2\Pi(1-\Pi)]}\right\}^2}{(\Pi_1-\Pi_0)^2}$$

Where the parameters: u= 1.96, critical value for significance level of 0.05, v= 1.65, critical value for 90% power, π_0 =0.06 and π_1= 0.22, representing proportion of unexposed and exposed from previous study.

Objective 2: To compare the mean GFR between exposed and unexposed participants:

A formula (shown below) for comparison of means was used to compare the mean GFR in the exposed and unexposed groups The mean and standard deviation for estimated GFR in TDF exposed patients used was mean change of 4 ml/min/1.73m^2

and a standard deviation of 1 for exposed and of 2 ml/min/1.73m^2 for unexposed patients. These figures were derived from a similar Spanish study (Laprise et al., 2013). This formula yielded a sample size of **28** for each group after inflating for non-responders; meaning only 28 participants are needed to show a difference of 4 ml/min between exposed and unexposed participants.

$$n = \frac{(u + v)^2 \times (\sigma_0^2 + \sigma_1^2)}{(u_1 - u_0)^2}$$

Where the parameters: u= 1.96, critical value for significance level of 0.05, v= 1.65, critical value for 90% power, change in mean (u_1-u_0) = 3.9ml/min, standard deviations σ_0 =4 and σ_1= 2.

Objective 3: To assess the determinants of the relationship between PTRD and TDF use:

A formula for logistic regression was used. The minimum sample size (N) based on logistic regression model was obtained using the formula suggested by Peduzzi et al,

N = 10k/p, where k is the number of independent variables and p is the number or events or prevalence of the condition of interest as determined from previous studies (Peduzzi *et al.*, 1996). The number of independent variables in this study were 7; age, sex, co-morbidities, body weight, TDF use, viral load and duration of ARV use. The prevalence was obtained from a Spanish study that determined the prevalence of proximal tubular dysfunction among infected patients as 15% (Labarga et al., 2009). Using the Peduzzi formula the sample size required was 467 total participants. Assuming a non - response rate of 10%, the N was inflated by the formula n ÷ (1-non-response rate). The estimated final sample size needed therefore was **518**, 259 exposed and 259 unexposed participants.

Objective 4:To determine the sensitivity and specificity of hypophosphatemia in determining PTRD: Buderer's formula (shown below) for incorporation of prevalence of disease into sample size calculation for sensitivity and specificity was used (Buderer, 1996). An overall sample size of **486** was needed to determine the diagnostic accuracy of hypophosphatemia in detecting proximal tubular renal dysfunction. This was calculated using the assumptions; a prevalence of 15% of proximal tubular dysfunction among HIV patients on ART, acceptable level of precision (L) of 0.05 and critical value of (v) 1.96 for significance level of 0.05 and an anticipated sensitivity of 95% and specificity of 95%.

$$n= \frac{v^2 * S_N * (1-S_N)}{L^2 * Prevalence} \quad \text{and} \quad n= \frac{v^2 * S_p * (1-S_p)}{L^2 * (1-Prevalence)}$$

Overall, the sample size calculated from Peduzzi formula and adjusted to account for non -responders was enough to answer all the research questions. The total sample size needed therefore was 518 with 259 exposed and 259 unexposed participants.

3.3.1 Sampling and recruitment

Participants for this study were selected through stratified random sampling. The required participants were first stratified according to which ART regimen they were using. A separate list of participants on TDF regimen and TDF-sparing regimen was generated and then computer-generated random numbers were used to select potential participants. The sampling frame used was the number of patients expected to attend clinic per month during the recruitment period (April to June) from the AMPATH medical records systems (AMRS).

3.4 Eligibility

Participants identified from the sampling technique above were checked for eligibility. The inclusion and exclusion criteria are listed below.

3.4.1 Inclusion criteria:

1. Consenting adults >18 years
2. HIV positive on first line ART regimen
3. Attending selected AMPATH outpatient clinics

3.4.2 Exclusion criteria:

1. Participants with an abnormal baseline creatinine at initiation of ART
2. Participants on hemodialysis or follow up for renal disease

3.5 Study procedures

3.5.1 Participant recruitment

Participants were recruited at the AMPATH outpatient HIV clinics. Those who did not meet the eligibility requirement were excluded beforehand. Participants who met the criteria were approached and requested to participate in the study. Figure 1 below shows a summary of the study flow algorithm.

3.5.2 Consenting

The consenting process was carried out by a Good Clinical Practice (GCP) trained research assistant or the principal investigator. Literate participants were provided with written consent form in English (Appendix 1) or Kiswahili (Appendix 2) as per their preference. Participants who could not read had the consent read to them in a language of their choice and a translator was sought when necessary. Data collection was carried out after written informed consent was given.

3.5.3 Clinical and Laboratory procedures

Participants' socio-demographic characteristics and disease status were collected by questionnaire (Appendix 3) and data collection sheet (Appendix 4). Blood pressure, Height and weight and blood sugar (Appendices 5,6,7) were measured before participants were taken to the lab.

Blood and urine specimens (Appendix 8) were collected from each participant for the lab tests needed; Urine B2M, urinary creatinine, urinary phosphate, urinary glucose and serum creatinine, serum phosphate and serum glucose (Appendix 9). All the phlebotomy procedures were carried out under sterile conditions.

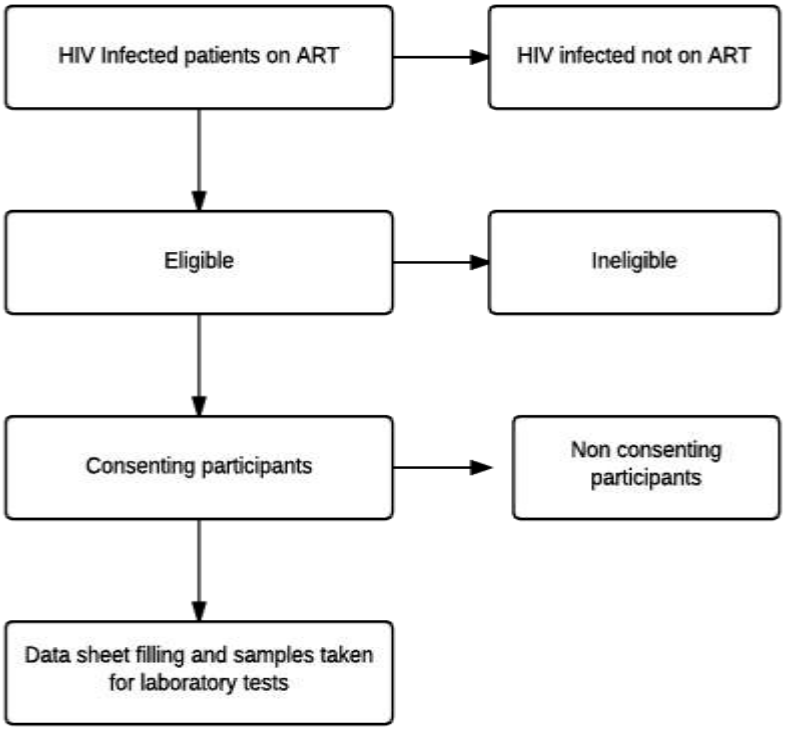

Figure 1: Study flow algorithm

3.6 Data management

3.6.1 Study variables

Dependent variables for this study were PTRD and estimated GFR which represented the overall renal function. PTRD was calculated from other renal function parameters assessed and was defined by any 2 of abnormalities as described in the operational definition. GFR was calculated from serum creatinine and age of the participants by CKD-EPI formula on an excel spreadsheet before being merged with the other variables (NKF, 2017).

The independent variables were use of TDF regimen or TDF-sparing regimen based first line regimen. Covariates were socio-demographic variables, co-morbidities, concomitant medications and HIV disease status.

Socio-demographic variables collected were age, gender, residence, level of education and occupation. Details on infectious and non-infectious co-morbidities and history of

alcohol or smoking and body mass index (BMI) were also collected. HIV disease status included information such as duration of ART use, WHO staging, most recent viral load and CD4 count at baseline.

Confounders determined a priori for this study were age, gender, co-morbidities, duration of ART, HIV status and concomitant medications. Data was collected on drugs known to cause proximal tubular toxicity including aminoglycoside antibiotics, antifungal agents such as amphotericin B, anti-retroviral drugs such as adefovir, anticancer drugs such as cisplatin and foscarnet (Kim and Moon, 2012).

3.6.2 Materials and data collection

Structured interviewer administered questionnaires and data collection sheets were used to collect data. The questionnaires collected data on symptoms presenting within the preceding 3 months, past medical illnesses and alcohol and smoking history.

Data collection sheets were used to collect data from the medical records system and participant charts. Laboratory results were recorded in data collection sheets. Details of HIV disease status were retrieved from patient charts and open MRS system. Once completed, questionnaires and data collection sheets were checked for errors and completeness then double entered into an Epi-data database.

3.6.3 Data cleaning

Data was cleaned during data collection, data entry and analysis. Data collection sheets and questionnaires were checked for completeness and errors at the end of each week during data collection. Data checks were included in the database to ensure data was error free during data entry.

Before analysis, the variables were checked for outliers, inconsistencies, missing data and distribution. Visual inspection of all continuous variables using scatter plots, box plots or histograms was done to identify outliers and distribution of the data. Data with skewed distribution was log transformed then analyzed as normally distributed if they conformed to normal distribution after transformation. Missing data was excluded from the multivariate and univariate analysis. There was no need to collect more data since the sample size had already been inflated to factor in missing data and non- responders.

3.6.4 Data entry

Data was entered into an Epi Data database (Christiansen and Lauritsen, 2010). Validation checks were incorporated into the database to ensure accuracy of data. Double data entry was done to ensure accuracy of the data.

3.6.5 Data protection and security

Questionnaires and data collection sheets were stored under lock and key in a secure cabinet with restricted access. Computers containing any data were password protected. Kaspersky antivirus 2015 and a firewall was installed in the computers containing the database (Kaspersky, 2015). Only the data manager and the principal investigator had access to the forms and database. Data was backed up on to an external hard disk which was stored securely in the care of the principal investigator. Questionnaires and data collection tools will be stored for up to 5 years.

3.6.6 Data analysis

Descriptive analysis was carried out to provide description of the population through means (standard deviations) or medians (interquartile range) and frequencies

(percentages) and presented as graphs or tables in the results section below. Inferential univariate analysis was carried out to determine whether the observed differences between the exposed (TDF) versus unexposed (TDF-sparing) groups was due to chance.

Objective 1 - Proportions were calculated for PTRD in TDF and TDF-sparing group then compared using chi square for statistical significance.

Objective 2: Mean and corresponding standard deviations were calculated for the estimated GFR and then Student T test was used to compare for statistical significance.

Wilcoxon rank sum test was for non-normally distributed continuous variables while Fishers' exact test was where frequencies were small.

