CHAPTER ONE: INTRODUCTION

1.0. Background

Sub- Saharan Africa endures over 60 % of the world's burden of the HIV disease (Banda, 2010). In 2007, it was estimated that over 22 million people were living with HIV in Sub- Saharan Africa according to Naicker (2009) and it remains the epicenter of the epidemic(Arendse, 2010). An estimated 66 % of adults, 86 % of children with HIV are in the Sub- Sahara; and 70 % of all AIDS deaths occurred in the same region(Naicker, 2009, MOHZ, 2010). In Zambia, approximately 82,700 people had HIV in 2009; the overall adult prevalence of the disease was 14 %, and 1.6 % of the adult population became newly infected with HIV each year (MOHZ, 2010).

Renal disease disproportionately affects patients living with HIV(Banda, 2010). HIV infected patients of African origin have a greater risk of renal diseases(Reid, 2008). Chronic kidney disease is three to four folds more frequent in Africa than in industrialized countries in non-HIV patients (Naicker, 2009) and(Brennan, 2011). From some outpatient renal screening, the prevalence of renal dysfunction in HIV was reported to be varying from 6 % to 50 % according to Mulenga (2008). Mulenga, (2008) described a prevalence of renal dysfunction of 34 % among HIV infected outpatients commencing Highly Active Antiretroviral Therapy (HAART) and added that depending on the criteria used to define renal dysfunction, the prevalence may be up to 10 times higher in hospitalized HIV infected patients(Mulenga, 2008, Banda, 2010).

Kidney disease remains an important cause of morbidity and mortality among persons living with HIV in the HAART era and they exhibit a higher risk of renal insufficiency, proteinuria, and EndstageRinal disease (ESRD) compared to the general population (Kalayjian, 2011). Death within 90 days of initiation of Antiretroviral Therapy (ART) was found to be more common amongst HIV/ AIDS patients who had preexisting renal insufficiency than those who had no renal insufficiency and the risk of death increased with the severity of the preexisting renal insufficiency (Mulenga, 2008).

Nephrotoxicity has been shown to be an important complication of HIV infection, particularly in patients with preexisting renal dysfunction(Brennan, 2011). Nephrotoxicity and renal tubular injury may be induced by the antiretroviral drug TenofovirDysoproxilFumarate (TDF) which is a Nucleoside Reverse Transcriptase Inhibitor (NRTI) (Gallant, 2005, Spaulding, 2011, Ro¨ ling, 2006, Patel, 2010).

However, in June, 2007 Zambia became one of the first African countries to include TDF in their first line of ART regimen following recommendation by World Health Organization (WHO) as TDF demonstrated comparable efficacy when compared with other first- line regimens containing Stavudine (D4T), Zidovudine (AZT) or Abacavir (ABC) and also because TDF had the additional advantage of low toxicity and availability as a once daily (Bygrave, 2011, WHO, 2012). Initially, D4T- based regimen or AZT- based regimen with Lamivudine (3TC) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine (NVP) or Efavirenz (EFV), were recommended as first line ARV agents. It was then changed to TDF which substituted D4T- based regimen and AZT- based regimen as the preferred NRTI alongside 3TC for patients with a creatinine clearance of 50mL/minute or more(MOHZ, 2010). But, since creatinine clearance was often not calculated, TDF was routinely prescribed in patients with serum creatinine of 120µmol/L or less and ABC was prescribed instead of TDF for those with impaired renal function (MOHZ, 2010).

This study, therefore, was aimed at determining whether patients on treatment with TDF- based regimen develop renal dysfunction at one year of therapy compared to those on non- TDF- based regimen (these patients were on either Stavudine- based regimen or Zidovudine- based regimen). The study was done at Centre for Infectious Disease Research in Zambia (AIDC).

1.1. Statement of the problem

The Ministry of Health changed to TDF- based regimen from D4T- based regimen or AZT-based regimen as first line of ART regimen because of its high potency against HIV and hepatitis B infections, its favorable resistance profile, its good tolerability and safety, and its availability as a co-formulation with other antiretroviral agents in once-daily pills(MOHZ, 2010, WHO, 2013, Thompson, 2012). However, many studies from industrialized countries have found TDF to be associated with significant nephrotoxicity, including proximal tubulopathy and impaired glomerular filtration(Manosuthi, 2010, Tourret, 2013, Winston, 2006, Poizot-Martin, 2013, Pontrelli, 2012). We are uncertain whether this is still the case in our setting and the likelihood of normal functioning kidneys being damaged in one year of treatment with TDF in our setting. Concerns are that some patients develop renal dysfunction so severe that they are removed from TDF- based regimen and switched to drug combinations with less renal toxicity, moreover, the study at UTH found mortality to be more common among patients with renal insufficiency(Mulenga, 2008). Therefore, this study was aimed at determine whether HIV/AIDS patients with normal kidney function at initiation of treatment with TDF- based ARV develop renal dysfunction after an arbitrary period of one year.

1.2. Justification

Since TDF is part of the first line of ART regimen that is given for long periods of time and is associated with nephrotoxicity; we need to know whether the renal dysfunction occurs within one year of treatment with TDF. This would help clinicians and other health- care staff to monitor renal function and improve care for HIV/AIDS patients on TDF- based regimen by early detection of TDF- induced renal dysfunction and thus prevent progression to severe renal dysfunction and allow patients benefit fully from TDF- based regimen.

CHAPTER TWO: LITERATURE REVIEW

2.0. Literature review

2.0.1. Overview of antiretroviral drugs

HIV treatment requires a lifelong medication therapy to suppress the virus in addition, they receive other agents to relieve adverse effects of antiretroviral treatment and prevent opportunistic infections (Cocohoba, 2008). Currently, nearly 10 million people are on ART worldwide and the initial saddening discrepancy in access between developed and developing countries are progressively being sealed (Lafeuillade, 2014). WHO strongly recommended that the first-line of ART consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI); tenofovir (TDF) and lamivudine (3TC) or emtricitabine (FTC) as the two NRTI plus efavirenz (EFV) or nevirapine (NVP) as the NNRTI to be administered as a fixed-dose combination to initiate ART (WHO, 2013, Ford, 2011, WHO, 2012, Reynes, 2013). In cases were TDF + 3TC (or FTC) + EFV (or NVP) combination was contraindicated or unavailable, one of the following options was recommended: zidovudine (AZT) + 3TC + EFV or AZT + 3TC + NVP (WHO, 2013). WHO also urged countries to discontinue stavudine (D4T) use in first-line regimens because it was associated with metabolic toxicities (WHO, 2013).

The WHO recommended second-line ART consist of two NRTIs and a ritonavir-boosted protease inhibitor (PI); AZT + D4T as NRTIs was recommended in second- line after failure on a TDF + 3TC (or FTC)– based first-line regimen while after failure on an AZT or D4T + 3TC–based first-line regimen, TDF + 3TC (or FTC) as the NRTI backbone was recommended in second-line regimens (WHO, 2013). Heat-stable fixed-dose combinations of (atazanavir/ ritonavir) ATV/r and (lopinavir/ ritonavir) LPV/r were the preferred boosted PI options for second-line ART (WHO, 2013).

For third-line regimens was recommended to include new drugs with minimal risk of cross-resistance to previously used regimens, such as integrase inhibitors and second-generation NNRTIs and PIs (WHO, 2013).

2.0.2. HIV replication cycle and site of action of antiretroviral drugs

Though HIV life cycle presents with many potential opportunities for therapeutic intervention only few have been exploited and targeted by ARVs (Hazuda, 2012). The first step in HIV replication is entry of the virus; attachment inhibitors, chemokine receptor antagonists and fusions inhibitors are examples of drugs intended to counteract this process (Hazuda, 2012). Reverse transcription of the HIV single- stranded Ribonucleic Acid (RNA) into double stranded Deoxyribonucleic Acid (DNA) follows entry of the viral core into the CD4 cells and the process is achieved by the viral enzyme Reverse Transcriptase (RT); a multifunctional enzyme with RNA- dependent DNA polymerase, RNase- H, and DNA- dependent DNA polymerase activities (Hazuda, 2012). The two distinct classes of ARVs designed against the RT are: the NRTI which are analogs of native nucleoside substrates and NNRTI which bind to a non- catalytic allosteric site on the RT enzyme (Hazuda, 2012). Nearly half of all approved antiretroviral drugs is accounted for by the 12 licensed NRTI (emtricitabine, zidovudine, lamivudine, abacavir, stavudine, tenofovir, didanosine, and zalcitabine) and NNRTI (Efavirenz, nevirapine, delavirdine, etravirine) which differ amongst themselves with respect to their site and molecular mechanism of interaction on the reverse transcriptase but both affect the DNA polymerase and block the generation of full- length viral DNA (Hazuda, 2012, WHO, 2013). Integration of the HIV viral DNA and the host DNA follows and is catalyzed by the HIV viral enzyme integrase which catalyzes the 3′ end processing of viral DNA as well as strand transfer and integration of viral genome into the host chromosome (Hazuda, 2012). Targeted to block the viral integrase are the integrase inhibitor (Raltegravir and Elvitegravir) antiretroviral drugs (Bushman, 2011, Hazuda, 2012). The final process involves the assembly and maturation of the virus on the inner plasma membrane and this entails proteolysis of the viral polyproteins which is responsible for production of infectious viral particles (Hazuda, 2012). Protease inhibitors (Ritonavir, lopinavir, indinavir, fosamprenavir, atazanvir, darunavir, nelfinavir, saquinavir and tipranavir) are a class of antiretroviral drugs targeted against the viral protease responsible for the cleavage of the viral gag and gag- pol polypeptide precursors during maturation of the virion(Hazuda, 2012).