Objective 3: Simple logistic regression was used to compare association between TDF exposure and PTRD, crude odds ratio with 95% confidence intervals have been presented. Each of the covariates was checked for association with TDF exposure and PTRD. Factors found to be significantly related to both PTRD and TDF exposure that was not on the causal pathway were included in the final logistic regression model as confounders. The a priori determined confounders; age, sex, co-morbidities were included in the final model regardless of their association with TDF exposure and PTRD.

Multiple logistic regression analysis was carried out to determine the factors affecting the association between TDF exposure and PTRD. Likelihood ratio test was used to determine statistical significance and confidence intervals for adjusted odds ratios have been presented.

Objective 4: Sensitivity, specificity and predictive values were calculated comparing serum hypophosphatemia to the gold standard for PTRD defined as any 2 of proximal tubular abnormalities. Receiver operating characteristics (ROC) curves were plotted for sensitivity and 1-specificity to compare overall accuracy of the gold standard and hypophosphatemia.

Results have been presented in tables, graphs and chart.

3.7 Ethical consideration

Ethical approval was obtained from the Institutional Research and Ethics Review Board (IREC) of Moi University and MTRH and AMPATH administration (Appendix 11). Informed consent was obtained from each participant enrolled into study. Participants were free to withdraw from the study, there were no monetary incentives provided to participate. Results of the participants were communicated back to the primary clinician for the necessary action.

The samples were drawn from patients already attending HIV clinic and this study did not expose them to new risks such as disclosure of HIV status. Adequate measures were taken to protect confidentiality including: all specimens, questionnaires, reports, and other records were de-identified. All records were kept locked. Interviews and clinical evaluations were completed in private examination rooms.

CHAPTER FOUR: RESULTS

4.1 Participants recruited and characteristics

4.1.1 Participants recruited

A total of 539 participants were approached for recruitment, 529 met the inclusion criteria while 10 were excluded because 3 refused to consent, 6 had known diabetes or overt hypertension and 1 participant was on a second line regimen (Figure 2). Out of 516 included in the final analysis, 261 were on TDF regimen while 255 were in the TDF-sparing group.

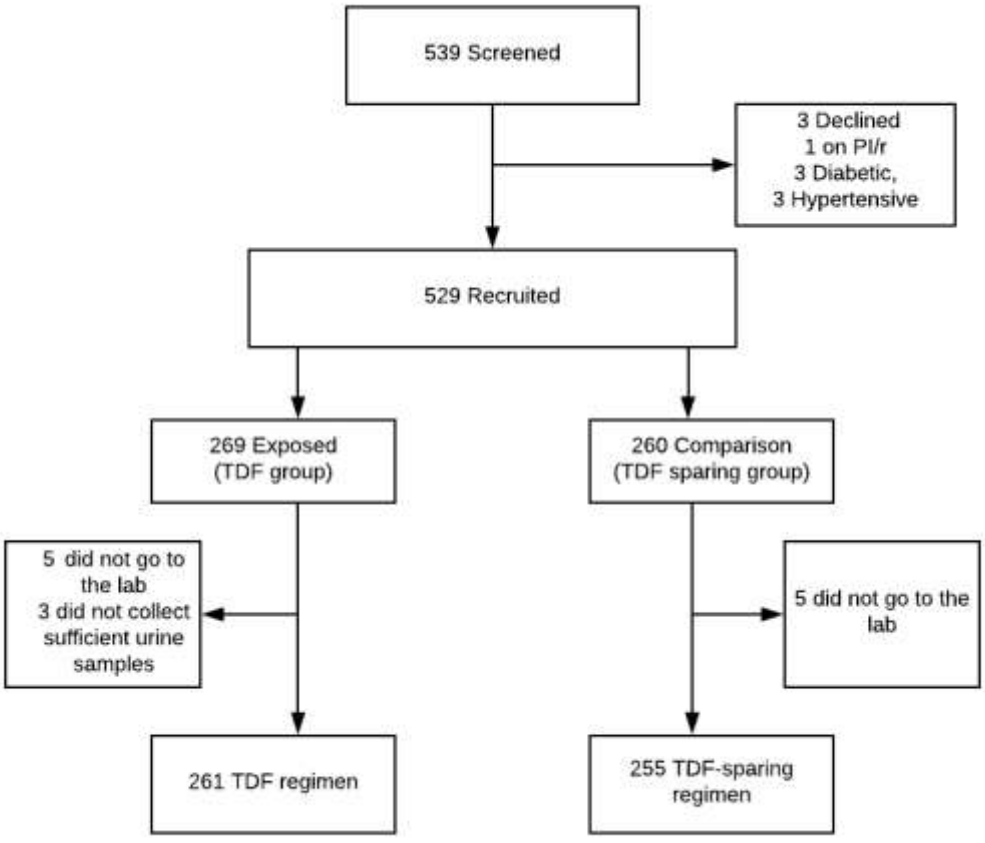

Figure 2: Recruitment schema

4.1.2 Handling of missing data

Missing data was excluded from the multivariable analysis and this was not expected to introduce any bias to the analysis because it was a small percentage missing 13/519 (2.5%). Out of the 13, 10 participants did not go to the lab while 3 did not collect enough urine samples.

4.2 Sociodemographic characteristics

The mean age (SD) of all the 519 participants was 41.5 (12.6) years. Most of the participants fell into the age category of 40 years or more. (60%). TDF-sparing regimen users were significantly ($p < 0.001$) older than those on TDF regimen, mean (SD) 43.9 (12.2) vs 39.2 (12.6) years respectively. Overall female to male ratio was 1.5:1, with majority of the participants being female 313/519 (60.3%). The TDF users had 168/264 (63.3%) female participants compared to 145/255 (56.9%) participants in the TDF-sparing group. The gender distribution in both the groups was not significantly different, $p = 0.115$.

Employment rates in the TDF regimen group were 155/264 (58.7%) and 154/255 (60.4%) in the TDF-sparing group. TDF-sparing regimen users were mostly in employment that did not require special skills such as casual labour (43.5%) while TDF users were mostly unemployed (34%). The variation in the type of employment was statistically significant between the two groups, $p=0.02$. Only 113/519 (21.8%) of participants were unemployed.

A total of 317/519 (61.1%) participants resided in Urban areas while 202/519 (38.9%) were rural residents. Half (50%) of the 519 participants were married, with 25% of the rest being single while 25% were widowed/divorced or separated. About 121/264 (45.8%) in the TDF regimen groups and 140/255 (54.9%) in the TDF-sparing group

were married. Majority (45%) of all the 519 participants had attained secondary school education level. The participants did not have statistically significant differences in residence, marital status and level of education. Table 1 below shows the overall sociodemographic characteristics, comparison by group and p values.

Table 1: Sociodemographic characteristics and comparison by TDF use

Participant characteristics		**Total (% or SD) N=519**	**TDF n=261 (%)**	**TDF-sparing n=255 (%)**	**P value**
Age	18 – 24 years	79 (15.4)	49 (18.9%)	30 (11.8%)	0.001
	25 – 40 years	127 (24.5%)	77 (29.2%)	50 (19.6%)	
	>40 years	312 (60.1%)	135 (51.9%)	175 (68.6%)	
Age	mean (SD)	41.5 (12.6)	39.2 (12.6)	43.9 (12.1)	0.001
Gender	Male	206 (39.7%)	96 (36.4%)	110 (43.1%))	0.115
	Female	310 (60.3%)	165 (63.6%)	145 (56.9%)	
Occupation	Unemployed	112 (21.8%)	89 (34.1%)	86 (33.7%)	0.02
	Skilled	175 (33.9%)	69 (26.5%)	43 (16.9%)	
	Unskilled	195 (37.8%)	84 (32.2%)	111(43.5%)	
	Did not report	34 (6.6%)	19 (7.2%)	15 (5.9%)	
Residence	Rural	202 (38.9%)	95 (36.0%)	107 (42.0%)	0.163
	Urban	314 (61.1%)	166 (64.0%)	148 (58.0%)	
Marital status	Single	127 (24.7%)	72 (27.7%)	55 (21.6%)	0.209
	Married	260 (50.3%)	120 (45.8%)	140 (54.9%)	
	Separated or widowed	127 (24.7%)	68 (26.1%)	59 (23.1%)	
	Did not report	1 (0.3%)	1 (0.4%)	2 (0.4%)	
Level of education	None/Primary	186 (36.2%)	102 (39.4%)	84 (32.9%)	0.087
	Secondary	232 (44.7%)	110 (41.7%)	122 (47.8%)	
	Post-secondary	91(17.7%)	48 (18.6%)	43 (16.9%)	
	Did not report	7 (1.4%)	1 (0.4%)	6 (2.4%)	

4.3 Clinical characteristics

Participants in the TDF-sparing group had more preexisting comorbidities compared to the TDF-regimen group,13.3% versus 6.1% p=0.02. TDF users had higher mean systolic blood pressure (120.2 vs 116.1 mmHg p<0.01), longer duration of ART use (8.0 vs 4.6 years p<0.01), lower baseline CD4 counts (253 vs 328 cells/mm3 p=0.05) and majority were WHO stage 3 (40.0% vs 34.5% p=0.04) compared to TDF sparing group. There were no statistically significant differences between the groups on clinical presentation, family history of medical illnesses, BMI categories, viral load and random blood sugar. Table 2 below presents the overall clinical characteristics and comparison by TDF use.

Table 2: Clinical characteristics and comparison by TDF use

Clinical characteristics		**Total N=516 (%)**	**TDF n= 261 (%)**	**TDF sparing n=255 (%)**	**P value**
Medical complaints	None	482 (92.8%)	243 (93.2%)	236 (92.6%)	0.582
	Body swelling	22 (4.2%)	10 (3.8%)	12 (4.7%)	
	Change in urine	11 (2.1%)	7 (2.7%)	4 (1.6%)	
	Did not report	4 (0.8%)	1 (0.4%)	3 (1.2%)	
Pre-existing comorbidities	None	460 (89.0%)	243 (92.8%)	217 (85.1%)	0.02
	Hypertension	50 (9.6%)	16 (6.1%)	34 (13.3%)	
	Diabetes	1 (0.2%)	0 (0.4%)	0(0.0%)	
	Kidney disease	2 (0.4%)	0 (0.0%)	2 (0.8%)	
	Did not report	4 (0.8%)	1 (0.4%)	3 (1.2%)	
Family history of medical illnesses	None	422 (81.9%)	215 (82.6%)	207 (81.2%)	0.569
	Yes	90 (17.3%)	45 (17.1%)	45 (17.7%)	
	Did not report	4 (0.8%)	1 (0.4%)	3 (1.2%)	
Drug history	None	409 (79.4%)	206 (79.2%)	203 (79.6%)	0.556
	Other nephrotoxic	103 (19.9%)	54 (20.5%)	49 (19.2%)	
	Did not report	4 (0.8%)	1 (0.4%)	3 (1.2%)	
BMI Categories kg/m^2	Underweight <18.5	67 (12.9%)	34 (12.9%)	33 (12.9%)	0.864
	Normal (18.5–24.9)	302 (58.6%)	150 (57.6%)	152 (59.6%)	
	Overweight (≥ 25)	147 (28.5%)	77 (29.6%)	70 (27.5%)	
Systolic Blood pressure (SBP)	Mean (SD) mmHg	118.3 (15.4)	116.1 (14.9)	120.2 (15.6)	0.001
Diastolic Blood pressure (DBP)	Mean (SD) mmHg	72.6 (10.5)	71.6 (10.3)	73.6 (10.6)	0.03
Duration of ART use	Mean (SD) years	6.3 (3.5)	4.6 (3.4)	8.0 (2.7)	0.001
HIV-1 viral load	Undetectable	420 (80.9%)	217 (82.2%)	203 (79.6%)	0.237
	Detectable	94 (18.1%)	42 (15.9%	52 (20.4%)	
	Did not report	5 (1.0%)	5 (1.9%)	0 (0.0%)	
CD4 categories at baseline	Median (IQR) $cells/mm^3$	283.5 (288.5)	305 (328)	276 (253)	0.05
Random blood sugar	Median (IQR) mmol/l	4.9 (0.9)	5.0 (0.8)	4.9 (0.9)	0.575
WHO stage	1	183 (35.5%)	102 (39.0%)	81 (31.8%)	0.041
	2	98 (19.1%)	54 (20.8%)	44 (17.3%)	
	3	192 (37.2%)	90 (34.5%)	102 (40.0%)	
	4	42 (8.1%)	14 (5.3%)	28 (11.0%)	
	Did not report	1 (0.2%)	1 (0.4%)	0.0(0%)	

4.4 Laboratory results

4.4.1 Proximal tubular renal dysfunction (PTRD) (Objective 1)

Proportion of participants with hypophosphatemia was 62/516 (12%), phosphate wasting 16/516 (3.1%), metabolic acidosis 200/516 (38.8%) and non-diabetic glucosuria was 1/516 (0.2%) in the overall group. These parameters were not significantly different in the comparison groups. Participants on TDF use were 2.51 times more likely to have B2 microglobulin in urine compared to those on TDF sparing regimen Unadjusted Odds Ratio (UOR) 2.51 (95% CI 1.50 to 4.21). Tubular proteinuria was also more likely among TDF-regimen users compared to TDF-sparing group UOR 3.45 (95% CI 1.88 to 6.32). Dipstick proteinuria was not significantly different in the two groups UOR 2.5 (95%CI 0.77 to 8.11).

The proportion of PTRD was 26/261 (10.0%) for the TDF-regimen group compared to 8/255 (3.1%) for the TDF-sparing group. The likelihood of PTRD was significantly higher in TDF vs TDF-sparing group, UOR 3.42 (95%CI 1.50 to 7.76). TDF users were 3.42 times more likely to have PTRD than TDF-sparing regimen users. Table 3 represents the results for the tests to determine tubular renal function.

Table 3: Renal function and comparison of renal function assessment parameters among participants by TDF use

Renal function parameter		**Total N=516**	**TDF-regimen n=261**	**TDF-sparing regimen n=255**	**Unadjusted Odds Ratio (95% CI)**
PTRD	**No**	**482 (93.4%)**	**235 (90.0%)**	**247(98.9%)**	**1**
	Yes	**34 (6.6%)**	**26 (10.0%)**	**8 (3.1%)**	**3.41 (1.50 to 7.76)**
Hypophosphatemia	No	454 (88.0%)	237 (90.8%)	217 (85.1%)	1
	Yes	62 (12.0%)	24 (9.2%)	38 (14.9%)	0.57 (0.33 to 1.00)
Phosphate wasting	No	500 (96.9%)	254 (97.3%)	246 (96.5%)	1
	Yes	16 (3.1%)	7 (2.7%)	9 (3.5%)	0.75 (0.27 to 2.06)
Metabolic acidosis	No	316 (61.2%)	152 (58.2%)	164 (64.3%)	1
	Yes	200 (38.8%)	109 (41.8%)	91 (35.7%)	1.29 (0.91 to 1.84)
Non-diabetic glucosuria	No	515 (99.8%)	261 (100.0%)	254 (99.6%)	1
	Yes	1 (0.2%)	0 (0.0%)	1 (0.4%)	0
B2M in urine	<0.3mg/dl	435 (84.3%)	205 (78.5%)	230 (90.2%)	1
	>0.3mg/dl	81 (15.7%)	56 (21.5%)	25 (9.8%)	2.51 (1.50 to 4.21)
Tubular proteinuria	No	451 (87.4%)	212 (81.2%)	239 (93.7%)	1
	Yes	65 (12.6%)	49 (18.8%)	16 (6.3%)	3.45 (1.88 to 6.32)
Proteinuria	No	502 (97.3%)	251 (96.2%)	251 (98.4%)	1
	Yes	14 (2.7%)	10 (3.8%)	4 (1.6%)	2.5 (0.77 to 8.11)

4.4.2 Estimated glomerular filtration rate (Objective 2)

Global renal function was determined by serum creatinine and glomerular filtration rate. The mean estimated GFR (SD) was 112.8 (21.5) vs 109.7 (21.9) ml/min/1.73m^2 for the TDF and TDF-sparing group respectively with UOR 1.00 (95% CI 0.99 to 1.01). Although 55/516 (10.7%) of the participants had elevated serum creatinine, this was not significantly different in the two groups UOR 1.00 (95%CI 0.99 to 1.01). Table 4 shows the estimated GFR and serum creatinine categories with comparison by TDF use.

The mean estimated GFR was found to be 93.3 vs 112.5ml/min/1.73m^2 (p=0.001) for participants who had PTRD vs those who did not have PTRD.

Table 4: Overall Renal function assessed by Estimated Glomerular filtration rate

Renal function parameter		**Total N=516**	**TDF-regimen n=261**	**TDF – sparing regimen n=255**	**Unadjusted Odds Ratio (95% CI)**
Estimated GFR ml/min/1.73m^2	**mean (SD)**	**111.3 (21.7)**	**112.8 (21.5)**	**109.7 (21.9)**	**1.00 (0.99 to 1.01)**
Serum creatinine umol/l	<80.0 (Normal)	461 (89.3%)	236 (90.4%)	2(8.2%)	1
	>80.0 Elevated)	55 (10.7%)	25 (9.6%)	30 (11.8%)	0.79 (0.45 to 1.39)

4.4.3 Factors associated with PTRD (Objective 3)

TDF-regimen users were 3.41 times more likely to have PTRD compared to TDF-sparing group UOR 3.41 (95%CI 1.50 to 7.76). This relationship remained positive, Adjusted OR, (AOR) 3.0, 95% CI 1.12 to 7.75, after adjustment of other factors in the multivariate analysis. Participants with BMI within normal range (18.5 to 24.9 kg/m^2) were 69% less likely to have PTRD compared to underweight participants AOR 0.31(95% CI 0.12 to 0.78). Increasing age, female gender, CD4 < 350 cells/mm^3, concomitant use of nephrotoxic medication, detectable viral load and increasing duration of ART use did increase the likelihood of PTRD in both univariate and multivariate analysis. Table 5 below presents the results for the univariate (UOR) and multivariate (AOR) logistic regression analysis.

Table 5: Factors associated with PTRD

Participant characteristics		**Unadjusted OR (95%CI)**	**Adjusted OR (95%CI)**
TDF use	No	1	1
	Yes	3.41 (1.52 to 7.69)	3.00 (1.16 to 7.75)
Age	Years	1.02 (0.99 to 1.05)	1.03 (0.99 to 1.06)
	Male	1	1
Gender	Female	0.73 (0.36 to 1.47)	0.81 (0.35 to 1.83)
Co-morbidities	None	1	
	Yes	0.67 (0.25 to 1.77)	0.63 (0.13 to 3.14)
	< 350 cells/mm^3	1	1
CD4 at start	> 350 cells/mm^3	0.70 (0.33 to 1.49)	0.71(0.31 to 1.59)
	Underweight <18.5	1	1
	Normal (18.5–24.9)	0.38 (0.16 to 0.90)	0.31 (0.12 to 0.78)
BMI (kg/m^2)	Overweight (≥ 25)	0.37 (0.13 to 1.01)	0.35 (0.11 to 1.08)
Concomitant medication	None	1	1
	Nephrotoxic	0.67 (0.25 to 1.77)	0.81 (0.28 to 2.31)
	Undetectable	1	1
Viral load	Detectable	1.42 (0.62 to 3.24)	1.41 (0.57 to 3.51)
Duration of ART	Years	0.89 (0.81 to 0.98)	0.92 (0.81 to 1.03)

4.4.4 Diagnostic accuracy of hypophosphatemia (Objective 4)

The diagnostic accuracy of hypophosphatemia (serum phosphate < 0.85mmol/l) in determining tubular toxicity was compared to gold standard methods. The gold standard method for determining tubular toxicity from a spot urine specimen is presence of 2 or more tubular abnormalities such as normoglycemic glucosuria, metabolic acidosis, Beta 2 microglobulinuria, and fractional excretion of phosphate >20%.

The sensitivity of hypophosphatemia was 12.9% (95% CI 6.1 to 24.4%), while specificity was 88.8% (95% CI 85.6 to 91.4%). Predictive values are largely dependent on disease prevalence, the overall PTRD prevalence of 6.6% was in these

study participants. The positive and negative predictive value were 12.9% (6.1 to 24.4%) and 94.3% (91.6 to 93.9%) respectively (see table 6 below).

The ratio between the probability of hypophosphatemia in the presence of PTRD and the probability of a hypophosphatemia in the absence of PTRD is indicated by the positive likelihood ratio 2.1 (95% 1.09 to 4.05).Positive likelihood ratio < 10 in this study indicates poor value of hypophosphatemia in increasing certainty about the presence of PTRD. The negative likelihood ratio of 0.86 (95% CI 0.71 to 1.04) is not significant as it overlaps 1(Table 6).

Receiver operating characteristics curves were plotted to evaluate diagnostic utility of hypophosphatemia as a continuous variable (Figure 3). The ROC curve further demonstrates the **non-discriminative** ability of hypophosphatemia in diagnosing PTRD (area under the ROC curve = 0.38). ROC curve for fractional excretion of phosphate has an area of 0.74 which indicates more discriminative ability of fractional excretion of phosphate in determining PTRD. Serum creatinine (ROC area 0.61) performed better than phosphate but no better than FePO4.