2.0.3. Adverse effects of antiretroviral drugs

The widespread use of Highly Active Antiretroviral Therapy (HAART) has dramatically reduced immunodeficiency events and increased the life expectancy of HIV- infected individuals (Paula, 2013, Vinikoor, 2014). However, various adverse effects have been reported in treatment with antiretroviral drugs and they have ranged from metabolic syndromes, neuropathies, cardiovascular disorders and nephropathies (Paula, 2013, Tanaka, 2013). In fact, adverse effects have been reported in treatment with different classes of antiretroviral drugs; NRTI which makes the backbone of ART have been associated with long term toxicity and cross- resistance (Boyd, 2013, Judd, 2010). Zidovudine and Stavudine have been associated with severe mitochondrial toxicity, anaemia and lipoatrophy; didanosine on the other hand has been associated with neuropathies which made WHO recommend phasing out usage of these drugs even in low- and middle- income countries (Boyd, 2013, WHO, 2013).

The current WHO recommended drug tenofovir has been associated with nephrotoxicity including acute and chronic renal failure, proximal tubular dysfunction, nephrogenic diabetes insipidus and nephrotic syndrome (Thompson, 2012, Calza, 2012, Judd, 2010, Tanaka, 2013, Tourret, 2013, Labarga, 2009, Cooper, 2010). In fact, a dose-dependent effect of plasma tenofovir concentrations on kidney tubular dysfunction and glomerular filtration was identified in a study done in France were renal toxicity increased with plasma tenofovir concentration (Poizot-Martin, 2013).

2.0.4. Occurrence of renal dysfunction in HIV/AIDS patients on TDF

HIV patients suffer a wide range of renal diseases some of which include Acute Kidney Injury (AKI) (Wyatt, 2006), Chronic Kidney Disease (CKD) (Arendse, 2010), HIV- associated glomerular disease (Arendse, 2010), and adverse effects due to treatment of HIV (Ro¨ ling, 2006). Based on the criteria employed in defining kidney disease, variable prevalence of these diseases in patients with HIV have been reported in Sub- Saharan Africa. In South Africa, the prevalence was estimated at 6 %; in Nigeria 38 %; in Côte d'Ivoire 26 %; in Tanzania 28 %; in Kenya 25 %; 20–48.5 % in Uganda and 33.4 % in Zambia (Naicker, 2009). Young, (2007) in his conclusion owed the discrepancies in findings from clinical trials and observational studies to the

differences in the clinical and sociodemographic characteristics of the study populations, the method used for estimation of eGFR, as well as in the criteria used in defining renal dysfunction; or in the case of observational studies to residual confounding.

In Mulenga, (2008) cohort study aimed at examining the association between baseline renal insufficiency and mortality among adults initiating antiretroviral therapy (ART) in urban African setting done in Lusaka from 18 primary care facilities. CL_{cr} calculated by the Cockcroft- Gault equation was used to determining kidney function and renal insufficiency was classified using the Kidney Disease Outcome Quality Initiative (K/DOQI). KDOQI classified renal dysfunction as follows:$CL_{cr} \geq 90$ mL/min was considered normal; CL_{cr} of 60 - 89 mL/min (K/DOQI stage 2) was mild renal insufficiency; 30 - 59 mL/min as moderate insufficiency (K/DOQI stage 3); and < 30 mL/min as severe insufficiency (K/DOQI stage 4 and 5). Secondary analyses used to measure renal function were serum creatinine levels alone and GFR calculated by the Modification of Diet in Renal Disease (MDRD) equation. A SCr ≤120umol/L was normal; 121 – 150umol/L was mild; 151 – 200umol/L was moderate and SCr ≥ 200umol/L was severe. K/DOQI was still used in categorizing MDRD estimates of GFR (Mulenga, 2008). Out of the 25,249 eligible participants in the study population, 33.5 % had renal insufficiency prevalent at baseline. Comparing with the secondary methods; baseline SCr was elevated in 3.8 % of participants. When GFR was calculated by the MDRD equation 12.4 % had renal insufficiency. Considering these results, different methods of measuring renal function yield different prevalence of renal insufficiency.

However, a very high prevalence of renal dysfunction was observed in a cross-section study done in Mwanza Tanzania on 355 participants without known preexisting renal disease or risk factors aside from HIV infection. The prevalence of renal dysfunction was 85.6 % in their study population (Msango, 2011). But, this high prevalence may be attributed to the method they used in defining renal dysfunction. Anyone with eGFR below 90 ml/min per 1.73 m^2 or proteinuria orwith microalbuminuria was considered to have renal dysfunction.

The criteria used by Msango, (2011) to define renal dysfunction were too wide; it should have been restricted to one criterion. Their criteria might have raised their estimated prevalence by making the study prone to more confounders and imprecision as earlier suggested by Young (2007). For example, after conservatively defining renal dysfunction as eGFR below 90 ml/min per 1.73 m^2 the prevalence of renal dysfunction reduced from 85.6 % to 63.7 % which was quite a huge reduction. They concluded that early diagnosis and regular monitoring for renal dysfunction in HIV-positive patients is essential for improving prognosis and medication dosing. They recommended that routine clinical follow-up visits should be planned every 3 to 6 months to allow for the monitoring of the CD4 T- cell count, creatinine and transaminases. They were worried about the risk of undiagnosed HIV-associated renal dysfunction in resource- limited settings in which routine laboratory testing was often not available (Msango, 2011).

Zimmermann, (2006) made similar conclusions in their cohort of 5 patients with Acute Renal Failure (ARF) and 22 others patients in medical literature. In these 27 patients, the glomerular filtration rate was calculated from the 24 hour Creatinine Clearance (CL_{cr}) rate and/or the Cockroft-Gault equation. They characterized Fanconi syndrome as abnormalities in proximal renal tubular function resulting in glycosuria, with normal serum glucose levels, phosphaturia, aminoaciduria, and decreased serum bicarbonate levels (Zimmermann, 2006).Zimmermann, (2006) showed very high rate of TDF related acute renal failure in the 27 participant and the laboratory findings improved dramatically when TDF was discontinued. However, they did not properly explain their method of selecting the participants of their cohort. Since there was no randomization in their participant selection the validity of their conclusions that TDF was associated with their cases of renal failure just because the laboratory findings resolved in a mean of 7.5 months after TDF was discontinued was rendered doubtable. In fact, it suffices to say that all the five cohort participants had other underlying risk factor for TDF nephrotoxicity as well as morbidity and mortality; hepatitis C, diabetes mellitus and advancement in age (Fernandez-Fernandez, 2011, Vinikoor, 2014).

Mauss (2005) compared renal function between patients on TDF- based (n= 82) regimen against patients on non- TDF- based regimen (n= 92) in a cross- sectional study. The glomerular filtration rate (GFR) was calculated on the basis of CL_{cr} in urine collected over 24 hours. The other marker was serum cystatin C which is a cationic low molecular weight cysteine proteinase, which is an established renal clearance marker. In addition, GFR was estimated using the modified Modification of Diet in Renal Disease Study (MDRD) formula. The MDRD formula is given by: $186 \times SCr\ (mg/dl)^{1.154} \times age\ (years)^{0.203} \times 1.212$ in male black patient. The calculated value is multiplied by 0.742 for female patient. The result gives a normal range of 90–120ml/min $1.73m^2$ (Mauss, 2005).

In Mauss (2005) study, patients on tenofovir showed significantly lower mean eGFR compared to patients on non- TDF- based regimen (97 ± 49mL/min 1.73 m^2 verses 107 ± 39mL/min $1.173m^2$) and cystatin C clearance (86 ± 21 mL/min 1.73 m^2 verses 97 ± 20mL/min 1.73 m^2); $P < 0.05$ (Mauss S, 2005). Mauss, (2005) concluded that even though the eGFR was still in the normal range, treatment with TDF- based regimen was associated with lower eGFR.The study by Mauss (2005) had merit as their control group was larger than the study group and also because the baseline characteristics of the study participants were not significantly different. The demerit of the study was that the researcher did not clearly state the antiretroviral regimen on which the participants in the control group were but simply reported as non- TDF- based regimen (Mauss, 2005).