Table 6: Evaluating the diagnostic accuracy of hypophosphatemia in determining PTRD

Statistic	Value	95% Confidence interval
Prevalence of PTRD	6.6%	4.7 to 9.2%
Test sensitivity	23.5%	11.4 to 41.6%
Test specificity	88.8%	85.6 to 91.4%
Positive predictive value	12.9%	6.1 to 24.4%
Negative predictive value	94.3%	91.6 to 93.9%
Positive likelihood ratio	2.10	1.09 to 4.05
Negative likelihood ratio	0.86	0.71 to 1.04
Accuracy	84.5%	81.1% to 87.5%

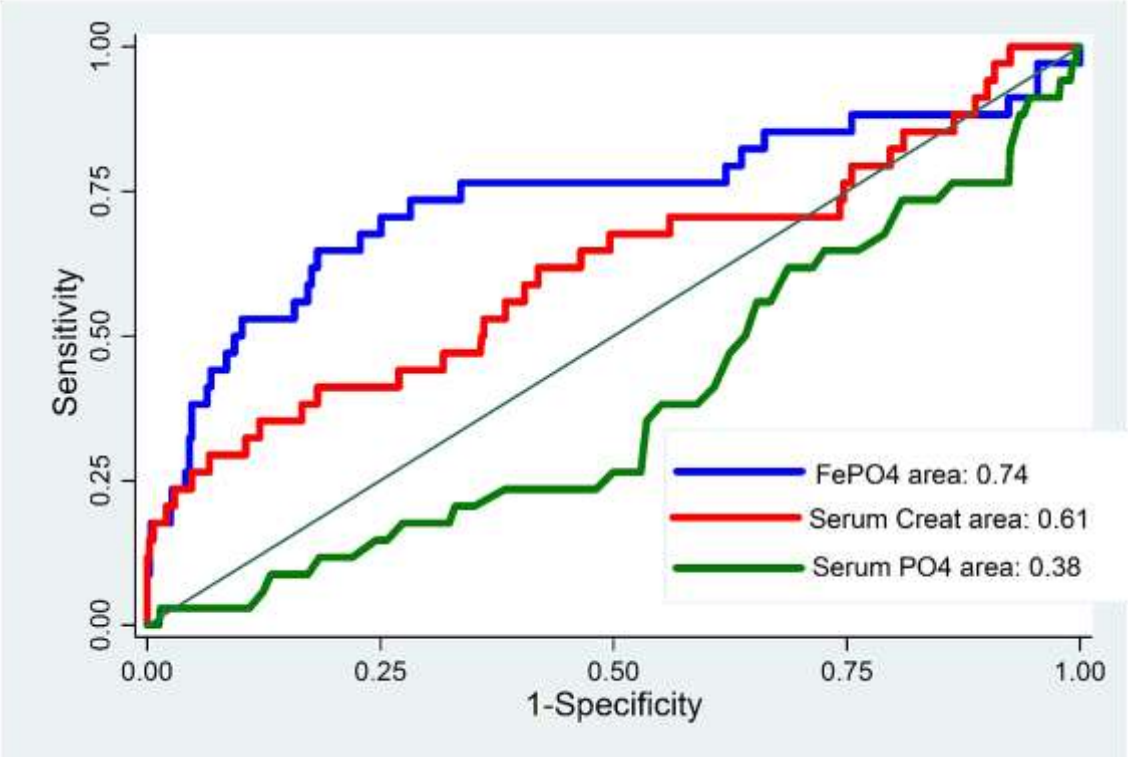

Figure 3: Receiver operating characteristics curves

CHAPTER FIVE: DISCUSSION

5.1 Key findings

Participants in the TDF-sparing regimen group were older in age and therefore had used ART for a longer duration compared to the TDF group. The age difference also explains why the TDF-sparing group had more comorbidities and higher mean systolic blood pressure. The TDF group had higher proportion of PTRD compared to TDF-sparing users (10.0% vs 3.0% $p<0.001$). The mean estimated GFR was 112.8 vs 109.7 mL/min/1.73 m^2 for TDF vs TDF-sparing regimen in this study which was not significantly different($p=0.80$). Tubular proteinuria was more likely among TDF users compared to TDF-sparing regimen users. Despite the presence of high proportion of PTRD, TDF users had relatively preserved GFR.

TDF users were 3 times more likely to have PTRD. Body Mass Index (BMI) greater than 18.5 was associated with less likelihood of proximal tubulopathy. There were no other factors significantly associated with PTRD. There was no significant confounding by age, gender, comorbidities, baseline CD4, concomitant medication, viral load or duration of ART use in this study. This study demonstrated TDF use and BMI as significant determinants of PTRD.

The present study reports a high specificity (88.8%) and a low sensitivity (23.5%) with a low positive likelihood ratio (2.10) of hypophosphatemia in detecting proximal tubulopathy. The study also demonstrates poor discriminatory ability for serum phosphate levels by ROC curves. Fractional excretion of phosphate had a better discriminative ability for detecting PTRD compared to hypophosphatemia.

5.2 Interpretation

5.2.1 Proportion of proximal tubular renal dysfunction

TDF use in first line regimens has increased since the release of WHO 2013 guidelines. The release of the 2015 guidelines further recommended test and treat strategy for all HIV infected persons. Several studies showed a high frequency of renal tubular dysfunction in TDF-treated patients in comparison with patients receiving other ART regimens. The present study reports a significantly higher proportion (10% VS 3%) of renal tubular dysfunction among use of TDF versus a TDF-sparing regimen. Higher proportion of tubular dysfunction have also been reported in Ghana (35% vs 6%), Germany (17% vs 3%), Spain (22% vs 6%) and France (31% vs 15%) (Chadwick *et al.*, 2015; Dauchy et al., 2011; Labarga et al., 2009; Mauss *et al.*, 2005). These studies however found much higher prevalence among those on TDF compared to the present study probably because the variations in the populations of study. Such variations may include concomitant use of second line regimens and race such as in the Spanish, French and German cohort. These studies done in Europe studied Caucasian and did not exclude use of second line regimens which are known to worsen TDF toxicity (Dauchy et al., 2011; Labarga et al., 2009; Mauss et al., 2005).

Further differences in the studies can be explained by immigration of populations at high risk to developed countries. The distribution of apolipoprotein 1 (*APOL1)* risk alleles is highest among individuals from West Africa compared to East and South Africans. East Africans, which is the sample form which this study was drawn, have least expression of APOL1 (Jose *et al.*, 2018). Therefore, West Africans have higher rates of kidney disease compared to East Africans 20% vs 14% (Kaze *et al.*, 2018).

A cross-sectional study of HIV-infected persons ART in Uganda found more proteinuria by those using TDF compared non-TDF use (22.0 % vs 11.3 %), P < 0.001. The same Ugandan study however found the higher proportion of hypophosphatemia among the Non-TDF ART compared to TDF-ART group (16.6% vs 10.6 %), P = 0.012 (Salome *et al.*, 2016). This Ugandan study did not find similar results to this because of differences in outcome determination. The present study used a more definitive test to determine proximal tubulopathy while the research in Uganda used on proteinuria and phosphate. These can parameters are not specific to TDF toxicity.

5.2.2 Estimated Glomerular filtration rates by TDF use

The non-significant difference in estimated GFR found in this study (112.8 vs 109.7 mL/min/1.73 m^2 p=0.8) has also been demonstrated in Ghana (99 vs 96 mL/min/1.73 m^2 p=0.21), Spain (109 vs 119 mL/min/1.73 m^2 p=0.1), Germany (106 vs 104 p=0.375) and Canada (104.9 vs 103.5mL/min/1.73 m^2 p>0.05). A cohort of Taiwanese HIV infected patients also demonstrated non-significant annual decline in estimated GFR between persons on TDF and TDF-sparing 2.7 vs 1.8 mL/min/1.73 m^2 p =0.567.

Guidelines on TDF use have been based on several studies that showed no significant renal dysfunction among TDF users compared to TDF-sparing regimens such as the present study. These non-significant results may be the result of short-term duration of TDF use among the participants in this study. TDF toxicity may be remain subclinical for several years before global function as measured by estimated GFR is impaired. Evidence of tubular dysfunction in the absence of change in estimated GFR has been demonstrated in this study.

Statistically significant differences in mean estimated GFR (102 vs 105 mL/min/1.73 m^2 p=0.01) were described in a Ugandan study. The difference in the Ugandan study was however very small and may not be clinically significant in making decisions. This difference may have resulted from the use of different way of estimating GFR by the Ugandan study where the authors used Cockcroft-Gault formula.

Although there was a statistically significant lower mean estimated GFR for those who had PTRD vs those who did not (93.3 vs 112.5ml/min/1.73m^2p<0.001), the level of GFR was not clinically significant. According to the National Kidney foundation, these GFR falls in the mild loss to normal range (NKF, 2017).

5.2.3 Factors associated with proximal tubular renal dysfunction

Previous studies from sub-Saharan Africa identify lower CD4 cell counts, older age, gender as risk factors for greater significant renal impairment (Nelson *et al.*, 2007) (Bygrave et al., 2011; L. Mulenga *et al.*, 2014). A similar cohort of HIV infected persons in Tanzania found factors predictors of renal dysfunction in multivariate analysis include female gender (OR 3.0, 95% CI;1.8–5.1, p<0.0001), BMI) <18.5 (OR 2.3 95% CI;1.3–4.1, p=0.004), CD4+ T-cell count <200 cells/mm^3 (OR 2.3 95% CI; 1.1–4.8, p=0.04), and WHO clinical stage II or above (OR 1.6 [1.2–2.3], p=0.001)(Msango *et al.*, 2011). This contrasts greatly with this study which only described normal BMI (OR 0.35, P<0.001) as a significant related protective factor.

TDF use was significantly associated with increased likelihood of tubular toxicity in this study. This finding of significant Odds Ratio (95% CI) in this study was comparable to studies in Ghana 3.43 (95% CI 1.10–10.69%), Spain 21.6 (95% CI

4.1–113%) and Zambia 3.11 (95% CI 2.52–3.87%) which showed that TDF users had significantly more tubular toxicity than TDF-sparing regimen (Chadwick et al., 2015; Labarga et al., 2009). This was an expected finding because this study TDF causes tubular injury as previously described in literature.

5.2.4 Sensitivity and specificity of hypophosphatemia

Hypophosphatemia was identified for validation in this study because it could have a double origin in TDF toxicity; reduced proximal reabsorption of phosphate and decreased vitamin D activation (Tourret et al., 2013). This means that it is the most likely electrolyte abnormality to be identified in TDF toxicity. A study of 15 Caucasian HIV-infected persons with presumed TDF toxicity reported serum phosphate and urine phosphate wasting as more sensitive markers for TDF-induced proximal tubulopathy (Waheed et al., 2015). In this study, hypophosphatemia however had very low sensitivity (12.9%) for determining PTRD although it had high specificity (88%) which may have resulted from the use of non-fasting phosphate levels.

Similar findings to our have been attributed to the multifactorial origin of hypophosphatemia in HIV infected persons which means that even those who were not on TDF could have hypophosphatemia (Pedrosa Naudín *et al.*, 2015). The effect of TDF in relation to phosphoremia have also been described as time dependent with stabilization of the loss after 6 months of TDF use (Badiou *et al.*, 2006). These studies, including the present study, suggest that hypophosphatemia as a marker of renal toxicity related to TDF in HIV infected persons is inconclusive. Furthermore, hypophosphatemia might be useful if followed up from baseline before any therapy is

started (Pedrosa Naudín et al., 2015). A cross sectional study carried out on TDF users in Tokyo to compare the diagnostic

accuracy of tubular markers with a collection of abnormalities found an AUC of fractional excretion of phosphate of 0.76 (95%CI 0.61 to 0.91). This was comparable to this study which found an AUC of 0.74.

5.3 Strengths and limitations

5.3.1 Strengths

This study used gold standard methods to determine PTRD (Nishijima *et al.*, 2013). Beta 2 microglobulin was used to determine tubular proteinuria and this improved the likelihood to detect early subclinical toxicity.

The study data was very robust because a large sample size was used. This sample size allowed sufficient power for all the objectives and any exploratory sub-group analysis.

5.3.2 Limitations

The use of non-fasting serum phosphate and spot urinary phosphate levels may have led to the underestimation of the participants with tubular dysfunction in this study. This may have also led to decreased sensitivity of phosphate as a proxy for PTRD.

5.4 Generalizability

Stratified random sampling that was used in this study improved the external validity of the results. The population studied is comparable to most in African HIV programs and therefore results can be generalized. The sample size was sufficiently large enough to estimate the prevalence of the PTRD of interest with adequate precision.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

In conclusion, there was significant proximal tubulopathy in HIV patients on TDF compared to TDF-sparing regimen. There was no significant difference in the mean estimated GFR in the 2 groups. The median duration of ART use was 6 years in the two groups therefore these findings could vary over longer duration of time.

TDF use was associated with PTRD and normal BMI which was found to be protective. Other factors such as age, sex, duration of ART use, Viral load and presence of comorbidities were not significantly associated with PTRD despite being apriori selected as risk factors. Serum phosphate levels are not useful as a proxy for detecting tubular dysfunction although fractional excretion of phosphate maybe a useful proxy.

The clinical significance of these findings in terms of progression to renal failure or bone loss may not be clear in the short term.

6.2 Recommendations

Periodic screening of tubular function parameters should be recommended to patients receiving TDF. A subsequent study to establish the clinical significance of tubular dysfunction in terms of progression to chronic kidney disease and bone loss should be carried out.

REFERENCES

Anderson, P. L., Kiser, J. J., Gardner, E. M., Rower, J. E., Meditz, A., & Grant, R. M. (2010). Pharmacological considerations for tenofovir and emtricitabine to prevent HIV infection. *Journal of Antimicrobial Chemotherapy*, *66*(2), 240-250.

Arribas, J. R., Pozniak, A. L., Gallant, J. E., DeJesus, E., Gazzard, B., Campo, R. E., ... & Toole, J. J. (2008). Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *47*(1), 74-78.

Arthur, G. C., & John, H. E. (2011). *Textbook of Medical Physiology*. Philadelphia: Elsevier Saunders.

Atta, M. G., & Fine, D. M. (2009). Editorial comment: tenofovir nephrotoxicity--the disconnect between clinical trials and real-world practice. *AIDS Read*, *19*(3), 118-119.

Badiou, S., De Boever, C. M., Terrier, N., Baillat, V., Cristol, J. P., & Reynes, J. (2006). Is tenofovir involved in hypophosphatemia and decrease of tubular phosphate reabsorption in HIV-positive adults?. *Journal of Infection*, *52*(5), 335-338.

Bakris, G. L., Fonseca, V. A., Sharma, K., & Wright, E. M. (2009). Renal sodium–glucose transport: role in diabetes mellitus and potential clinical implications. *Kidney international*, *75*(12), 1272-1277.

Boron, W. F. (2006). Acid-Base Transport by the Renal Proximal Tubule. *Journal of the American Society of Nephrology*, *17*(9), 2368-2382.

Brennana, A., Evans, D., Maskew, M., Naicker, S., Ive, P., & Sanne, I. (2011). Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. *AIDS*, *25*, 1603 - 1609.

Buderer, N. M. F. (1996). Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Academic Emergency Medicine*, *3*(9), 895-900.

Bygrave, H., Kranzer, K., Hilderbrand, K., Jouquet, G., Goemaere, E., Vlahakis, N., . . . Ford, N. (2011). Renal safety of a tenofovir-containing first line regimen: experience from an antiretroviral cohort in rural Lesotho. *PLoS One*, *6*(3), e17609.

Campbell, L. J., Dew, T., Salota, R., Cheserem, E., Hamzah, L., Ibrahim, F., . . . Post, F. A. (2012). Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. *BMC Nephrol*, *13*, 85.

Cassetti, I., Madruga, J. V. R., Suleiman, J. M. A., Etzel, A., Zhong, L., Cheng, A. K., ... & Study 903E Team. (2007). The safety and efficacy of tenofovir df in combination with lamivudine and efavirenz through 6 years in antiretroviral-naïve HIV-1—infected patients. *HIV clinical trials*, *8*(3), 164-172.

CDC. (2018). Pre-Exposure Prophylaxis (PrEP). Retrieved Mar 19/2018, from https://www.cdc.gov/hiv/risk/prep/index.html

Chadwick, D. R., Sarfo, F. S., Kirk, E. S., Owusu, D., Bedu-Addo, G., Parris, V., ... & Phillips, R. (2015). Tenofovir is associated with increased tubular proteinuria and asymptomatic renal tubular dysfunction in Ghana. *BMC nephrology*, *16*(1), 195.

Chapman, T., McGavin, J., & Noble, S. (2003). Tenofovir disoproxil fumarate. *Drugs*, *63*(15), 1597-1608.

Christiansen, T., & Lauritsen, J. (2010). (Ed.) EpiData - Comprehensive Data Management and Basic Statistical Analysis System. Odense Denmark, EpiData Association, 2010

Chua, A. C., Llorin, R. M., Lai, K., Cavailler, P., & Law, H. L. (2012). Renal safety of tenofovir containing antiretroviral regimen in a Singapore cohort. *AIDS Res Ther*, *9*(1), 19.

Coca, S., & Perazella, M. A. (2002). Rapid communication: acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. *The American journal of the medical sciences*, *324*(6), 342-344.

Cockcroft, D. W., & Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*, *16*(1), 31-41.

Conti, M., Moutereau, S., Zater, M., Lallali, K., Durrbach, A., Manivet, P., ... & Loric, S. (2006). Urinary cystatin C as a specific marker of tubular dysfunction. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *44*(3), 288-291.

Cooper, R. D., Wiebe, N., Smith, N., Keiser, P., Naicker, S., & Tonelli, M. (2010). Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clinical Infectious Diseases*, *51*(5), 496-505.

Crum-Cianflone, N., Ganesan, A., Teneza-Mora, N., Riddle, M., Medina, S., Barahona, I., & Brodine, S. (2010). Prevalence and factors associated with renal dysfunction among HIV-infected patients. *AIDS patient care and STDs*, *24*(6), 353-360.

Dauchy, F. A., Lawson-Ayayi, S., de La Faille, R., Bonnet, F., Rigothier, C., Mehsen, N., ... & Dupon, M. (2011). Increased risk of abnormal proximal renal tubular function with HIV infection and antiretroviral therapy. *Kidney international*, *80*(3), 302-309.

Del Palacio, M., Romero, S., & Casado, J. L. (2012a). Proximal tubular renal dysfunction or damage in HIV-infected patients. *AIDS Rev, 14*(3), 179-187.

Del Palacio, M., Romero, S., & Casado, J. L. (2012b). The use of biomarkers for assessing HAART-associated renal toxicity in HIV-infected patients. *Curr HIV Res, 10*(6), 521-531.

Delaney, W. E., Ray, A. S., Yang, H., Qi, X., Xiong, S., Zhu, Y., & Miller, M. D. (2006). Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. *Antimicrobial agents and chemotherapy*, *50*(7), 2471-2477.

Eknoyan, G., & Levin, N. W. (2002). K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis, 39*(2 Suppl 1), S1-266.

Ezinga, M., Wetzels, J. F., Bosch, M. E., Van Der Ven, A. J., & Burger, D. M. (2014). Long-term treatment with tenofovir: prevalence of kidney tubular dysfunction and its association with tenofovir plasma concentration. *Antivir Ther, 19*(8), 765-771.

Fernandez-Fernandez, B., Montoya-Ferrer, A., Sanz, A. B., Sanchez-Nino, M. D., Izquierdo, M. C., Poveda, J., ... & Ruiz-Ortega, M. (2011). Tenofovir nephrotoxicity: 2011 update. *AIDS research and treatment, 2011*.

Fux, C. A., Simcock, M., Wolbers, M., Bucher, H. C., Hirschel, B., Opravil, M., . . . Furrer, H. (2007). Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. *Antivir Ther, 12*(8), 1165-1173.

Gallant, J. E., & Deresinski, S. (2003). Tenofovir Disoproxil Fumarate. *Clinical Infectious Diseases, 37*(7), 944-950.

Gallant, J. E., Parish, M. A., Keruly, J. C., & Moore, R. D. (2005). Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clinical Infectious Diseases, 40*(8), 1194-1198.

Gallant, J. E., Staszewski, S., Pozniak, A. L., DeJesus, E., Suleiman, J. M., Miller, M. D., ... & 903 Study Group. (2004). Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *Jama, 292*(2), 191-201.

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A. K., & Vernekar, S. N. (2010). Markers of renal function tests. *North American Journal of Medical Sciences, 2*(4), 170-173.

Grant, P. M., & Cotter, A. G. (2016). Tenofovir and bone health. *Current Opinion in HIV and AIDS, 11*(3), 326.

Grim, S. A., & Romanelli, F. (2003). Tenofovir disoproxil fumarate. *Ann Pharmacother, 37*(6), 849-859.

Gupta, S. K., Anderson, A. M., Ebrahimi, R., Fralich, T., Graham, H., Scharen-Guivel, V., ... & Wyatt, C. M. (2014). Fanconi syndrome accompanied by renal function decline with tenofovir disoproxil fumarate: a prospective, case-control study of predictors and resolution in HIV-infected patients. *PloS one*, *9*(3), e92717.

Han, W. K., Bailly, V., Abichandani, R., Thadhani, R., & Bonventre, J. V. (2002). Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int*, *62*(1), 237-244.

Haverkort, M. E., van der Spek, B. W., Lips, P., Slieker, W. A., Ter Heine, R., Huitema, A. D., & Bronsveld, W. (2011). Tenofovir-induced Fanconi syndrome and osteomalacia in two HIV-infected patients: role of intracellular tenofovir diphosphate levels and review of the literature. *Scandinavian journal of infectious diseases*, *43*(10), 821-826.

Ichimura, T., Bonventre, J. V., Bailly, V., Wei, H., Hession, C. A., Cate, R. L., & Sanicola, M. (1998). Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *Journal of Biological Chemistry*, *273*(7), 4135-4142.

Islam, F. M., Wu, J., Jansson, J., & Wilson, D. P. (2012). Relative risk of renal disease among people living with HIV: a systematic review and meta-analysis. *BMC public health*, *12*(1), 234.

Izzedine, H., Harris, M., & Perazella, M. A. (2009). The nephrotoxic effects of HAART. *Nature Reviews Nephrology*, *5*(10), 563.