Similar findings and inferences were made in the study by Young (2007) in which they assessed temporal trends in estimated CL_{cr} and GFR and the incidence of moderate to potentially life threatening renal insufficiency between patients who initiated either a TDF-containing HAART regimen (TDF-exposed group) and TDF-sparing HAART regimen (TDF-unexposed group). They concluded that TDF was associated with small but significant reduction in CL_{cr} and eGFR(Young, 2007). Though the findings of Young (2007) had merit of a large sample size, the demerit was due to the baseline characteristics of the participants in the two groups being

significantly different. These differences might have affected the results that were obtained and subsequently the conclusions.

Gallant, (2005) compared the changes in renal function between patients treated with TDF-based regimen (n= 344) and those treated with other nucleotide analogues reverse- transcriptase inhibitors (NRTI) - based regimen (n= 314) in an observational cohort study. They determined renal dysfunction by the CL_{cr} method which estimates CL_{cr} by factoring in SCr, age and sex of patient. They calculated the change in the CL_{cr} in the two groups at intervals of 3 months for 1 year. Findings showed that patients on TDF- based regimen had CL_{cr} significantly decreased by 4 % after 1 year compared to patients on other NRTI, $P< 0.001$. Ultimately, they concluded that clinical trials which usually did not show the association between TDF may not represent "real world" scenarios, because patients with renal insufficiency or risk factors for renal insufficiency were often excluded which made data from clinical cohorts somewhat conflict (Gallant, 2005).

To the contrary, the study by Antoniou, (2005) gave conflicting conclusions. The study showed lower incidence of TDF- associated nephrotoxicity in their retrospective cohortof 172 patients who received TDF for a median of 16 months (range 3–25 months). Nephrotoxicity was defined as grade 1 increase if SCr increased by $\geq$ 44mmol/L or greater than 1.5× increase from baseline. The incidence of grade 1 increase in SCr was 4 % while the incidence of 1.5× increase in serum creatinine was 8.7 % (Antoniou, 2005). In addition, only four patients discontinued TDF because of suspected nephrotoxicity, and three out of the four appeared to have developed features consistent with Fanconi syndrome. They concluded from their findings that TDF-mediated nephrotoxicity at therapeutic doses was unlikely and that TDF was generally a well-tolerated ARV agent from a renal standpoint (Antoniou, 2005). However, Antoniou (2005) might have overlooked TDF- mediated nephrotoxicity in their conclusion because they used increase in SCrin defining renal dysfunction but, SCr only increased dramatically when eGRF reduced below 60mL/min/1.73m^2(Fernandez-Fernandez, 2011)

Similar findings to Antoniou (2005) were observed in a cross-sectional study by Banda done in Lusaka which included 300 HIV infected and uninfected participants and aimed at determining the prevalence and risk factors associated with renal dysfunction among hospitalized HIV infected patients at UTH (Banda, 2010). The following criteria were used by Banda to determine renal function: **R**isk was 1.5× increased SCr from normal or eGFR decrease by 25 % or urine output less than 0.5ml/kg per hour for 6 hours. **I**njury was 2× increased SCr from normal, or eGFR decrease by 50 % or urine output less than 0.5ml/kg per hour for 12hours. **F**ailure was 3× SCr increased from normal or GFR decrease by 75 % or urine output less than 0.5ml/kg per hour for 24hours. **L**oss and **E**ndstagewere outcome parameters and the criterion was abbreviated as **RIFLE**. Chronic Kidney Disease was evidence of kidney damage that persisted for 3 months or more (Banda, 2010). Their findings showed a prevalence of renal dysfunction of 42 % among HIV positive participant to 27 % among the uninfected. It was concluded that treatment with TDF- based regimen was not associated with renal dysfunction in hospitalized HIV patients (Banda, 2010). However, prevalence of ARF in Banda's study was high because combinations of three methods were used to determine the renal function namely: SCr, GFR and urine output as explained by the RIFLE criteria. In addition, the prevalence they reported was for both HIV infected and uninfected hospitalized participants whose reasons for hospitalization were not stated.

Similar conclusions were reached by Brennan (2011) who did a cohort analysis of 890 HIV-infected adults who received TDF at the Themba Lethu Clinic, South Africa. The study was aimed at estimating the relationship between renal dysfunction and nephrotoxicity. They defined nephrotoxicity as any decline in kidney function from baseline (acute or chronic) which could be secondary to a toxin including drugs. Findings showed that patients with renal dysfunction were at highest risk of death by 48 months compared to patients with normal renal function. They concluded that much of the incident renal dysfunction in TDF patients was likely related to preexisting renal disorder which might be exacerbated by TDF. It was recommended that with expanded use of TDF, screening for renal dysfunction prior to initiation and dose adjustment was necessary to help improve ART outcomes (Brennan, 2011). However, on the conflicting findings in studies, Fernandez (2011) suggested that the mismatched results between clinical trials and

case reports may be explained because clinical trials have strict inclusion and exclusion criteria while in contrast to routine clinical practice where patients may have associated conditions, medications, or background that may predispose to tenofovir nephrotoxicity(Fernandez-Fernandez, 2011).

2.0.5. Pathophysiology of TDF associated renal dysfunction in HIV/AIDS patients

Tenofovir is an acyclic nucleotide phosphonatediester analog of adenosine monophosphate and like other NRTIs, tenofovir inhibits HIV-1 reverse transcriptase by competing with the natural substrate deoxy- adenosine 5′- triphosphate one of the nucleotide pools used by virus in generating cDNA(Kohler, 2009). Owing to the fact that it is an analog of nucleosides, tenofovir and other NRTIs is likely to inhibit mammalian DNA polymerases, including mitochondrial DNA polymerase-γ, and induce oxidative stress (Kohler, 2009). TDF is eliminated from the kidneys by Glomerular filtration and active proximal tubular secretion, which helps to maintain metabolic balance such as stable pH as the kidneys filters blood (Zimmermann, 2006). Common in HIV is HIV associated nephrotoxicity (HIVAN), TDF has reputed to have an additive or synergistic toxic effect on the kidney with HIVAN (Kohler, 2009). Tenofovir is likely to causes renal proximal tubular mitochondrial ultrastructural abnormalities that are parallel to mitochondrial DNA depletion in the peroximal tubular cells (Kohler, 2009, Palacio, 2012).Tenofovir is normally eliminated by active secretion through the renal tubules, therefore, impaired or delayed elimination of tenofovir would lead to its accumulation and increased tenofovir abundance in the proximal tubules and hence its phosphorylation in those cells could create an imbalance in nucleotide pools, thereby disrupting mitochondrial biogenesis (Kohler, 2009) . Accumulation of TDF in the proximal renal tubular cells may result in renal toxicity, renal tubular acidosis and, ultimately, renal failure characterized by a decline in eGFR and hypophosphataemia (Fernandez-Fernandez, 2011, Kohler, 2009, Tourret, 2013).

2.1. Biochemistry and physiology of creatinine

Creatine is synthesized in the kidneys, liver and pancreas by two enzymatically mediated reactions. In the first reaction, arginine and glycine are transamidated to guanidinoacetate. In the second reaction, guanidinoacetate is methylated to creatine with the methyl group donated by S-

adenosylmethionine(Burtis, 2008). Creatine is reversibly phosphorylated to Creatine phosphate (phosphocreatine) by creatine kinase using ATP as phosphate donor. The interconvert ion of phosphocreatine and creatine is a particular feature of the metabolic processes of muscle contraction (Burtis, 2008). Phosphocreatine functions as a store of high- energy phosphate in muscles and the amount of phosphocreatine is proportional to muscle mass (Murray, 2003).

Creatine kinase
Phosphocreatine + ADP ↔ Creatine + ATP (3.a)

Creatinine is a nonprotein nitrogenous metabolite produced from phosphocreatine in muscles by an irreversible nonenzymatic dehydration and dephosphorylation of phosphocreatine (Murray, 2003). Creatinine is a waste product of creatine and is a cyclic anhydrous form of creatine that is produced as the final product of decomposition of phosphocreatine. It is produced endogenously and realized into body fluids at a constant rate and its plasma concentration is maintained within narrow limits predominantly by glomerular filtration. Consequently; both plasma concentration of creatinine and renal creatinine clearance are used as makers of glomerular filtration rate and their measurement is used as diagnostic indicators of kidney function (Burtis, 2008).

2.2 Hypothesis

It was hypothesized that HIV patients on treatment with TDF- based regimen at UTH did not have a threefold higher likelihood of developing renal dysfunction at one year of therapy compared to HIV/AIDS patients on non- TDF- based regimen (D4T- based regimen or AZT-based regimen).

2.3. General objective

The general objective was to determine if HIV/AIDS patients treated with TDF- based regimen at UTH develop renal dysfunction at one year of treatment more than those on non- TDF- based regimen.