Jose, S., Hamzah, L., Jones, R., Williams, D., Winston, A., Burns, F., ... & Post, F. A. (2018). Chronic kidney disease risk in African and Caribbean populations with HIV. *The Journal of infectious diseases*, *218*(11), 1767-1772.

Kalyesubula, R., & Perazella, M. A. (2011). Nephrotoxicity of HAART. *AIDS Research and Treatment*, *2011*.

Karras, A., Lafaurie, M., Furco, A., Bourgarit, A., Droz, D., Sereni, D., ... & Molina, J. M. (2003). Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clinical Infectious Diseases*, *36*(8), 1070-1073.

Karras, A., Lafaurie, M., Furco, A., Bourgarit, A., Droz, D., Sereni, D., ... & Molina, J. M. (2003). Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus. *Clinical Infectious Diseases*, *36*(8), 1070-1073.

Kaze, A. D., Ilori, T., Jaar, B. G., & Echouffo-Tcheugui, J. B. (2018). Burden of chronic kidney disease on the African continent: a systematic review and meta-analysis. *BMC nephrology*, *19*(1), 125.

Kearney, B. P., Mathias, A., Mittan, A., Sayre, J., Ebrahimi, R., & Cheng, A. K. (2006). Pharmacokinetics and safety of tenofovir disoproxil fumarate on coadministration with lopinavir/ritonavir. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *43*(3), 278-283.

Kim, S. Y., & Moon, A. (2012). Drug-induced nephrotoxicity and its biomarkers. *Biomolecules & therapeutics*, *20*(3), 268.

Kinai, E., & Hanabusa, H. (2005). Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. *AIDS*, *19*(17), 2031-2033.

Kirkwood R Betty, & Sterne A Jonathan. (2003). *Essential Medical Statistics* (Vol. 2). Oxford: Blackwell publishing company.

Kohler, J. J., Hosseini, S. H., Green, E., Abuin, A., Ludaway, T., Russ, R., ... & Lewis, W. (2011). Tenofovir renal proximal tubular toxicity is regulated by OAT1 and MRP4 transporters. *Laboratory investigation*, *91*(6), 852.

Kyaw, N. T. T., Harries, A. D., Chinnakali, P., Antierens, A., Soe, K. P., Woodman, M., ... & Fernandez, M. (2015). Low incidence of renal dysfunction among HIV-infected patients on a Tenofovir-based first line antiretroviral treatment regimen in Myanmar. *PLoS One*, *10*(8).

Kyaw, N. T. T., Harries, A. D., Chinnakali, P., Antierens, A., Soe, K. P., Woodman, M., ... & Fernandez, M. (2015). Low incidence of renal dysfunction among HIV-infected patients on a Tenofovir-based first line antiretroviral treatment regimen in Myanmar. *PLoS One*, *10*(8), e0135188.

Lacey, M. J. (2014). Impact of Pharmaceutical Innovation in HIV/AIDS Treatment During the Highly Active Antiretroviral Therapy (HAART) Era in the US, 1987–2010.

Laprise, C., Baril, J. G., Dufresne, S., & Trottier, H. (2012). Association between tenofovir exposure and reduced kidney function in a cohort of HIV-positive patients: results from 10 years of follow-up. *Clinical infectious diseases*, *56*(4), 567-575.

Levey, A. S., Stevens, L. A., Schmid, C. H., Zhang, Y., Castro, A. F., Feldman, H. I., . . . Coresh, J. (2009). A New Equation to Estimate Glomerular Filtration Rate. *Annals of internal medicine, 150*(9), 604-612.

Lucas, G. M., Lau, B., Atta, M. G., Fine, D. M., Keruly, J., & Moore, R. D. (2008). Chronic kidney disease incidence, and progression to end-stage renal disease, in HIV-infected individuals: a tale of two races. *The Journal of infectious diseases*, *197*(11), 1548-1557.

Lucas, G. M., Ross, M. J., Stock, P. G., Shlipak, M. G., Wyatt, C. M., Gupta, S. K., . . . Kalayjian, R. C. (2014). Clinical Practice Guideline for the Management of Chronic Kidney Disease in Patients Infected With HIV: 2014 Update by the HIV Medicine Association of the Infectious

Diseases Society of America. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *59*(9), e96-e138.

Lyseng-Williamson, K. A., Reynolds, N. A., & Plosker, G. L. (2005). Tenofovir disoproxil fumarate: a review of its use in the management of HIV infection. *Drugs*, *65*(3), 413-432.

Malik, A., Abraham, P., & Malik, N. (2005). Acute renal failure and Fanconi syndrome in an AIDS patient on tenofovir treatment—case report and review of literature. *Journal of Infection*, *51*(2), E61-E65.

Markowitz, M., Zolopa, A., Squires, K., Ruane, P., Coakley, D., Kearney, B., . . . Lee, W. A. (2014). Phase I/II study of the pharmacokinetics, safety and antiretroviral activity of tenofovir alafenamide, a new prodrug of the HIV reverse transcriptase inhibitor tenofovir, in HIV-infected adults. *J Antimicrob Chemother*, *69*(5), 1362-1369.

Mauss, S., Berger, F., & Schmutz, G. (2005). Antiretroviral therapy with tenofovir is associated with mild renal dysfunction. *AIDS*, *19*(1), 93-95.

Molina, J. M., Clumeck, N., Orkin, C., Rimsky, L. T., Vanveggel, S., & Stevens, M. (2014). Week 96 analysis of rilpivirine or efavirenz in HIV-1-infected patients with baseline viral load </= 100 000 copies/mL in the pooled ECHO and THRIVE phase 3, randomized, double-blind trials. *HIV Med*, *15*(1), 57-62.

Msango, L., Downs, J. A., Kalluvya, S. E., Kidenya, B. R., Kabangila, R., Johnson, W. D., . . . Peck, R. N. (2011). Renal Dysfunction among HIV-Infected Patients Starting Antiretroviral Therapy in Mwanza, Tanzania. *AIDS*, *25*(11), 1421-1425.

Mulenga, L. B., Kruse, G., Lakhi, S., Cantrell, R. A., Reid, S. E., Zulu, I., . . . Chi, B. H. (2008). Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. *AIDS*, *22*(14), 1821-1827.

Mulenga, L., Musonda, P., Mwango, A., Vinikoor, M. J., Davies, M. A., Mweemba, A., ... & Wandeler, G. (2014). Effect of baseline renal function on tenofovir-containing antiretroviral therapy outcomes in Zambia. *Clinical infectious diseases*, *58*(10), 1473-1480.

Naesens, L., Balzarini, J., Bischofberger, N., & De Clercq, E. R. I. K. (1996). Antiretroviral activity and pharmacokinetics in mice of oral bis (pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl) adenine, the bis (pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl) adenine. *Antimicrobial agents and chemotherapy*, *40*(1), 22-28.

Naicker, S. (2009). End-stage renal disease in sub-Saharan Africa. *Ethn Dis*, *19*(1 Suppl 1), S1-13-15.

Nelson, M. R., Katlama, C., Montaner, J. S., Cooper, D. A., Gazzard, B., Clotet, B., . . . Rooney, J. F. (2007). The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS*, *21*(10), 1273-1281.

Nishijima, T., Shimbo, T., Komatsu, H., Takano, M., Tanuma, J., Tsukada, K., ... & Oka, S. (2013). Urinary beta-2 microglobulin and alpha-1 microglobulin are useful screening markers for tenofovir-induced kidney tubulopathy in patients with HIV-1 infection: a diagnostic accuracy study. *Journal of infection and chemotherapy*, *19*(5), 850-857.

NKF. (2017). National Kidney Foundation GFR Calculator from https://www.kidney.org/professionals/kdoqi/gfr_calculator

Pedrosa Naudín, M., Briones Cuesta, E., Dueñas Gutierrez, C., Güemes Garcia, M., & Machín Morón, M. (2015). PS-034 Hypophosphatemia in HIV patients treated with antiretroviral treatment. *European Journal of Hospital Pharmacy*, *22*(Suppl 1), A149-A150.

Peduzzi, P., Concato, J., Kemper, E., Holford, T. R., & Feinstein, A. R. (1996). A simulation study of the number of events per variable in logistic regression analysis. *Journal of clinical epidemiology*, *49*(12), 1373-1379.

Pitisci, L., Demeester, R., & Legrand, J.-C. (2014). Prevalence and European AIDS Clinical Society (EACS) criteria evaluation for proximal renal tubular dysfunction diagnosis in patients under antiretroviral therapy in routine setting. *Journal of the International AIDS Society*, *17*(4Suppl 3), 19564.

Pollak, M. R. (2008). Kidney disease and African ancestry. *Nat Genet*, *40*(10), 1145-1146.

Post, F. (2014). Adverse events: ART and the kidney: alterations in renal function and renal toxicity. *J Int AIDS Soc*, *17*(4 Suppl 3), 19513. doi: 10.7448/ias.17.4.19513

Post, F. A., Moyle, G. J., Stellbrink, H. J., Domingo, P., Podzamczer, D., Fisher, M., ... & Branco, T. (2010). Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1–infected adults: 48-week results from the ASSERT study. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *55*(1), 49-57.

Post, F. A., Wyatt, C. M., & Mocroft, A. (2010). Biomarkers of impaired renal function. *Curr Opin HIV AIDS*, *5*(6), 524-530.

Pozniak, A. (2008). Tenofovir: what have over 1 million years of patient experience taught us?. *International journal of clinical practice*, *62*(8), 1285-1293.

Rector, F. C., Jr. (1983). Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am J Physiol*, *244*(5), F461-471.

Reid, A., Stohr, W., Walker, A. S., Williams, I. G., Kityo, C., Hughes, P., . . . Gibb, D. M. (2008). Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. *Clin Infect Dis, 46*(8), 1271-1281.

Rodriguez-Novoa, S., Alvarez, E., Labarga, P., & Soriano, V. (2010). Renal toxicity associated with tenofovir use. *Expert Opin Drug Saf, 9*(4), 545-559.

Rodriguez-Nóvoa, S., Alvarez, E., Labarga, P., & Soriano, V. (2010). Renal toxicity associated with tenofovir use. *Expert Opinion on Drug Safety, 9*(4), 545-559.

Röling, J., Schmid, H., Fischereder, M., Draenert, R., & Goebel, F. D. (2006). HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy—Induced Nephropathy. *Clinical Infectious Diseases, 42*(10), 1488-1495.

Rosenvinge, M. M., Gedela, K., Copas, A. J., Wilkinson, A., Sheehy, C. A., Bano, G., . . . Sadiq, S. T. (2010). Tenofovir-linked hyperparathyroidism is independently associated with the presence of vitamin D deficiency. *J Acquir Immune Defic Syndr, 54*(5), 496-499.

Salome, T., Kasamba, I., Mayanja, B. N., Kazooba, P., Were, J., Kaleebu, P., & Munderi, P. (2016). The effect of Tenofovir on renal function among Ugandan adults on long-term antiretroviral therapy: a cross-sectional enrolment analysis. *AIDS Res Ther, 13*(1), 28.

Slocum, J. L., Heung, M., & Pennathur, S. (2012). Marking renal injury: can we move beyond serum creatinine? *Transl Res, 159*(4), 277-289. doi: 10.1016/j.trsl.2012.01.014

Some, F., Koech, M., Chesire, E., & Kigen, G. (2017). Reversal of tenofovir induced nephrotoxicity: case reports of two patients. *Pan Afr Med J, 27*, 126. doi: 10.11604/pamj.2017.27.126.10033

Stohr, W., Reid, A., Walker, A. S., Ssali, F., Munderi, P., Mambule, I., . . . Hakim, J. (2011). Glomerular dysfunction and associated risk factors over 4-5 years following antiretroviral therapy initiation in Africa. *Antivir Ther, 16*(7), 1011-1020.

Szczech, L. A., Gupta, S. K., Habash, R., Guasch, A., Kalayjian, R., Appel, R., . . . Winston, J. A. (2004). The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int, 66*(3), 1145-1152.

Tourret, J., Deray, G., & Isnard-Bagnis, C. (2013). Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? *J Am Soc Nephrol, 24*(10), 1519-1527.

Traynor, J., Mactier, R., Geddes, C. C., & Fox, J. G. (2006). How to measure renal function in clinical practice. *BMJ, 333*(7571), 733-737. doi: 10.1136/bmj.38975.390370.7C

Ustianowski, A., & Arends, J. E. (2015). Tenofovir: What We Have Learnt After 7.5 Million Person-Years of Use. *Infectious Diseases and Therapy, 4*(2), 145-157.

Verhelst, D., Monge, M., Meynard, J. L., Fouqueray, B., Mougenot, B., Girard, P. M., . . . Rossert, J. (2002). Fanconi syndrome and renal failure induced by tenofovir: a first case report. *Am J Kidney Dis, 40*(6), 1331-1333.

von Kleist, M., Metzner, P., Marquet, R., & Schutte, C. (2012). HIV-1 polymerase inhibition by nucleoside analogs: cellular- and kinetic parameters of efficacy, susceptibility and resistance selection. *PLoS Comput Biol, 8*(1), e1002359. doi: 10.1371/journal.pcbi.1002359

Waheed, S., Attia, D., Estrella, M. M., Zafar, Y., Atta, M. G., Lucas, G. M., & Fine, D. M. (2015). Proximal tubular dysfunction and kidney injury associated with tenofovir in HIV patients: a case series. *Clinical Kidney Journal, 8*(4), 420-425. doi: 10.1093/ckj/sfv041

WHO. (2013). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection.

WHO. (2015). Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV.

Zhuo, J. L., & Li, X. C. (2013). Proximal Nephron. *Comprehensive Physiology, 3*(3), 1079-1123

Zimmermann, A. E., Pizzoferrato, T., Bedford, J., Morris, A., Hoffman, R., & Braden, G. (2006). Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. *Clinical Infectious Diseases, 42*(2), 283-290

Zolopa, A., Sax, P. E., DeJesus, E., Mills, A., Cohen, C., Wohl, D., . . . Szwarcberg, J. (2013). A randomized double-blind comparison of coformulated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate versus efavirenz/emtricitabine/tenofovir disoproxil fumarate for initial treatment of HIV-1 infection: analysis of week 96 results. *J Acquir Immune Defiyc Syndrom, 63*(1), 96-100.

APPENDICES

Appendix 1: Informed Consent (English)

Introduction: My name is Dr Mercy J Karoney. I am a licensed medical doctor currently pursuing a Master's degree in Internal medicine at Moi University. I would like to request you to participate in this study that will screen you for abnormal kidney function. This is a consent form that gives you information about this study.

You are free to ask questions about this study at any time. If you agree to take part in this study, we will ask you to sign this consent form. If you chose not to participate in this study, you will continue receiving care without any discrimination. You are also free to withdraw from this study at any time without fear of discrimination.

Purpose of the study

The study is being done on people living with HIV who are on ART to determine if the ART drug they are using is affecting their renal function. You have been selected to participate because you are either on ART regimen that contains Tenofovir Disoproxil Fumarate (TDF) or you are on a regimen not containing TDF and you will be used to compare.

TDF is a drug used to treat HIV infection that is very commonly used. This drug has been found to cause kidney abnormalities although this effect has not been well established in our setting because few studies have been done. This study will be important in finding out how many people are suffering from the effects of Tenofovir so that we can do tests to make sure we prevent these side effects

Procedures for the study

If you decide to join this study, you will need to be seen in the clinic at least once. You will also be required to fill in a questionnaire. This will contain your basic details: age, sex, occupation; health assessment and risk assessment questionnaire. The evaluations required will take about 30 minutes to complete. We will draw 10ml (1 tablespoon) of blood and collect about 20ml (2 tablespoons) of urine from you to carry out the tests. The samples collected from you will only be used for study related testing and will be stored in a secure place (with usual protections of your identity)

until the tests are done. Your kidney function and whether Tenofovir has affected this kidney function will be determined from these blood and urine tests. Any leftover samples will be destroyed afterwards. Results of testing performed on these samples will be given to you. If you are found to have any abnormality you will be referred to a physician for further review. You will not be required to pay any money for the study.

The study team will assign a code number to all of your study-related records. This number (not your name or other information that could be used to identify you) will be used for laboratory tests related to this study. Only the study staff will have access to your information. Any publication of this study will not use your name or identify you personally. Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

Your consent

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

Name……Sign ……Date……

Investigator/ Research assistant

Name……Sign……Date…….

Appendix 2: Informed consent (Swahili)

Utangulizi

Jina langu ni Daktari Mercy Karoney. Mimi ni daktari aliyefuzu nakusajiliwa na bodi ya madaktari wa Kenya (Kenya Medical Practitioners and Dentists Board). Mimi ni msomi wa shahada ya juu (Masters) ya udaktari (Internal medicine)katika chuo kikuu cha Moi University. Ningependa kukuomnba ushiriki kwenye utafiti utakaochnguza kama figo yako infanya kazi vizuri. Hii ni idhini inayotoa taarifa zaidi kuhusu uchunguzi huu.

Uko huru kuuliza maswali kuhusu utafiti huu wakati wowote. Kama unakubali kushiriki katika utafiti huu, utahitajika kutia sahihi kwenye fomu ya idhini. Ikiwa hutakubali kushiriki katika uchunguzi huu, utapata matibabu yako ya kawaida wala hutabaguliwa. Uko huru kuondoka kwenye utafiti huu wakati wowote pia bila ubaguzi.

Sababu ya utafiti huu

Utafiti huu unafanywa kati ya watu wanaoishi na virusi vya ukimwi na ambao wanatumia madawa ya virusi au ARV, kudhibitisha kama haya madawa ya virusi yanaweza kuathiri figo. Umechaguliwa kushiriki kwa sababu unatumia ARV ambayo imepatikana kuwa an athari kwa figo, Tenofovir Disoproxil Fumarate (TDF) au unatumia madawa yasiyokuwa na TDF ili kulinganisha.

TDF ni dawa inayotumika kutibu maambukizi ya HIV na hutumika sana. Dawa hii imepatikana kusababisha athari kwa figo ingawa athari hii haijadhibitishwa katika mazingira yetu kwa sababu tafiti chache zimefanyika. Utafiti huu utakuwa muhimu katika kutafuta jinsi watu wengi wanateseka kutokana na athari za tenofovir ili tuweze kufanya vipimo kuhakikisha kuwa tunazuia madhara haya.

Taratibu ya utafiti

Kama ungependa kushiriki katika utafiti huu, baada ya kusoma na kutiwa sahihi kwenye idhini, utajaza hojaji. Hojaji ambayo utajaza una maelezo yako ya msingi: umri, jinsia, kazi; tathmini ya afya na tathmini ya hatari.

Kama utajiunga na utafiti huu, utahitajika kuonekana katika kliniki angalau mara moja. Itachukua nusu saa kukamilisha vipimo vyote. Tutachukua damu kidogo kama kijiko kimoja (10ml) mkojo (20ml) kutoka kwako ili tufanye vipimo vya kudhamini hali ya figo na kama una athari ya figo kutokana na kutumia tenofovir. Ikiwa utapatikana kuwa na athari yoyote kutokana na vipimo, utatumwa kwa daktari wa ili upate usaidizi zaidi. Sampuli zitakazochukuliwa zitatumika tu kwa ajili ya utafiti na zitahifadhiwa katika mahali salama (pamoja na ulinzi wa wa utambulisho wako) hadi ukamilisho. Kama figo zako zinafanya kazi au kama Tenofovir imeathiri figo itajulikana kutoka vipimo hivi vya damu na mkojo.

Sampuli yoyote itakayobaki haitahifadhiwa kwa matumizi ya baadaye. Hautahitaji kulipa chochote katika utafiti huu.

Jina lako halitatumika kwa rekodi au sampuli zozote ili kulinda utambulisho wako. Timu ya utafiti itakupa kodi ya siri ya kutambulisha rekodi zako zote. Uchapishaji yoyote kutokana na utafiti huu pia hautatumia jina lako au utambulisho wako. Juhudi zitafanywa kuweka rekodi zako siri. Hatuwezi kuthibitisha usiri kabisa kwani maelezo yako ya kibinafsi inaweza kuwasilishwa kwa amri ya sheria. Hakutakuwa na njia ya kuunganisha sampuli yako na wewe kwani tutatumia nambari wala hatutafichua maelezo yoyote utakayotupa.

Idhini yako

Nimeelezwa ipasavyo ya kwamba ninashiriki katika uchunguzi wa usomi utakayochunguza kama nimeathirika kwa kutumia ARV. Mchunguzi pia amenieleza kuwa sitakosa matibabu yangu ya kawaida iwapo nishiriki katika uchunguzi au nisiposhiriki. Pia nimeelezwa kuwa sitahitajika kulipia chochote kinachohusiana na uchunguzi huu.

Jina……Sahihi……Tarehe……

Msaidizi

Jina……Sahihi……Tarehe……

Appendix 3: Questionnaire (Interviewer administered)

Please answer the following questions as best as you can. In the first part you will be asked about your basic details followed by health assessment questions. Please tick your answer in the space provided. Do not hesitate to ask any questions or for clarifications

1. Basic information

Date of Birth (Dd/Mm/Yy)	**Occupation**
Gender Male Female	**Residence**
Marital Status Please tick the appropriate answer Single ☐ Married ☐ Separated ☐ Divorced ☐ Widowed ☐	**Level of education** Please tick the appropriate answer Primary: Incomplete ☐Complete ☐ Secondary: Incomplete ☐ Complete ☐ Tertiary: Incomplete ☐ Complete ☐ Adult education ☐ Never been to any formal school ☐

Medical history

Do you have any of these symptoms? Or have you ever been diagnosed with any of the conditions listed below	**Yes**	**No**
Body swelling		
Reduced urination		
Darkening of urine (‘cola’ colored urine)		
High blood pressure (hypertension)		
High blood sugar (diabetes)		
Kidney disease		
Heart disease		
Anybody in your nuclear family with the above diseases?		