2.4. Specific objectives

The specific objectives were to determine the baseline CL_{cr} of HIV patients on TDF and on non-TDF- based regimen when matched by sex and to compare the CL_{cr}at one year of treatment in the two groups.

CHAPTER THREE: MATERIALS AND METHODS

3.0. Study design

The study was an analytical cross-sectional study. It involved analysis of data obtained from files of HIV/AIDs patients' files. The data was collected between December, 2013 and March, 2014.

3.1. Population

Participants were HIV- infected patients aged 16 years old and above who began ARTin the period between 30th September, 2007 and 30th January, 2013. Selected participants' SCr and demographics like age, sex and body weight were obtained and used in calculating their CL_{cr}using the Cockcroft- Gault equation

3.2. Study site

The study was conducted at the University Teaching Hospital at the Centre for Infectious Disease Research in Zambia (AIDC).

3.3. Selection criteria

3.3.1. Inclusion criteria

Participants were HIV/AIDS patients aged 16 years old and above that started ART on TDF-based regimen or D4T- based regimen or AZT- based regimenfrom the period between 30th September, 2007 and 30th January, 2013.

3.3.2. Exclusion criteria

Files of HIV- infected patients aged below 16 years old were excluded from the study. HIV-infected patients whose files had missing necessary information were also excluded. HIV-infected patients with record of preexisting renal disease at the time they were initiated on therapy were also excluded from the study. HIV- infected patients with record of hypertension, diabetes, and hepatitis B or C virus co-infection were all excluded from the study.

3.4. Sample size

Based on an expected HIV prevalence of 34 % in outpatient adults and 6 % renal dysfunction prevalence in non TDF exposed HIV patients, using α= 0.05, 228 participants were required in order to have 80 % power to detect an Odds Ratio of 3.0 for renal dysfunction in TDF exposed HIV patients. An additional 20 % of participants were included in case of loss to follow up; making a total of 274 participants in each group.

The following formula was used to calculate the sample size:

$$N = \frac{[u\sqrt{\pi_1(1-\pi_1)+\pi_0(1-\pi_0)} + v\sqrt{2\pi(1-\pi)}]^2}{(\pi_0-\pi_1)^2} \qquad (3.1)$$

Were N was the size of each group, π_0 and π_1 were proportions, π was the average of the proportions; u was 1.28 for 90 % power and 0.84 for 80 % power, v was the Z statistic which was equal to 1.96 for α equal to 0.05. TDF is associated with renal dysfunction in HIV outpatients. We expected 6 % renal dysfunction among TDF unexposed HIV patients, 18 % renal dysfunction among TDF exposed HIV patients and 50 % TDF use among HIV patients in the study population.

$$N = \frac{[u\sqrt{\pi_1(1-\pi_1)+\pi_0(1-\pi_0)} + v\sqrt{2\pi(1-\pi)}]^2}{(\pi_0-\pi_1)^2}$$

$$= \frac{[0.84\sqrt{0.06(0.94)+0.18(0.82)} + 1.96\sqrt{0.24\times0.88}]^2}{(0.18-0.06)^2}$$

$$= 114$$

We required 114 participants in each arm and a total of 228 participants in the study population.

3.5. Determination of serum creatinine at UTH chemical pathology laboratory

3.5.1. Analytical methodology

Plasma creatinine is commonly measured using either chemical or enzymatic methods. The chemical method is based on the reaction between creatinine and alkaline picrate to produce a colored compound creatinine picrate; the reaction is also called the Jaffe reaction (Burtis, 2008).

The time period selected for the research was between 2007 and 2013; around that period the machine that was in use in chemical pathology laboratory at UTH in the period was the Beckman Coulter AU400. The machine measured serum creatinine based on the fact that creatinine reacts with picric acid in an alkaline medium to form creatinine picrate which is a yellow- orange colored compound (Coulter, 2006). The machine was a form of spectrophotometer and it determined the concentration of substances in solutions by determining their absorbance at specific wavelengths (Coulter, 2006).

Figure 1: Picture of the Beckman Coulter AU400

When incident monochromatic light with intensity I_o is radiated from a source and passed through a square cell (cuvette) containing a solution of a compound that absorbs monochromatic light; the intensity of transmitted light I_s would be less than I_o as some of the incident light is absorbed. Beer's law speculates that the absorbance of monochromatic light by a solution is proportional to the absorptivity (a), the length of path (b) and the concentration (c) (Burtis, 2008).

$$A = abc \qquad (3.2)$$

Were (A) is absorbance, (a) absorptivity, (b) length of path and (c) concentration

The direct proportion between absorbance and concentration was established under specific conditions. Frequently a linear relationship existed and Beer's law only applied along the linearity range and within that range the concentration of a solution could be calculated based on the absorbance. Within the linearity range, a calibration constant was derived and used to calculate the concentration of an unknown solution by comparison with the calibrating solution (Burtis, 2008). From equation (3.2)

$$a = \frac{A}{bc} \qquad (3.3)$$

Therefore

$$\frac{A_c}{b_c c_c} = \frac{A_u}{b_u c_u} \qquad (3.4)$$

Where (c) and (u) represent calibrator and unknown and since the length of path (b) is same; solving for concentration of unknown we get:

$$c_u = \frac{A_u}{A_c} \times c_u \qquad (3.5)$$

The instrument used equation (3.5) to calculate the concentration of the unknown sample from the calibrator.

3.5.2. Specific performance characteristic of the Beckman Coulter AU400

The reagents used in determination of SCr on the Beckman Coulter AU400 were designated reagent 1 and reagent 2. Reagent 1 contained 120mmol/L sodium hydroxide while reagent 2 contained 2.9mmol/L picric acid (Coulter, 2006). Creatinine reacted with picric acid in an alkaline medium to form creatinine picrate which is a yellow- orange colored compound. The rate of change in the absorbance at 520nm and at 800nm was directly proportional to the concentration of creatinine(Coulter, 2006).

$$\text{Creatinine} + \text{picric acid} \xrightarrow{(NaOH)} \text{creatinine picrate complex} \qquad (3.b)$$

The machine samples 20µL of serum specimen which it adds to 120uL of reagent 1 and 90uL of diluent. Then 120uL of reagent 2 was added to the mixture. The machine measured the primary and secondary absorbance. The primary absorbance was measured at 520nm while the secondary absorbance was measured at 800nm. The whole sequence of reactions had 27 measuring points however for creatinine, the machine measured at point number 13 for primary absorbance and point number 24 for secondary absorbance. The test for serum creatinine by the Beckman Coulter AU400 was linear within the range of 18umol/L to 2200umol/L of creatinine and it was within this range that the measurement of serum creatinine obey Beer's law (Coulter, 2006).

3.5.3. Internal Quality Assurance of the Beckman Coulter AU400 at UTH chemical pathology laboratory

Internal Quality Assurance (IQA) was attained through maintenance of the machine and quality control. The machine was maintained on a daily, weekly, monthly and quarterly basis which is done according to the guidance in the Olympus AU400 user guide. Internal Quality Control (IQC) on the other hand gave the measure of imprecision of the method. IQC was done every morning and involved calibration of the machine and running of Quality Control (QC) samples which were all commercially prepared in a lyophilized form. Calibration was done using a multiple calibrator reagent which calibrated most tests done by the machine including creatinine. To reconstitute the multiple calibrator which came in a lyophilized form, 5mL of distilled water was added and allowed to dissolve at room temperature for 30 minutes. Aliquots were made and

stored at -18˚C where they remained stable for 21 days and used within that period. For Quality Control (QC) reagents; two commercially prepared reagents designated control 1 and the control 2 were used. These were prepared together with the calibrator. They also came in a lyophilized form and to reconstitute, 5mL of distilled water was added in each and allowed to dissolve for 30 minutes at room temperature before use. Aliquots of the controls were stored at -18˚C where they remained stable for 21days (UTH, 2012a) and (UTH, 2012b).

Calibration and QC of tests done for a particular day was done every morning. It was also done whenever a reagent on board was changed and in those cases calibration and QC were only done for the new reagent on board. After calibration and QC was done, the results of the QC were plotted on a Levey- Jennings chart. Whether the QC qualified was dependent on whether the result obtained in the QC for a particular day violated the Westgard rules after plotting on a Levey- Jennings chart. Running of patients' specimen only starts once the QC qualified and QC only qualified if none of the Westgard rules was violated (UTH, 2012a) and (UTH, 2012b).

3.5.4. External Quality Assurance at UTH chemical pathology laboratory

External Quality Assurance (EQA) at UTH chemical pathology laboratory was achieved by participation in an external proficiency testing scheme. EQA measured the closeness of the result obtained by a method to the reference value. Proficiency testing samples were sent from National Health Laboratory (NHLS) which is in the Republic of South Africa; once every three months. The samples were handled in the same manner as patient samples. Record of result obtained from the proficiency testing samples were kept before sending a copy to the scheme provider who gave feedback to the laboratory on the performance of the method (UTH, 2012b).

3.6. Determination of presence of renal dysfunction

Renal dysfunction was determined using CL_{cr}which was calculated from serum creatinine; weight and age of a participant using the Cockcroft- Gault equation. The age, sex and serum creatinine of the participants were obtained from the files of selected participants. The Cockcroft- Gault equation factored in the age; the body weight and the serum creatinine in calculating the CL_{cr} making it very sensitive to any changes in GFR (Fernandez- Fernandez,

2011). SCr may not rise above the upper limit of normal until the eGFR goes below 60mL/min/1.73m ^{2} which increases chances of overlooking the renal injury using SCr alone (Fernandez, 2011). Though CL_{cr} calculated using the Cockcroft- Gault equation is sensitive to changes in eGFR, it may underestimate the extent of renal dysfunction if the muscle mass is lower than the age of patient and this is usually the case in HIV (Fernandez- Fernandez, 2011). The $CL_{cr}$was calculated using the Cockcroft- Gault equation:

$$\text{Estimated } CL_{cr} \text{ (mL/min)} = \frac{(140 - \text{age [years]} \times \text{weight [kg]})}{(0.815 \times \text{SCr (umol/ L)})} \quad (3.6)$$

Multiplied by 0.85 for females

In this study, renal dysfunction was defined by K/DOQI criterion. According to K/DOQI criterion; renal dysfunction calculated from Cockcroft- Gault equation was classified as follows: $CL_{cr} \geq$ 90mL/min was considered no renal dysfunction; CL_{cr} of 60 - 89mL/min was mild renal dysfunction (K/DOQI stage 2); CL_{cr} of 30 - 59mL/min as moderate dysfunction (K/DOQI stage 3); and CL_{cr} lower than 30mL/min was severe dysfunction (K/DOQI stage 4 and 5).

3.7. Data collection tools and variables

The age, sex, patients' weight at initiation of therapy; at six months of therapy; and at one year of therapy, the serum creatinine at initiation of therapy; at 6 months of therapy; and at 12 months were collected from patients' files. Collecting information from files began in December, 2013 and finished in March, 2014. In collecting the data, a Microsoft Excel spreadsheet was obtained from AIDC which had tabulated data with columns reflecting the patient's ART number, names, date of initiation of therapy, drugs the patient was taking, their sex and age. The spreadsheet was then separated into cases which only included patients on treatment with TDF- based regimen; and controls which included patients on either D4T- based regimen of AZT- based regimen. See Appendix: 3.1.

Using the spreadsheet, a total of five hundred and forty nine (549) participants were systematically randomly selected. One thousand three hundred and ninety six (1396) HIV/AIDS

patients were on treatment with TDF- based regimen while four thousand four hundred and fifty three (4,453) patients were either on AZT- based regimen or D4T- based regimen. Out of the total of 1,396 on TDF- based regimen, two hundred and seventy five (275) participants were systematic randomly selected making the study group. The selection of participants in the study group started from position twenty six (26) and the participants were selected at intervals of five from. Two hundred and seventy four (274) participants with sex matching with the participants in the study group were selected from the 4,453 making the control group.

A list of matched selected cases and controls was made. See Appendix: 3.2. The list of matched participants was then printed and used to find the files of the patients from which information was obtained. Only about 20 % of files were found by this method because some files could not be found. This necessitated the change of sampling method to randomly selecting and including any file of patient found in drawers. Files of patients on treatment with TDF- based were included in the study group. An equivalent number of patient files with corresponding sex on treatment with either D4T- based regimen or AZT- based regimen were included in the control group. Each drawer was checked only once to avoid selecting the same patients' file more than once.

Information was collected from forms called clinical follow up forms which were in the patients' files. These forms gave the date the patient was initiated on therapy, age of the patient, weight of the patient on each visit, sex of the patient and the drug combination on which the patient was. SCr concentrations at date of initiation of therapy, 6 months of therapy and 1 year of therapy were obtained from the result scripts enclosed in the patients' files. The information obtained from the patients' files was entered into a data collection sheet. See Appendix: 3.3.

3.7.1. Variables

The variables shown in the table below were the variables of interest which were obtained from the patients files. The dependent variable was renal dysfunction. The independent variables were: treatment with TDF, age and sex.

Table 3.1: Variable of interest

Type of Variable	Definition of the Variables	Units
Dependent Variables • Renal dysfunction	$CL_{cr} \geq 90$mL/min is normal dysfunction, CL_{cr} of 60 - 89mL/min is mild, CL_{cr} of 30 - 59mL/min moderate; $CL_{cr} < 30$mL/min is severe renal dysfunction	mL/min
Independent variables • TDF exposure	Treatment with TDF based regimen	
• Sex	Sex-(Gender)	
• Age	Age-(Age at visit)	Years

3.7.2. Data handling

Primary data was saved as it was originally collected in a separate file and was not to be altered. A copy of the primary data was made and all the changes to format the data in a manner analyzable by data analysis tools was made. In formatting the data, a variable definition key was made which give description of the acronyms used in the table during analysis. See Appendix: 3.4.

3.7.3. Data analysis

The data was entered into STATA software version 12 for analysis. Univariate analysis was done in order to describe the distribution of single variables in the cases and controls. Multivariate analysis was done in order to describe the relationship of multiple variables to each other.

3.8. Ethical consideration

Permission to access the data from the database was obtained from the UTH management and ethical approval was done by Excellence in Research Ethics and Science (ERES). To assure anonymity, neither the names nor addresses of the participants were used but participant were given accessioning numbers which were used for identification. All possible patient identifiers which may include race of the patient were not obtained or used. And since data from the database was used, there was no contact with the patients themselves.

3.9. Study limitations

The study was limited because renal dysfunction was determined using CL_{cr}calculated using the Cockcroft- Gault equation which may underestimate the renal dysfunction in case of weight loss in a patient (Fernandez-Fernandez, 2011). A Randomized Clinical Trial (RCT) can be suggested for further findings as the design used in this study could not determine causal relationships.

CHAPTER FOUR: RESULTS

4.0. Descriptive results

There were a total of 549 participants in the study. The study group and the control group were comparable with 275 participants exposed to TDF- based regimen verses 274 participants exposed to non TDF- based regimen (D4T or AZT). Out of the total number of 275 participants in the study group, 131participants were male while the remaining 144 participants were female. Out of the total number of 274 participants in the control group, 131 participants were male while the remaining 143 participants were female.

4.1. Characteristics of participants at baseline and after 1 year of exposure to ART

The study participants were significantly older than the control participants. The median age of participants in the study group was 29 years with lower quartiles at 19 years and upper quartile 35 years verses the median age of 20 years with lower quartiles at 17 years and upper quartile at 30 years in the control group, $P<0.001$. See Table: 4.1.

The median baseline CL_{cr} was not significantly different between the study group and control group; both were in the normal CL_{cr} range; 99.39 mL/min verses 104.58 mL/min, $P= 0.0574$. On the other hand, the median 1 year CL_{cr} in the study group was significantly lower than the median CL_{cr} in the study group; 93.87 mL/min verses 115.07 mL/min, $P< 0.001$. See Table: 4.1.

Table 4.1: Summary statistics of the study population

	Control			Cases			
Variables	25 %	50 %	75 %	25 %	50 %	75 %	P-Value
Age (years)	17	20	30	19	29	35	0.0001
Baseline weight(Kg)	46	52	60	48	55.5	64	0.0053
1 year weight(Kg)	48.5	54.5	63	50.9	58	66	0.004
Baseline SCr(µmol/L)	59	67	75.1	63	71.8	86	0.0001
1 year SCr(µmol/L)	54.2	63.3	73	66	78.9	94	0.0001
Baseline CL_{cr}(mL/min)	90.1	104.5	118.2	78.9	99.39	120.6	0.0574
1 year CL_{cr}(mL/min)	96.1	115.1	132.4	79.5	93.87	115.9	0.0001

4.2. Severity of renal dysfunction categorized by exposure to TDF

Using the K/DOQI criteria of classifying renal dysfunction, the cases of renal dysfunction were identified and graded in the study group and control group at baseline and after 1 year of therapy. A total of 162 participants out of a total number of 549 participants in the study population had renal dysfunction at baseline which translated into a prevalence preexisting renal dysfunction of 29.5 % in the study population. A total of 170 participants out of a total number of 549 participants in the study population had renal dysfunction after 1 year of treatment which translated into a prevalence of renal dysfunction of 31 % in the study population after 1 year of ART. See Table: 4.2.

Table 4.2: Severity of Renal Dysfunction Categorized by Exposure to TDF

		Baseline Renal dysfunction		1 year Renal dysfunction	
		Count	Percent	Count	Percent
			-- %--		-- %--
Control	No renal dysfunction	207	75.55	229	83.58
	Mild renal dysfunction	60	21.9	38	13.87
	Moderate renal dysfunction	7	2.55	7	2.55
	Severe renal dysfunction	0		0	
Case	No renal dysfunction	180	65.45	150	54.55
	Mild renal dysfunction	81	29.45	108	39.27
	Moderate renal dysfunction	14	5.09	14	5.09
	Severe renal dysfunction	0		3	1.09
	Total	549		549	

4.3. Comparison between the baseline and 1 year creatinine clearances in the controls group

Paired t- test was done to compare the baseline CL_{cr} to the CL_{cr} after 1 year of treatment in the control group. The median CL_{cr} in the control group significantly increased at 1 year compared to the baseline CL_{cr} by a mean of 10.75mL/min with 95 % Confidence Interval (95 % CI) ranging from 8.36mL/min to 13.14mL/min and $P< 0.001$. See Table: 4.3.

Table 4.3: Paired t- test Results for Baseline and 1 year CL_{cr} in control group

Variable	Obs	Mean	Std. Dev.	[95 % Interval]	Confidence
Baseline CL_{cr}	274	106.61	26.54	103.45	109.76
1 year CL_{cr}	274	117.36	30.99	113.67	121.05
Diff	274	-10.75	20.09	-13.14	-8.36

mean(diff)= mean(Baseline CLcr - 1 year CLcr); t= -8.8603; degrees of freedom= 273

Ho: mean(diff)= 0

P(|T|> |t|)< 0.001

4.4. Comparison between the baseline and 1 year creatinine clearances in the study group.

Paired t- test was done to compare the baseline CL_{cr} to the CL_{cr} after 1 year of treatment in the study group. Results showed that the CL_{cr} after 1 year significantly decreased by a mean of 4.29mL/min from the baseline CL_{cr}, 95 % CI ranging from 1.59mL/min to 7mL/min and *P*= 0.0019. See Table: 4.4.

Table 4.4: Paired t- test results for baseline and 1 year creatinine clearance in the study group

Variable	Obs	Mean	Std. Dev.	[95 % Interval]	Confidence
Baseline CL_{cr}	275	102.08	29.09	98.63	105.54
1 year CL_{cr}	275	97.79	28.29	94.43	101.15
Diff	275	4.29	22.76	1.59	7.00

mean(diff)= mean(Baseline CLcr - 1 year CLcr); t= -3.1289; degrees of freedom= 274

Ho: mean(diff)= 0

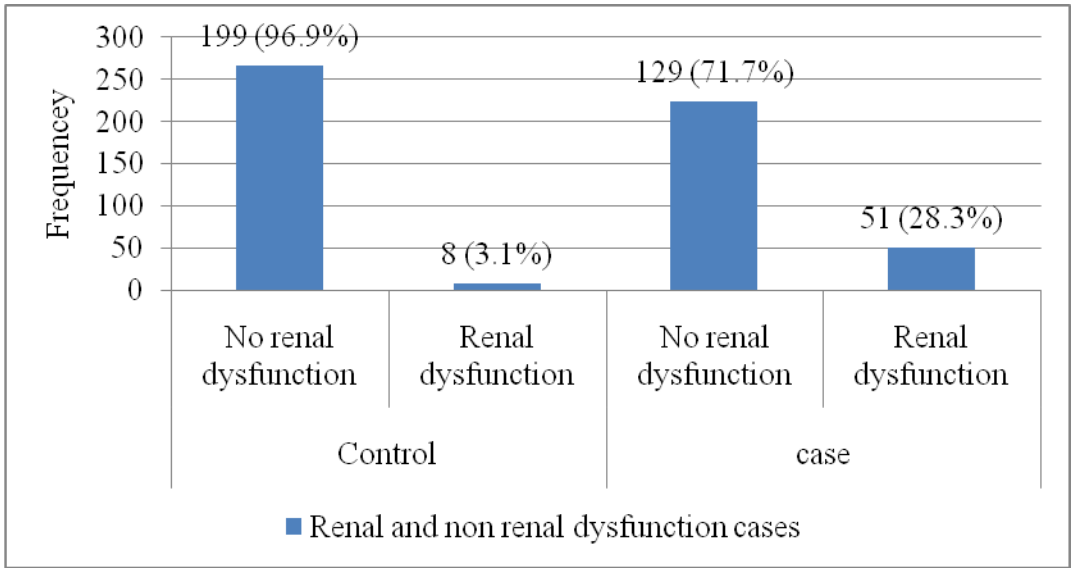
P(|T|> |t|)= 0.0019

4.5. Frequency of developing renal dysfunction (renal dysfunction) after 1 year of ART in the study population

The frequency of developing abnormal CL_{cr}(renal dysfunction) after one year of ART from having normal CL_{cr} (no renal dysfunction) at baseline was compared between the study group and control group. Results showed a larger number of participants in the study group developed

renal dysfunction compared to participants in the control group; 51 out of 180 in the study group verses 8 out of 207 in the control group. See Figure: 4.1.

Figure 4.1: Frequency of developing abnormal CL_{cr}(renal dysfunction) after 1 year of ART in the study population



The chi- square test (χ^2- test) was done to assess the strength of evidence that exposure to TDF really affected the probability of patients developing renal dysfunction after 1 year in participants who had no renal dysfunction at baseline. The model was statistically significant with χ^2= 44.6114 and P< 0.001. These findings showed that the participants in the study group had a higher probability of developing renal dysfunction after 1 year from having no renal dysfunction at baseline compared to the participants in the control group. See Table: 4.5.

Table 2.5: Frequency of Renal Dysfunction Development (RD) in Cases and Controls after 1 year of treatment

TDF Exposure	RD after 1 year		Total Patients
	Yes	No	
Cases	51(28.3 %)	129(71.7 %)	180
Controls	8(3.1 %)	199(96.9 %)	207
Total	59(15.2 %)	328(84.8 %)	387

4.6. Comparison of likelihood of developing renal dysfunction after 1 year of ART in the study population.

To determine the odds of developing renal dysfunction from having no renal dysfunction at baseline, logistic regression was done. The final model in logistics included age; gender and the outcome CL_{cr} at one year in relation to exposure to TDF- based regimen. The model was statistically significant for exposure to TDF and age but not significant for gender. The odds of patient developing renal dysfunction after one year of therapy with TDF- based regimen controlled for age and sex was 8.77 (95 % CI 3.97 to 19.34). See Table: 4.6.

Increase in age also significantly increased the odds of developing renal dysfunction after one year of treatment with TDF- based regimen, $P< 0.001$. The odds of developing renal dysfunction at one year of treatment with TDF- based regimen increased by 1.07 per unit increase in age with 95 % CI ranging from 1.03 to 1.11. However, gender showed no significant association with developing renal dysfunction after one year of treatment with TDF- based regimen, $P= 0.12$. See Table: 4.6.

Table 4.6: Logistic regression model with developing renal dysfunction after 1 year of ART as the dependent variable

Independent Variables	Odds Ratio	Std. Err.	*P*	[95 % Confidence Interval	
Age	1.07	0.02	0.001	1.03	1.11
Gender	1.62	0.50	0.122	0.88	2.98
TDF exposure	8.77	3.54	0	3.97	19.34
Constant	0.01	0.00	0	0.00	0.02
N= 387					

CHAPTER FIVE: RESEARCH FINDINGS DISCUSSION

5.0. Discussion

The study aimed at determining whether HIV/AIDS patients treated with TDF- based regimen at UTH develop renal dysfunction at one year of treatment more than those treated with D4T-based regimen or AZT- based regimen. Findings of this study showed a decrease in the median creatinine clearance after 1 year of therapy by a mean of 4.39mL/min compared to the baseline CL_{cr}, $P< 0.001$ in the study group which meant a reduction in kidney function after 1 year of treatment with TDF. In addition to that, findings in this study also showed an association between treatments with TDF- based regimen and renal dysfunction where HIV/AIDS patients on treatment with TDF- based regimen were 8.77 times more likely to develop renal dysfunction after 1 year of therapy compared to those on D4T- based regimen or AZT- based regimen containing regimen, $P< 0.001$. This evidence of TDF being associated with renal dysfunction has been accentuated in many studies including studies by Mauss (2005), Gallant (2005), Fernandez-Fernandez (2011), Zimmermann (2006), Poizot- Martin (2013), Thompson (2012), Calza (2012), Judd (2010), Tanaka (2013), Labarga (2009) and Tourret (2013) all of which talked of the association of TDF with renal dysfunction. Mauss (2005) showed that patients on tenofovir had significantly lower mean eGFR and cystatin C clearance compared to patients on non- TDF-based regimen which lead them to conclude that even though the eGFR was still in the normal range, treatment with TDF- based regimen was associated with lower eGFR a finding coinciding to this study in that patients on TDF had a reduction in CLcr which entails a reduction in eGFR. Gallant (2005) alongside found that patients on TDF- based regimen had CL_{cr} significantly decreased by 4 % after 1 year of treatment compared to patients on other NRTI which is equally coinciding with the findings of this study. Significant correlation between Ctrough-TDF and the decrease in GFR highlights a toxic concentration-dependent effect of TDF on glomerular filtration also found by Poizot- Martin (2013) and is concurrent with previous studies reporting a tubular nephropathy in patients on a TDF-based regimen. However, the findings of this study disagree with Banda's (2010) study, the controversy can be ascribed to the fact that Banda used a combination of SCr, eGFR and urine output as explained by RIFLE as criteria of determining renal dysfunction which was too wide because it encompassed three different methods at once as opposed to the K/ DOQI criterion in this study, in fact, this also explains why Banda found a

baseline prevalence of renal dysfunction of 42% vs. the 29.5% of this study. However, it is noteworthy that the prevalence baseline renal dysfunction of 29.5% in this study somewhat agrees with the 34.5% prevalence found in Mulenga's (2008) who also used the CL_{cr} in determining renal dysfunction and K/ DOQI criterion in classifying it. Therefore, with the evidence from the research findings, the null hypothesis that HIV/AIDS patients on treatment with TDF- based regimen at UTH do not have a threefold likelihood of developing renal dysfunction at one year of therapy compared to those on D4T- based regimen or AZT- based regimen was rejected.

5.1. Conclusion

Since the paired t- test, the chi-square test and the logistic regression model were all significant with $P< 0.001$. It can be concluded that adults with HIV/AIDS on treatment with TDF- based regimen are 8.77 times more likely to develop renal dysfunction after one year of therapy from having no renal dysfunction at beginning of therapy compared to those on non- TDF - based regimen. Therefore, the null hypothesis that adults with HIV/AIDS on treatment with TDF- based regimen at UTH do not have a threefold likelihood of developing renal dysfunction at one year of therapy compared to HIV/AIDS patients on D4T- based regimen or AZT- based regimen was rejected.

5.2. Recommendations

The study showed that treatment with TDF- based regimen increased the likelihood of developing renal dysfunction after one year of therapy. It showed that an HIV/AIDS patient on treatment with TDF- based regimen was 8.77 times more likely to develop renal dysfunction after one year of therapy compared to an HIV/AIDS patient on D4T- based regimen or AZT- based regimen. Therefore, it can be recommended that patients on treatment with TDF- based regimen get frequent renal checkup and follow up during their treatment to increase their benefit of treatment with TDF or possibly change to a non TDF- based regimen where possible. In addition, a Randomized Control Trial (RCT) is suggested to determine the causal relationship between TDF and renal dysfunction in our setting.

APPENDICES

Appendix 3.1: Participant Selection Spreadsheet

ART #	First name	Surname	Date therapy started	Drug name	Other disease condition	Sex	Age	Match #
DMD	Jjj	Ccc	16-Jan-08	TDF/3TC	TB	Male	28	1
DMD	Uuu	Xxx	12-Dec-11	TDF/3TC		Female	18	2
DMD	Fff	Bbb	2-May-07	TDF/3TC		Female	33	3
DMD	Nnn	Vvv	12-Feb-12	3TC/D4T(30mg)	TB	Male	28	1
DMD	Zzz	Aaa	1-Dec-09	AZT/3TC		Female	18	2
DMD	Ggg	Mmm	30-Jun-10	AZT/3TC		Female	33	3

Appendix 3.2: Matched Participants Sheet

Match #	ART #	Name	ART#	Name	Gender	Age
1	DMD	JjjCcc	DMD	NnnVvv	Male	28
2	DMD	Uuu Xxx	DMD	ZzzAaa	Female	18
3	DMD	FffBbb	DMD	GggMmm	Female	33

Appendix 3.3: Data Collection Sheet

P.N	Age	Gender (F/M)(0/1)	Weight(Kg)			SCr(umol/L)			TDF/AZT/D4T 1/2/3
			baseline	6mon	12mon	baseline	6mon	12mon	
1	28	1	55.6	58	60.9	65.1	67.4	80.2	1
2	18	0	63.4	60.5	61	63.5	72.6	80.5	1
3	33	0	82.4	79.1	77.3	69.	67.5	65	1
4	28	1	44.1	45	44.4	50.82	56	69	3
5	18	0	68.4	70	69	84.3	79	83	2
6	33	0	70.1	70	73.3	71	64.3	93.91	2
7									
8									
9									
10									
N									

Appendix 3.4: Variable definition Key

Variable Identifier	Variable Name
1. PN	Participant's Number
2. Age(years)	Age in years
3. Gender(0/1)	male/female
4. W. base	Baseline weight
5. W. base	Weight at 6 months
6. C. base	Baseline creatinine
7. C.6mon	Creatinine at 6 months
8. C.12mon	Creatinine at 1 year
9. CL. base	Baseline creatinine clearance
10. CL.6mon	Creatinine clearance at 6 months
11. CL.12mon	Creatinine clearance at 1 year
12. TDF EXPO(1/0)	TDF exposure (Yes/No)
13. KdPr12mYN	Renal Dysfunction after 1 year of TDF therapy(Yes/No)
14. KP(0)	No kidney dysfunction(CL≥90mL/min)
15. KP(1)	Mild kidney dysfunction (CL of 60-89mL/min)
16. KP(2)	Moderate kidney dysfunction(CL of 30-59mL/min)
17. KP(3)	Severe kidney dysfunction (CL<30mL/min)
18. KdPrbase	kidney dysfunction at baseline
19. KdPr6m	kidney dysfunction at 6 months
20. KdPr12m	kidney dysfunction at 1 year

REFERENCES

1. ANTONIOU T, R. J., CHIRHIN S, YOONG D, GOVAN V, GOUGH K, RACHLIS A AND LOUTFY M (2005) Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. *HIV medicine,* 6**,** 284-290.

2. ARENDSE G, W. N., OKPECHI I AND SWANEPOEL C (2010) The acute, the chronic and the news of HIV-related renal disease in Africa. *Kidney International* 78**,** 239-245.

3. BANDA J, M. A., SIZIYA S, MWEENE M, ANDREWS B ANS LAKHI S (2010) Prevalence and Factors Associated with Renal Dysfunction in HIV Positive and Negative Adults at the University Teaching Hospital, in Lusaka. *Medical Journal of Zambia,* 37**,** 136-142.

4. BOYD, A. A. A. M. (2013) antiretroviral regimen sparing agents from the nucleoside(tide) reverse transcriptase inhibitor class: a review of the recent literature. *AIDS RESEARCH AND THERAPY,* 10**,** 1- 9.

5. BRENNAN A, E. D., MASKEW M, NAICKER S, IVE P, SANNE I, MAOTOE T AND FOX M (2011) Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. *AIDS,* 25**,** 1603-1609.

6. BURTIS C, A. E., BURNS D, SAWYERS B (2008) (Ed.6) *TietzFundermentals of Clinical Chemistry,* Philadelphia, Saunders Elservier.

7. BUSHMAN, C. R. A. D. (2011) HIV DNA integration. *Cold Spring Harbor Prospectives in Medicine,* 2.

8. CALZA, L. (2012) Renal toxicity associated with antiretroviral therapy. *HIV Clinical trials,* 13**,** 189- 211.

9. COCOHOBA, J. (2008) Antiretroviral drug interactions and adverse side effects. *Advanced studies in pharmacy,* 5**,** 105- 113.

10. COOPER R, W. N., SMITH N, KEISER P, NAICKER S AND TONELLI M (2010) Systematic Review and Meta-analysis: Renal Safety of TenofovirDisoproxilFumarate in HIV-Infected Patients. *Clinical Infectious Diseases,* 51**,** 496-505.

11. COULTER, B. (2006) Clinical chemistry reagent guide. *Olympus Life and Material Science Europa GmbH.*

12. FERNANDEZ-FERNANDEZ B, M.-F. A., SANZ A, SANCHEZ-NIˇ M, IZQUIERDO M, POVEDA J, SAINZ-PRESTEL V, ORTIZ-MARTIN N, PARRA-RODRIGUEZ A, SELGAS R, RUIZ-ORTEGA M, EGIDO J, AND ORTIZ A (2011) Tenofovir Nephrotoxicity: 2011 Update. *AIDS Research and Treatment,* 2011**,** 11.

13. FORD N, C. A., AND MOFENSON L (2011) Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and metaanalysis. *AIDS,* 25**,** 2301- 2304.

14. GALLANT J, P. M., KERULY J, AND MOORE R (2005) Changes in Renal Function Associated with TenofovirDisoproxilFumarate Treatment, Compared with Nucleoside Reverse-Transcriptase Inhibitor Treatment. *Clinical Infectious Diseases* 40**,** 1194-1198.

15. H. BYGRAVE, K. K., K. HILDERBRAND, G. JOUQUET, E. GOEMAERE, N. VLAHAKIS, L. TRIVIN˜O, L. MAKAKOLE, AND NATHAN FORD (2011) Renal Safety of a Tenofovir-Containing First Line Regimen: Experience from an Antiretroviral Cohort in Rural Lesotho. *Open Access,* 6.

16. HAZUDA, A. E. A. D. (2012) HIV-1 Antiretroviral Drug Therapy. *ColdspringHabor Laboratory Press,* 2**,** 1- 23.

17. JUDD A, B. L., STO¨HR W, DUNN D, BUTLER K, LYALL H, SHARLAND M, SHINGADIA D, RIORDAN A AND GIBB D (2010) Effect of tenofovirdisoproxilfumarate on risk of renal abnormality in HIV-1-infected children on antiretroviral therapy: a nested case–control study. *AIDS* 24**,** 525–534.

18. KALAYJIAN, R. C. (2011) Renal Issues in HIV Infection. *current HIV/AIDS report,* 8**,** 164-171.

19. KOHLER J, H. S., HOYING- BRANDT A, GREEN E, JOHNSON D, RUSS R, TRAN D, RAPER M, SANTOIANNI R AND LEWIS W (2009) Tenofovir renal toxicity targets mitochondria of renal peroximal tubules. *Laboratory investigation,* 89**,** 513- 519.

20. LABARGA P, B. P., MARTIN-CARBONERO L, RODRIGUEZ-NOVOA S, SOLERA C, MEDRANO J, RIVAS P, ALBALATER M, BLANCO F, MORENO V, VISPO E AND SORIANO V (2009) Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS,* 23**,** 689–696.

21. LAFEUILLADE A, W. M., GOUGEON M, KINLOCH-DE LOES S, HALFON P AND TISSOT-DUPONT H (2014) Highlights from the 2014 International Symposium on HIV & Emerging Infectious Diseases (ISHEID): from cART management to the end of the HIV pandemic. *AIDS Research and Therapy* 11.

22. MANOSUTHI W, M. W., LUEANGNIYOMKUL A, PRASITHSIRIKUL W, TANTANATHIP P, SUNTISUKLAPPON B, NARKKSOKSUNG A, NILKAMHANG S AND SUNGKANUPARPH S (2010) Renal impairment after switching from stavudine/ lamivudine to tenofovir/lamivudine in NNRTIbased antiretroviral regimens. *AIDS Research and Therapy* 7**,** 1-8.

23. MAUSS S, B. F. A. S. G. (2005) Antiretroviral therapy with tenofovir is associated with mild renal dysfunction. *AIDS* 19**,** 93–99

24. MOHZ, M. O. H. Z. (2010) Adult and adolesecent antiretroviral therapy protocol 2010. 9.

25. MSANGO L, D. J., KALLUVYA S, KIDENYA B, KABANGILA R, JOHNSON W, FITZGERALD D AND PECKA R (2011) Renal dysfunction among HIV-infected patients starting antiretroviral therapy. *AIDS* 25**,** 1421-1425.

26. MULENGA L, K. G., LAKHI S, CANTRELL R, REID S, ZULU I, STRINGER E, KRISHNASAMI Z, MWINGA A, SAAG M, STRINGER J, CHI B (2008) Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. *AIDS*, 12**,** 1821-1827.

27. MURRAY R, D. J., MAYES P AND RODWELL V (Ed.) (2003) *Harper's Illustrated Biochemistry,* Toronto, McGraw-Hill campanies.

28. NAICKER, J. F. A. S. (2009) HIV and kidney disease in sub-Saharan Africa. *Nature reviews nephrology***,** 591- 598.

29. PALACIO M, R., AND CASADO J (2012) Proximal Tubular Renal Dysfunction or Damage in HIV- infected Patients. *AIDS*, 14.

30. PATEL K, P. A., RANJAN R, PATEL R AND PATEL J (2010) Tenofovir- associated Renal dysfunction in clinical practice: An observational corhort from Western india. *Indian Journal of Sexually transmitted diseases and AIDS* 30**,** 30- 34.

31. PAULA A, F. M. A. P. A. (2013) Metabolic syndrome in HIV-infected individuals: underlying mechanisms and epidemiological aspects. *AIDS Research and Therapy* 10**,** 1-8.

32. POIZOT-MARTIN I, S. C., ALLEMAND J, OBRY-ROGUET V, PRADEL V, BREGIGEON S, FAUCHER O, AND LACARELLE B, PHARMD, (2013) Renal Impairment in Patients Receiving a Tenofovir-cART Regimen: Impact of Tenofovir Trough Concentration. *Journal of Acquired Immunal Deficiency Syndrome* 62**,** 375-380.

33. REID A, S. H. W., WALKER W, WILLIAMS I, KITYO C, HUGHES P, KAMBUGU A, GILKS C, MUGYENYI P, MUNDERI P, HAKIM J, AND GIBB D (2008) Severe Renal Dysfunction and Risk Factors Associated with Renal Impairment in HIV-Infected Adults in Africa Initiating Antiretroviral Therapy. *Clinical Infectious Diseases 46:1271–81,* 46**,** 1271-1281.

34. REYNES J, T. R., PULIDO F, SOTO-MALAVE R, GATHE J, QAQISH R, TIAN M, FREDRICK L, PODSADECKI T, NORTON M AN NILIUS A (2013) Lopinavir/Ritonavir Combined with Raltegravir or Tenofovir/Emtricitabine in Antiretroviral-Naive Subjects: 96-Week Results of the PROGRESS Study. *AIDS RESEARCH AND HUMAN RETROVIRUSES,* 29**,** 256- 265.

35. RO¨ LING J, S. H., FISCHEREDER M, DRAENERT R, AND GOEBEL F (2006) HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy–Induced Nephropathy. *Clinical Infectious Diseases,* 42**,** 1488- 1495.

36. SPAULDING A, R. G. A. S. N. (2011) Tenofovir or zidovudine in three-drug combination therapy with one nucleoside reverse transcriptase inhibitor and one non-nucleoside reverse transcriptase inhibitor for initial treatment of HIV infection in antiretroviral-naïve individuals. *The Cochrane Collaboration*.

37. TANAKA H, A. M., TOMODA Y, WADA T, YAGO K, SATOH M (2013) Evaluation of Renal Adverse Effects of Combination Anti-retroviral Therapy including Tenofovir in HIV- infected Patients. *Journal of Pharmaceutical Sciences* 16**,** 405-413.

38. THOMPSON M, A. J., HOY J, TELENTI A, BENSON C, CAHN P, ERON J,GUNTHARD HHAMMER S AND REISS P (2012) recommendation of the international antiviral society- USA panel. *JAMA,* 308**,** 387- 402.

39. TOURRET J, D. G. A. I.-B. C. (2013) Tenofovir Effect on the Kidneys of HIV-Infected Patients: A Double-Edged Sword? *Journal of the American Society of Nephrology* 24**,** 1519- 1527.

40. UTH (2012a) Standard Operating Procedures for Specimen analysis - Beckman Coulter AU400. *University Teaching Hospital Department of Pathology and Microbiology-Chemical Pathology SOPs.* Lusaka.

41. UTH (2012b) Standard Operating Procedures for Statistical Process Control (Quality Control). *University Teaching Hospital Department of Pathology and Microbiology-Chemical Pathology SOPs.* Lusaka.

42. VINIKOOR M, J. J., MWALE J, MARX M, GOMA F, MULENGA L, STRINGER J, ERON J AND CHI B (2014) Age at Antiretroviral Therapy Initiation Predicts immune Recovery, Death, and Loss to Follow-Up Among HIV-Infected Adults in Urban Zambia. *AIDS RESEARCH AND HUMAN RETROVIRUSES,* 30**,** 949- 955.

43. WHO (2012) Technical update on treatment optimization: pharmacological equivalence and clinical interchangeability between lamivudine and emtricitabine, a review of current literature.

44. WHO (2013) Consolidated Guidelines on The use of Antiretroviral Drugs for Treating and Preventing HIV infection Recommendation for Public Health Approach

45. WINSTON A, A. J., MALLON P.W.G, MARRIOTT D, CARR A, COOPER A, D AND EMERY S (2006) Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovirdisoproxilfumarate-containing highly active antiretroviral therapy. *HIV Medicine (2006),* 7**,** 105-111.

46. WYATT M, R. R. A., KLOTMAN P AND KLOTMAN M (2006) Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. *AIDS,* 20**,** 561- 565.

47. YOUNG, K. B., R. K. BAKER, A. C. MOORMAN, K. C. WOOD, J. CHMIEL, J. T. BROOKS AND HIV OUTPATIENT STUDY INVESTIGATORS (2007) Antiretroviral Therapy in the HIV Outpatient Study Renal Function in Tenofovir-Exposed and Tenofovir-Unexposed Patients Receiving Highly Active. *Journal of the International Association of Physicians in AIDS Care* 6**,** 178-187.

48. ZIMMERMANN A, P. T., BEDFORD J, MORRIS A, HOFFMAN R, AND BRADEN G (2006) Tenofovir-Associated Acute and Chronic Kidney Disease: A Case of Multiple Drug Interactions. *Clinical Infectious Diseases* 42**,** 283-290.