Drug history

Are you on any of these medicines? Have you been on any of these recently in the past month?	**Yes**	**No**
Septrin		
Paracetamol		
Aspirin		
Brufen		
Gentamicin		
Other aminoglycoside		
Other		
Don’t know the name of the medicine		
Herbal medication		
No other medication used recently		

2. What time did you have your last meal?

Appendix 4: Data collection form

1. Vital signs and anthropometry	Height (meters)	
	Weight (kg)	
	Blood pressure (mm/Hg)	
	Random blood sugar	
2. HIV history	Date of diagnosis	
	Date of enrollment	
	WHO stage	
	CD4+ at before ART initiation	
	Last viral load (cp/ml)	
3. Laboratory results	Urine B2M	
	Urine creatinine	
	Urine Phosphate (mmol/l)	
	Serum phosphate (mmol/l)	
	Serum creatinine (umol/l)	
	Dipstick glucose (present/absent)	
4. Calculated values	Urine B2M: urine creatinine	
	Basal Metabolic Index (kg/m^2)	
	Fractional excretion of phosphate	
	Estimated GFR/ overall renal function	
	PRTD (Present/absent)	

Appendix 5: Procedures for measuring blood pressure

Blood pressure was taken using an Omron M2 compact upper arm blood pressure monitor.

The participants sat in a quiet place, in a relaxed sitting position with no tight-fitting clothing on the upper arm, or any thick clothing such as a sweater. The blood pressure was taken with the participant upright, back straight and arm on the table so that the cuff was on the same level as the heart. The cuff was wrapped snugly on the right arm with the bottom of the cuff was at least 1cm above the elbow. The start button on the machine was pressed and automatically the cuff inflated, and the machine took a reading. The blood pressure results as well as a heart rate reading was displayed on the screen and then recorded by the research assistant. Any abnormally high or low recordings were repeated then also done on the other arm. In the case of many abnormality, the highest recording was taken.

High blood pressure readings were confirmed manually using a mercury sphygmomanometer. The blood pressure machines were calibrated every week.

Appendix 6: Procedures for measuring height and weight

Anthropometric measurements were taken by a calibrated weight and height machine. A balanced beam scale with height measuring scale was used. Calibration occurred at the beginning and end of each examining day and was corrected if weighing error was greater than 0.2kg. The results of checking the recalibrations were recorded in a logbook. Participants were asked to remove any heavy outer garments then stand at the center of the platform with weight evenly distributed to both feet.

The height bar was placed on the top of the participants head and the reading was taken from the scale. The weights on the beam balance were moved until they balanced, and the measurement taken.

Appendix 7: Procedures for taking specimens

The procedures were explained to the patient and verbal consent sought. Blood and urine specimen were collected from each participant. Universal precautions for venipuncture will be observed. A tourniquet was applied at a distal site about 5cm proximal to the selected site of venipuncture. The participant was asked to make a fist without pumping the hand. The phlebotomist put on a pair of clean gloves and cleaned the selected site thoroughly with methylated spirit starting with the center and working outward then allowed it to dry. The participant's arm was grasped firmly using the thumb to keep the skin taut and to anchor the vein.

A sterile Vacutainer® system was opened and the blood collection needle inserted gently into the lumen of the vein at an angle of 15- 30^{o}, then the other end attached to a Vacutainer® blood collection bottle. Blood was expected to flow freely into the bottle due to negative pressure.

Plain bottles not containing additive were used to collect 2ml of blood for creatinine and 2ml for phosphate determination. After adequate blood was collected, the tourniquet was released then the Vacutainer® needle removed gently and a dry swab was applied at the site under pressure. Pressure was applied for a whole minute then the site reassessed for continued bleeding. The area was then dressed in a dry gauze and tape.

Midstream urine specimen was collected in a sterile container.

Appendix 8: Procedure for measuring blood sugar

Blood sugar was measured using from the sample taken using venipuncture. An OptiumXceed™ blood glucometer was used. A new blood measuring strip (manufacturer specific) was inserted in the glucometer then checked to ensure it was ready. The machine works on the principle that glucose in the patient's blood reacts with chemicals in the test strip to produce a current that the machine measures and converts it to a glucose level reading.

A drop of blood was applied at the test pad of the blood measuring strip. The machine took about 5 seconds before displaying a result. In case of error, the process was repeated. The machine displays the result in mmol/L.

Appendix 9: Lab standard operating procedures

Procedure for measuring Serum creatinine and urinary creatinine

Equipment	Cobas Integra® 400 plus analyzer Computerized Workstation and Printer Centrifuge for separating blood samples 1000 µl pipettors Cobas cups
Reagents	Creatinine cassette Calibrators cfas Controls (2 Levels Precinorm and Precipath) Normal saline Cobas integra cleaner cassette
Principle	Buffered kinetic jaffe reaction without deproteinization: in alkaline solution creatinine reacts with picrate to form a yellow red product. Creatinine + picric acid alkaline ph = yellow red complex The rate of the dye formation (color intensity) is directly proportional to the creatinine concentration in the specimen.
Method	Blood in plain Vacutainer® bottles or urine in an acid were washed detergent free container and taken immediately to the lab. Blood specimens were stored for up to one day at 20 to 25^{o}C up to seven days at 4 to 8^{o}C and up to six months at -20 to -80^{o}C. Urine was stored up to 8 hours at 20 to 25^{o}C.The specimens are set onto a centrifuge and spun at 3000 rpm for 3 minutes to separate the serum from the cells. The supernatant was carefully suctioned using a micropipette and transferred to a sample cup. The Cobas Integra® 400 plus analyzer uses the Jaffe reaction to quantify creatinine. The analyzer read out this absorbance and based on its software it calculated the serum creatinine. It printed out the result on paper. Quality control checks are run daily.
Reference range	Female >18 years <80umol/l Male >18 year <106umol/l

Procedure for measuring Serum phosphate and urinary phosphate

Equipment	Cobas Integra® 400 plus analyzer Computerized Workstation and Printer Centrifuge for separating blood samples 1000 µl pipettors Cobas cups
Reagents	R_1: Reagent blank in vial A and B (Liquid). R_2: SR phosphate reagent in vial C (Liquid).
Principle	Endpoint method where inorganic phosphate forms an ammonium phosphomollybdate complex with ammonium molybdate in the presence of sulfuric acid
Method	Blood in plain Vacutainer® bottles or urine in an acid washed detergent free container was taken immediately to the lab. Blood specimens were stored for up to one day at 20 to 25^{o}C up to seven days at 4 to 8^{o}C and up to six months at -20 to -80^{o}C. Urine was stored up to 8 hours at 20 to 25^{o}C. The specimens were set onto a centrifuge and spun at 3000 rpm for 3 minutes to separate the serum from the cells. The supernatant was carefully suctioned using a micropipette and transferred to a sample cup. This method utilized ammonium molybdate as the color-forming reagent. Measurement of the final product occurs at 340 nm (secondary wavelength 700 nm). Inorganic phosphate formed an ammonium phosphomolybdate complex having the formula (NH4)3PO4 (MoO3)12 with ammonium molybdate in the presence of sulfuric acid. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration.
Reference range	0.87 – 1.45 mmol/L (2.7 -4.5 mg/dL) in blood

Procedure for urinalysis

Equipment	Uristix® strip
Principle	Techniques for measuring glucosuria was based on glucose oxidase test. Hydrogen peroxide was generated and reacted with horseradish peroxidase to produce nascent oxygen. It in turn oxidized orthotoluidine to produce the blue or purple color that is read. In the Tes-Tape process, oxidized orthotoluidine reacted with the yellow dye tartrazine to produce a greater range of color development.
Method	Midstream urine was collected in an acid washed non detergent containing container and stored at 20-25^{0}C. The edge of the Uristix®strip was run against the rim of the urine container to remove excess urine. The strip was held in a horizontal position and reactions read visually. The strip test area was compared to that on the Uristix® color chart. This was read after 60 seconds. The color at the center of the pad was compared to the corresponding color chart on the bottle label. Two observers read the color and a third person acted as a tie-breaker if there is disagreement. Normal and abnormal controls were run daily to ensure validity of results.
Reference range	Glucose in urine ≥ 1+

Procedure for measuring Beta 2 microglobulin in urine

Equipment	Microplate reader Data reduction software Multichannel dispenser or repeatable pipette for 100ul Vortex mixer Laboratory timing device Measuring cylinder Plastic container for storage of the wash solution
Reagents	Control positive and negatives Calibrators Sample buffer Enzyme conjugate containing B2 microglobulin antibodies TMB substrate Stop solution Wash buffer containing Tris detergent
Principle	Highly purified anti human Beta 2- microglobulin antibodies were bound to microwells. Beta 2 microglobulin, if present in diluted urine was bound to the respective antibody. Washing the microwells removed unbound unspecific serum and plasma components. During incubation with anti-beta 2 microglobulin enzyme conjugated immunologically mediated a conjugate/antibody/ antigen complexes were formed. Washing of the microwells removed the unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzed to form a blue colour. The addition of an acid stopped the reaction forming a yellow end-product. The intensity of this yellow colour was measured photometrically at 450nm. The amount of color was directly proportional to the concentration of antibodies present in the original sample.
Method	Urine in acid washed containers was stored for up to 5 days at 2 to 8^{o}C, up to seven days up to six months at -20^{o}C. Diluted the urine samples 1:10 before the assay, was added 100 ul of urine to 900ul sample buffer. Pipette 100ul of calibrators, controls and prediluted participant samples into the wells was carried out then incubated for 30 minutes at room temperature (20-28^{o}C). Washing 3 times with 300ul of water was done. Dispensed 100ul of enzyme conjugate into each well and incubated for 15 minutes at room temperature then washed 3 times with 300ul of water. One hundred microliters of TMB substrate was added into each well and incubated for 15 minutes then stop solution was added. The optical density was read at 450nm and results were calculated.

Appendix 10: Dissemination

The results of this study will be widely disseminated to AMPATH administration and clinicians, to Government and stakeholders and policy makers through regional meetings and reports.

Preliminary results will be communicated to AMPATH stakeholders through the monthly Tuesday meeting on work in progress updates. At the end of the study, a CME will be organized to relay the findings to clinicians around western Kenya. If funds will not be available, then the information will be communicated in the AMPATH newsletter after necessary approval has been sought.

The study findings will also be presented at least 1 local and 1 international conference and will be published in a peer reviewed journal.

Appendix 11: IREC Approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

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

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/21
Approval Number: 0001659

27th June, 2016

Dr. Mercy Karoney Jelagat,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 JUN 2016
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Dr. Karoney,

RE: FORMAL APPROVAL

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

"Proximal Tubular Renal Dysfunction among HIV Patients on Tenofovir Versus Tenofovir Sparing Regimen in Western Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1659*** on 27th June, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th June, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

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

Sincerely,

For

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD