ASSOCIATION BETWEEN LONG TERM TENOFOVIR-BASED THERAPY AND THE INCIDENCE OF RENAL DYSFUNCTION IN ADULT HIV-POSITIVE PATIENTS AT RONALD ROSS HOSPITAL: A RETROSPECTIVE COHORT STUDY

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DECLARATION

I, WANTAKISHA WABENGA ROBERT ENOCK hereby declare that this dissertation is my original work and has not been presented for any other awards at the University of Zambia or any other University.

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ABSTRACT

Background & Aim: Tenofovir disoproxil fumerate (TDF)-based antiretroviral therapy places HIV-infected patients at high risk of renal dysfunction. Therefore, we evaluated if the incidence of renal dysfunction in HIV positive adults patients on long term tenofovir-based regimen versus those on non-tenofovir use.

Methods: We performed a retrospective cohort analysis of 834 HIV positive patients at the counselling and testing center (CTC) at Ronald Ross General Hospital. Patients were evaluated at baseline and with every follow up visit for creatinine and creatinine clearance (Cockcroft-Gault formula) calculated. Patients' records in data management software called SMARTCARE from 2008 to 2014 were reviewed to compare renal function between patients on TDF-containing regimen (447 patients) with non-TDF containing regimen (387 patients). We evaluated Glomerular Filtration Rate (GFR) using creatinine clearance as defined by the Kidney Disease Outcomes Quality Initiative Classification (K/DOQI) by GFR.The effect of creatinine, urea and exposure to TB medication, Cotrimoxazole use and CD4 cell count on GFR were also follow up for 18 months. Univariate and Multivariate logistic regression was used to determine the factors associated with renal dysfunction. We report multivariable hazard ratios (Cox modeling) and binary outcomes with predictors retained if $P < 0.05$ and analyzed survival time. Renal function was categorized as CrCl <50 ml/min and ≥ 50 ml/min. Potential predictor variables for renal dysfunction included in the multivariable models were age, sex, weight, creatinine and treatment with tenofovir.

Results: The CD4 cell count, urea, anti-TB medication, Cotrimoxazole use, education level, employment status and creatinine clearance were similar at baseline between the two groups. There was no significant difference from exposure to TDF between groups on renal dysfunction development (OR: 2.52, 95% CI, 0.79-8.0, P-value=0.118) as a predictor of renal dysfunction in both univariate and multivariate analysis. Creatinine and creatinine clearance between groups at baseline and 18 months were not statistically different. The prevalence of mild and severe renal dysfunction among HIV-positive adults on TDF-based therapy was 18.4% and 0.2% respectively at 18 months during therapy. Those patients on TDF who were older than 50 years and presented with CrCl <50 ml/min and a CD4 cell count below 500 cells/uL at baseline were more likely to develop renal dysfunction. Twenty-four (5.5%) and 2 (0.5%) patients presented with mild and severe renal dysfunction respectively at baseline.

Further, Univariate and multivariate analysis showed that ages <49 years than >50 (AOR: 6.35 (2.18-18.4), p-value=0.001), and higher CD4+ cell count >500 cells/uL (AOR: 1.24 (0.97-1.07), P-value=0.051) were less likely associated with renal dysfunction. Age per year-increase (OR: 6.35, 95% CI -2.18-18.4, P<0.001) and CD4 cell count per 1 cell-increase/µl (OR: 3.03, 95% CI, 0.98-9.28, P=0.053) on univariate were important factors affecting renal dysfunction.

Conclusion: We found no evidence to suggest that TDF-based therapy is associated with decline in renal function and that it does not affect creatinine clearance. However, there is need for close renal monitoring of patients initiated on TDF by using creatinine clearance.

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ABBREVIATIONS

AIDS-Acquired Immunodeficiency Syndrome

ART- Antiretroviral Therapy

AKI-Acute Kidney Injury

ARVs-Antiretroviral Drugs

AZT-Zidovudine (also known as ZDV)

CD4-Cluster Differentiation

CrCl-Creatinine Clearance

CSF-Cerebral Spinal Fluid

CTC-Counselling and Testing Centre

CTX-Cotrimoxazole

D4T-Stavudine

DNA- Deoxyribonucleic Acid

ESRD-End stage Renal Disease

EFV-Efavirenz

FTC-Emtricitabine

GFR-Glomerular Filtration Rate

GRZ-Government of the Republic of Zambia

HAART- Highly Active Antiretroviral Therapy

HIV- Human Immunodeficiency Virus

HIVAN-HIV-Associated Nephropathy

HMIS-Health Management Information System

LPV/r-Lopinevir

IQR-Interquartile range

NNRTIs- Non-Nucleoside Reverse Transcriptase Inhibitors

NRTIs-Nucleoside Reverse Transcriptase Inhibitors

NVP-Nevirapine

MDRO- Modification of diet in renal disease

PIs- Protease Inhibitors

RNA-Ribonucleic Acid

RRGH-Ronald Ross General Hospital

RT-Reverse Transcriptase

SOP-Standard Operating Procedure

TDF-Tenovir Disoproxil Fumerate

TDF+FTC-Truvada

DEFINITIONS

First line regimen-This is the first regimen of ART treatment in HIV patients.

Second line regimen-This is the second regimen of ART treatment in HIV patients after first treatment failure.

Tubulointerstitial Nephrotic -A kidney disease marked by increased protein in urine, abnormal low blood protein (albumen) and fluid gathering in the tissues.

Nephrolithiasis - The presence of stones in the kidney.

Renal impairment- Renal impairment defined as GFR < 50 ml/min/1.73 m^2

Proteinuria - The presence of excess of serum protein in the urine.

Hypertension- Sustained elevation of systemic arterial blood pressure to a level likely to induce cardiovascular damage or other adverse consequences.

Glomerular filtration rate - This is basically the measure of the rate at which the kidney is excreting certain metabolites such as creatinine to determine renal function.

Nephropathy -Disease of the kidney which can be in various categories, i.e. kidney insufficiency, nephritic syndrome.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND ON HIV AND EPIDEMIOLOGY OF RENAL DYSFUNCTION

1.1.1 BURDEN OF HIV

In Africa, many of the countries have developed and expanded their responses to the Acquired Immunodeficiency Syndrome (AIDS) epidemic and increased access to antiretroviral treatment (Wools-Kaloustian et al., 2007, Struik et al., 2011). Despite this progress, Zambia remains one of the countries still highly affected with Human Immunodeficiency Virus (HIV). The effects are most seen in infants' morbidity and mortality rates.

Zambia, in southern Africa, is one of the countries affected with HIV/AIDS epidemic with HIV prevalence in adults standing at 13% (Central Statistical Office et al., 2014). More than one in every seven adults in the country are living with HIV and life expectancy at birth has fallen to just 49.4 years from 56 years (Central Statistical Office et al., 2014). In 2011, nearly 42,000 adults and 9,500 children were newly infected with HIV that is about 115 new infections each day in adults (UNAIDS, 2011). As of 2014, overall HIV prevalence was 13 percent, 15% in women and 11% in men (Central Statistical Office et al., 2014); however, it has been reported as considerably higher in some urban areas (Mulenga et al., 2008, Central Statistical Office et al., 2014).

The increased morbidity and mortality rates are partial consequences of high sero-prevelence of HIV infection (Quesada et al., 2015, Mulenga et al., 2008, Patel et al., 2010). With significant reductions in mortality and risk of progression to AIDS in the era of Highly Active Antiretroviral Therapy (HAART), complications of long-standing HIV infection and treatment have become increasingly important. Such complications include the nephrotoxic effects associated with HAART (Izzedine et al., 2009, Szczech, 2008, Thuppal et al., 2015, Quinn et al., 2010).

Early diagnosis and regular monitoring of renal dysfunction in HIV-positive patients is essential for prognosis, medication dosing, and treatment. The risk of undiagnosed HAART associated renal dysfunction is worrisome especially in resource-limited settings where routine laboratory testing is often not available (Banda et al., 2010, Quesada et al., 2015, Mauss et al., 2005, Della Negra et al., 2015). The World Health Organization (WHO) recommends calculating creatinine clearance (CrCl) for patients at initiation of tenofovir and

every six months "if feasible," though inability to test does not preclude tenofovir use (UNAIDS, 2009, Mizushima et al., 2014).

In high-resource countries, renal monitoring is recommended at HIV diagnosis and at least yearly, thereafter for patients with chronic kidney disease (CKD) extensive renal monitoring. Existing risk factors include African ethnicity, CD4+ T-cell (CD4) count <200 cells/mm3, or plasma viral load >4000 copies/mL (Wools-Kaloustian et al., 2007). In Sub-Saharan Africa, screening may even be more urgent because many patients have all of these risk factors, often first presented for care when they already have low CD4 counts and high viral loads.

Renal disease disproportionately affects patients living with HIV (Banda et al., 2010). Although Sub-Saharan Africa endures over 60% of the world's burden of HIV disease (UNAIDS, 2009), there is limited data in the region on HIV related kidney disease, with most available data coming from the developed countries (Banda et al., 2010, Struik et al., 2011). Kidney disease is a recognised complication in HIV infected patients whose presentation can be acute renal failure (ARF) or chronic kidney disease (CKD).

The few outpatient renal screening studies that have been done in Sub-Saharan Africa describe varying prevalence ranging between 6% to almost 50% (Banda et al., 2010, Gupta et al., 2011). Screening outpatient studies in South Africa reported 6% renal dysfunction, 33.5% in Zambia and 48.5% in Uganda (Fabian and Naicker, 2008, Mulenga et al., 2008). In hospitalised HIV infected patients in Zambia, prevalence is up to 10 times higher and carries high mortality (Banda et al., 2010).

In 2009, a prevalence of renal dysfunction of 33.5% among HIV infected outpatients commencing Highly Active Antiretroviral Treatment (HAART) in Lusaka, Zambia was found (Banda et al., 2010). It is estimated that more than 574 000 HIV infected patients may well have renal disease (Banda et al., 2010). Zambia like most Sub-Saharan countries has limited facilities offering renal replacement therapy (RRT) with only one public hemodialysis Unit with limited machines (Banda et al., 2010). The study thus investigates the incidence of renal dysfunction on patients exposed to TDF and whether creatinine and creatinine clearance can better predict associations to renal dysfunction among HIV infected patients exposed to tenofovir at Ronald Ross General Hospital (RRGH), in Mufulira.

1.1.2 MORTALITY AND RENAL DYSFUNCTION IN HIV INFECTED PATIENTS

Mortality rates are high in HIV patients with renal disease in Zambia (Banda et al., 2010, Mulenga et al., 2008, Freeman et al., 2015). They range from 25% to 80% depending on the cause of kidney injury and co-morbidities in the patient. Although reversible with renal replacement therapy, development of renal dysfunction carries high mortality in HIV infected patients compared to non-HIV infected patients. For instance, of the 700 patients admitted with acute renal failure (ARF) at the Johannesburg Hospital, almost 20% had HIV and mortality was 20% among the HIV infected patients (Izzedine et al., 2004).

At the University Teaching Hospital (UTH) Hemodialysis Unit in Lusaka, mortality was 34.4% among 58 hospitalised HIV infected patients with renal dysfunction in the first half of the 2009 (unpublished UTH audit 2009) (Banda et al., 2010). In other studies, established CKD at HAART initiation was associated with higher mortality independent of HIV related risk factors for death. HIV infected patients with kidney dysfunction were twice likely to die compared to those with normal kidneys (Banda et al., 2010).

1.1.3 KIDNEY AND NEPHRON FUNCTION

Kidneys are two-bean shaped organs, located on either side of the spine, just. The kidneys are linked directly with the circulatory and excretory systems. The functions of these complex organs are to clean the blood of toxins and regulate the amounts of other substances dissolved in blood. The actual cleaning occurs in tiny units inside the kidney called nephrons. Each kidney contains approximately 1 million nephrons (Ganong, 2003). Nephrons are the functional unit of the kidney; it is responsible for the actual filtration and purification of the blood.

The tubule of the nephron is surrounded by tiny blood vessels, called capillaries. By a process called diffusion, substances that the body can still use get reabsorbed (Ganong, 2003). The filtrate within the tubule of the nephron contains water, ions, glucose and other useful small molecules at high concentrations. The filtered blood in the capillaries contains these useful substances at low concentrations. As a result, these useful substances in the tubule diffuse back into the capillaries. The amount of each substance that is reabsorbed is controlled to maintain the perfect concentrations in the blood stream.

If the kidneys become damaged or diseased they do not function as they normally would. Wastes, other toxic substances, and water accumulate in blood and the proper concentration of ions and other substances is not maintained. (Ganong, 2003). This inability of the kidneys to function properly is called kidney failure. Kidney failure is a serious health concern which, if left untreated, may cause death within days or weeks.

Many drugs and their metabolites are excreted through the kidney-nephrons, especially via the proximal tubule. Given the high rate of blood flow through the proximal tubule, and, consequently, the high level of toxins it has to process, this portion of the nephrons is at particular risk of developing drug-related damage (Izzedine et al., 2009, Struik et al., 2011). Since the introduction of HAART, which has led to a dramatic decline in the mortality and morbidity associated with HIV infection, a variety of adverse renal effects of this treatment have been recognized as well (Wools-Kaloustian et al., 2007, Thuppal et al., 2015, Mitra et al., 2014).

Measuring blood creatinine is a useful and inexpensive method of evaluating renal function. Creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue (Ganong, 2003). Creatinine production is continuous and is proportional to muscle mass. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate which is estimated by the creatinine clearance (CrCl). Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine levels rises. If the serum creatinine level doubles, the GFR is considered to have been halved (Struik et al., 2011). A threefold increase is considered to reflect a 75% loss of kidney function (Mulenga et al., 2008, Izzedine et al., 2004, Ganong, 2003).

Increased serum creatinine levels are seen in: impaired renal function, chronic nephritis, and Urinary tract obstruction, Muscle disease such as gigantism, acromegaly, and myasthenia gravis, congestive heart failure, and shock. Decreased creatinine levels may be seen in: the elderly, persons with small stature, decreased muscle mass or inadequate dietary protein. Muscle atrophy can also result in decreased serum creatinine level (Wools-Kaloustian et al., 2007).

The only pathological condition that causes a significant increase in the serum creatinine level is damage to a large number of nephrons. The serum creatinine level does not rise until at least half of the kidney's nephrons are destroyed or damaged (Ganong, 2003). Because creatinine levels rise and fall more slowly than urea levels, creatinine levels are often preferred to monitor renal function on a long-term basis (Mizushima et al., 2014, Marrazzo et al., 2015, Brennan et al., 2011, Ganong, 2003).

1.1.4 MAJOR FACTORS AFFECTING RENAL DYSFUNCTION

There are various risk factors predisposing HIV infected patients to developing renal dysfunction. This development happens by and large due to the same reasons as in non HIV patients. However, certain factors are exclusive to HIV infected and the incidence is higher in HIV patients (Banda et al., 2010). The causes of renal disease in HIV patients can be community or hospital acquired but hospitalised patients have a higher risk of developing renal dysfunction (up to ten times more common) with worse outcomes (Banda et al., 2010, Kinai and Hanabusa, 2009).

Volume depleting conditions are a major cause of pre renal failure in HIV patients due to chronic gastroenteritis among HIV infected patients. Hypotension causes reduced mean arterial pressure (MAP) leading to Acute Tubular Necrosis (Izzedine et al., 2009). The causes of reduced MAP among hospitalised HIV infected patients include gastroenteritis, sepsis with shock and dehydration (Banda et al., 2010). Disordered renal regulations of salt and water balance such as adrenal insufficiency and tubular dysfunction are also contributory to volume depletion conditions and hypotension among HIV infected patients (Msango et al., 2011). However, hypotension was reported not to be significantly associated to renal dysfunction (Banda et al., 2010, Quesada et al., 2015).

Gastroenteritis is caused by opportunistic infections, malabsorption and drug treatments for HIV and related opportunistic infections. Opportunistic infections (OIs) causing chronic diarrhoea are cryptosporidiosis, isospora belli, microsporidiosis, mycobacterium avium complex (MAC) and malignancies affecting the gastrointestinal tract (Bygrave et al., 2011b, Mulenga et al., 2008). Infections are a leading cause of severe renal dysfunction in some studies among HIV infected patients. AIDS leads to impaired immunity in HIV infected patients (Gupta et al., 2011). In other studies, sepsis was associated with 52% renal dysfunction, the majority of which were AIDS associated infections (Banda et al., 2010).

Medications prescribed to HIV patients may also cause renal dysfunction (Izzedine et al., 2009). Some of these medications include HAART, medications for treatment or prophylaxis of OIs and herbal medications. For example, tenofovir disoproxil fumerate is a first line antiretroviral drug in Zambia and has been linked to renal dysfunction though the incidences are very low (Izzedine et al., 2009, Mulenga et al., 2008, Ministry Of Health, 2013). Amphotericin B is used for treatment of cryptococcus neoformans which is the most common cause of meningitis at UTH (Banda et al., 2010). Trimethoprim/Sulfamethoxazole, a drug used for pneumocystis carini pneumonia prophylaxis among HIV patients with CD4 count below 350cells/mm3 and treatment, can cause renal dysfunction (Banda et al., 2010).

HIV patients with CD4 counts below 200cells/mm3 are more predisposed to development of renal dysfunction (Msango et al., 2011, Della Negra et al., 2015, Kabbara and Ramadan, 2015). In France, a schini study, sixty nine episodes of renal dysfunction occurred among 39 patients with CD4 counts <200cell/mm3 compared to forty renal dysfunctions episodes among 30 patients with CD4 counts <200cells/mm3 (Bygrave et al., 2011b, Pichit and Somnue, 2010). Patients with higher viral load are significantly at risk of developing renal dysfunction among hospitalised patients (Banda et al., 2010). WHO stages III and IV of the disease have also been associated with renal dysfunction among HIV infected patients in Zambia (Bygrave et al., 2011a). Malnutrition, age, and severity of HIV disease were common risk factors in HIV patients with kidney dysfunction in a Zambian study (Banda et al., 2010).

1.1.5 ANTIRETROVIRAL THERAPY IN ZAMBIA

State provision of antiretroviral therapy (ART) began in Zambia in late 2002, although initially very few people could afford the monthly payments towards the drugs. Provision of free treatment started in June 2004.The delivery of the programme relies on the involvement of many Non-governmental Organizations (NGOs), churches and communities. Ultimately, Zambia aspires to provide universal treatment access, so that ARV therapy is equally available to everyone who is clinically eligible.

In Zambia figure 2 the choice is use of Tenofovir Disoproxil Fumerate (TDF) and Emtricitabine (FTC) with a Non-Nucleoside Reverse Transcriptase inhibitor efavirenz (EFV) or nevirapine (NVP) as first line treatment (Ministry Of Health, 2013). All classical HAART regimens have always contained two nucleoside Reverse Inhibitors (NRTIs) backbone usually containing a thymidine analogue, Zidovudine (AZT) or Stavudine (D4T). However, because of the toxicity problems of both substances as well as the problematic resistance

associated with failure of therapy, the preferred backbone used today of TDF/FTC or Abacavir (ABC) and Lamivudine (3TC) gives more treatment options in case of resistance. In the event of treatment failure, the entire regimen is changed from first line to second line combination.

The second line regimen includes two new Nucleoside Reverse Transcriptase inhibitors and one Protease inhibitors (PI). It is recommended when there is treatment failure to the first line regimen. It is important to assess the risk of ART on patient's renal function because it undermines the excretion process and thus increasing the risk to toxicity for such patients (Ministry Of Health, 2013). As these drugs are often taken for a long period of time, it is a concern that there may be a future epidemic due to HAART associated-renal complications in these patients on ART.

The decision about when to start treatment in adults and adolescents is particularly challenging. One school of thought is that clinical judgment should balance the potential benefit of treatment with the long-term toxicities and adherence challenges. Prevention largely depends on the quality of prescription and also requires specialist health practitioners who can recognize the potential for clinically important ART interactions which can occur as a result of various ART combinations over a long period of exposure.

The capacity to prescribe properly also depends on the availability of modern equipment to measure important patient parameters such as blood creatinine, urea, urinalysis and calculation of GFR (Wools-Kaloustian et al., 2007, Jones et al., 2004, Mitra et al., 2014, Pedrol et al., 2015). The high prevalence of clinically important ART interactions, side effects is likely to result in prolonged hospital admission due to renal problems. This in turn will stretch the economic resources of the health institution as much more medication will be required to manage increased cases of HAART–associated renal problems.

Furthermore, improved survival among patients with HIV due to ART is anticipated to result in an increase in the risk of chronic HAART-associated metabolic complications, such as diabetes and dyslipidaemia (Izzedine et al., 2009), which, in turn, can contribute to vascular damage and decreased renal function. Understanding the mechanism of HAART-related kidney disease is essential to put in place appropriate preventive measures for proper selection and dose adjustment/substitutions of antiretroviral agents and other drugs commonly used in patients with kidney disease which is an important component of patient management and care (Ministry Of Health, 2013).

1.2 STATEMENT OF THE PROBLEM

ART administration to HIV positive adults and adolescents in health institutions has been going on for some time now (since 2002) in Zambia. It has experienced a steady rise due to an increase in HIV positive patients eligible to start the treatment (Ministry Of Health, 2013).

The TDF plus FTC based treatment has been associated with renal abnormalities (Pichit and Somnue, 2010). Several studies have shown that TDF can cause mild to moderate renal dysfunction (Bygrave et al., 2011b, Szczech, 2008, Mitra et al., 2014, Freeman et al., 2015, Pichit and Somnue, 2010). With the increase of patients eligible to start on this combination in Zambia especially that over 71% ART patients are on this drug (Ministry Of Health, 2013, Central Statistical Office et al., 2014) it becomes very important to monitor renal function. Monitoring should be by using creatinine clearance instead of just measurement of blood creatinine and urea (UNAIDS, 2011, Ministry Of Health, 2013).

Of the 827 patients files on TDF and non TDF sampled at Ronald Ross General Hospital only 53 (6.3%) had they creatinine clearance done. If this is the case, the likelihood of missing patients with renal dysfunction who are ineligible for TDF increases. The guideline recommends non-use of TDF on patients with creatinine clearance $\leq$ 50ml/min. This is to say HIV patients not eligible for TDF are been initiated on it. This has even been recommended in the latest standard HAART treatment protocol for adults and adolescents in Zambia (HAART manual GRZ, 2013) that creatinine clearance be used to monitor renal function. In April 2011, Kidney Disease Deaths in Zambia reached 1,819 or 1.07% of total deaths (UNAIDS, 2011).

With mortality linked to renal dysfunction standing at 34.4% at University Teaching Hospital (UTH) (Banda et al., 2010) it is essential to calculate creatinine clearance. Creatinine clearance is the recommended parameter to be used in assessment of renal function though in a number of hospitals in Zambia this is not the case (Mulenga et al., 2008) Thus, it's difficult to known when possible the dose of patients develop renal damaging need to be adjusted or changed. This is expensive for institutions and nation at large to manage patients with dialysis especially that not enough dialysis machines exist. For instance there is just one dialysis unit for instance in Copperbelt. If we can reduce the problem by determining creatinine clearances why not promote it. This study aimed to provide statistical reverence of this to reduced future epidemics and improve patient management and care.

1.3 STUDY JUSTIFICATION

The increased number of HIV positive patients meeting the eligibility criteria for starting antiretroviral drugs, specifically the first line cannot be overemphasized. The TDF/FTC combination is in the first line treatment for whole newly eligible patients on ART in Zambia (Ministry Of Health, 2013). The study aimed to enlighten health practitioners, authorities and general public on the incidence of renal dysfunction on HIV positive patients on HAART since the revision of new treatment guidelines in Zambia.

In Mufulira for instance, over 4,000 patients are on this combination (TDF/FTC) and with an increase of patients been switch to second line due to many factors and renal abnormalities standing as one of the reasons. It then becomes vital to know amongst the population that begin a TDF/FTC combination how many are actually developing renal dysfunction and in the management was creatinine clearance done.

The last Hospital Performance assessment 2015 1st quarter reported a mild increase in number of renal cases by clinicians (Audit unpublished reports, 2015). If for example, some cases are been missed at initiation, how far good is the follow up so that those who develop renal abnormalities can be changed. This study aimed to improve patient care, identify the potential challengers been faced in patient management and further reduce the risk of having future epidemics. Furthermore, it presented information to authorities and health practitioners to known how certain cases of renal dysfunction are been missed on initiation and when possible renal dysfunction begins in patients on TDF/FTC regimen.

This information if available to clinicians can result in improved patient management and care. It is evident that over 95% of all eligible ART patients begin on this combination (Ministry Of Health, 2013, Central Statistical Office et al., 2014). Considering the renal abnormalities associated with this TDF-based therapy (Szczech, 2008, Freeman et al., 2015) it becomes important to the general public to know if the guidelines on patient management are been followed. The study also provided statistical information on the importance of evaluating renal function using creatinine clearance rather than just measurement of blood creatinine levels and urea. It further pointed to medical practitioners to appreciate the importance of determining the creatinine clearance in intervals on HIV positive patients before and during treatment. The study in turn informed and helped authorities to plan and put in place facilities which will help prescribers to design and monitor appropriate ART regimens and management for such patients.

CHAPTER 2: RESEARCH HYPOTHESIS AND AIMS

2.2 RESEARCH QUESTION

Does creatinine clearance predict renal function better than serum creatinine?

Does Tenofovir treatment of HIV positive patients aged 15 to 45 alter creatinine clearance?

2.3 HYPOTHESIS

Null hypothesis (Ho): There is no difference in development of renal dysfunction between HIV positive patients exposed to TDF and those not.

Alternative (Ha): There is a difference in development of renal dysfunction between HIV positive patients exposed to TDF and those not.

2.3 GENERAL OBJECTIVE

To determine if the incidence of renal dysfunction in adults HIV-positive patients with long term tenofovir-based regimen use is higher than in those without tenofovir use.

2.4 SPECIFIC OBJECTIVES

1. To compare blood creatinine in HIV-positive patients on tenofovir-based therapy and those without tenofovir-based therapy.

2. To calculate and compare creatinine clearance in HIV-positive patients on tenofovir-based therapy and those on non-tenofovir therapy.

3. To determine whether blood creatinine is a better biomarker predictor of renal dysfunction than creatinine clearance in HIV-positive patients on long term treatment.

CHAPTER 3: METHODS AND MATERIALS

3.1 STUDY DESIGN

This was a retrospective cohort study. Secondary data was used to extract baseline data for patients first from the data management software SMARTCARE, verified with patients files/nots, registers before HAART was initiated to them thereafter a follow up of similar variable at 6 months, 12 months and 18 months. The blood/serum creatinine results were used to calculate the creatinine clearance which was then used to estimate the GFR (renal function) for those exposed to TDF or not (Figure1).

3.2 STUDY SETTING/AREA

This study was conducted at Ronald Ross General Hospital, located in the Copperbelt province Mufulira, Zambia. The district is 411 kilometres away from the capital city Lusaka. It is a teaching hospital for nurses and also the main referral hospital for south eastern region covering 18 health centres and one district hospital. It has a bed capacity of 340. The health services in the district are mainly provided by hospitals, private clinics, traditional practitioners, health centres and health posts. Ronald Ross Hospital, a general Hospital (level 2) was chosen as the site for the study because of its effective use of SMARTCARE software and for convenience.

3.3 STUDY POPULATION

All HIV positive patients aged 15 years and above on antiretroviral therapy at Ronald Ross General Hospital ART database.

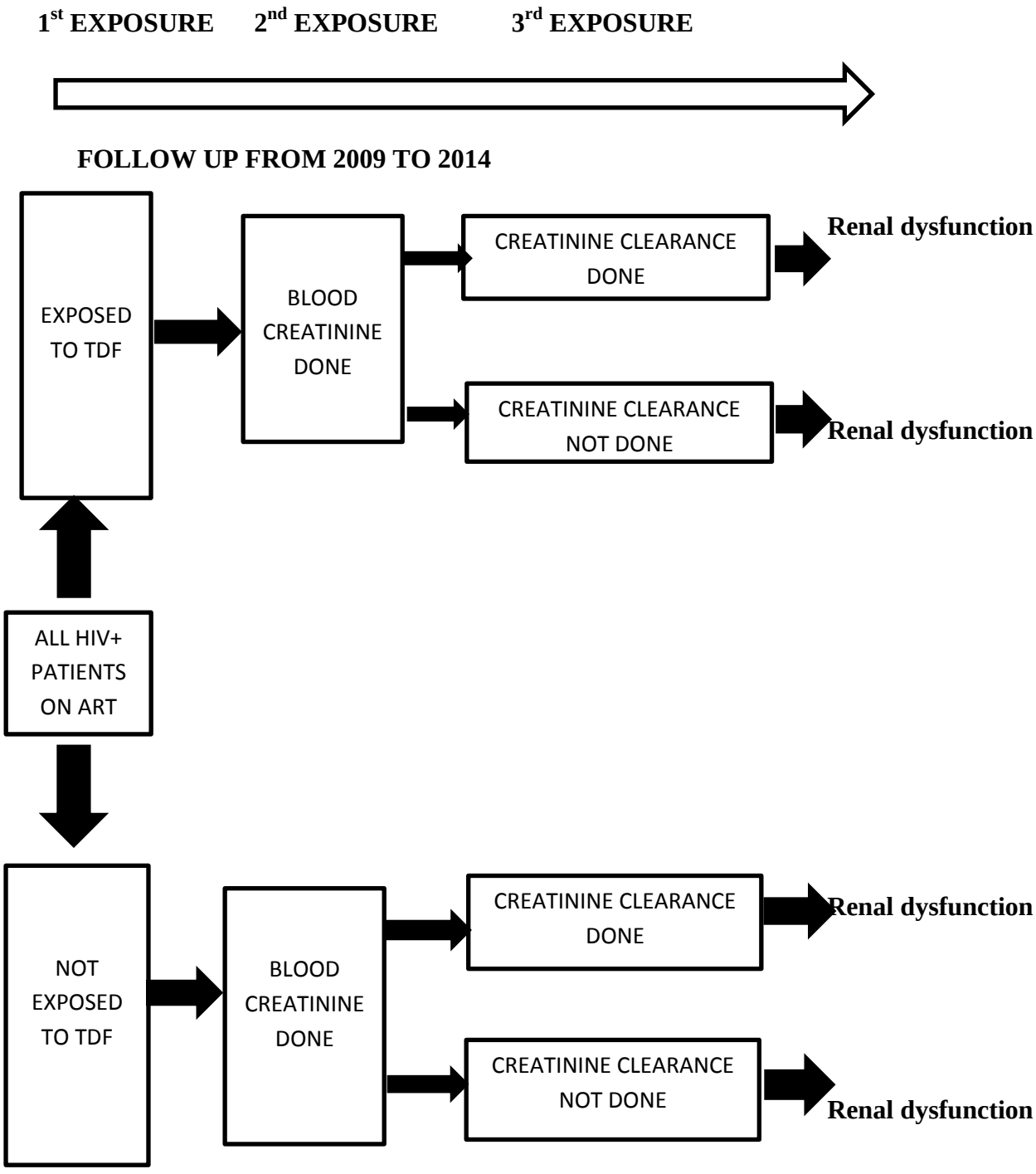

Figure 1: The cohort design

3.4 VARIABLES

Table 1: Outcome/Dependent and independent variables

	VARIABLE	INDICATOR	VARIABLE TYPE
OUTCOME	Renal dysfunction (Impairment)	Creatinine clearance ≤ 50 ml/min	Categorical (Paired)
INDEPENDENT	CD 4+ cell count	Availability	Continuous
	Age	Availability	Continuous
	Gender	Male Female	Categorical (binary)
	Cotrimoxazole use	Yes No	Categorical
	Serum/blood creatinine	Availability	Continuous
	Creatinine clearance	Availability	Continuous
	Urea	Availability/count	Continuous

3.5 STUDY PROCEDURE

3.5.1 SAMPLING PROCEDURE

In this study, study population included all HIV positive patients aged 15 and above on ART at Ronald Ross General Hospital. Two line groups of antiretroviral therapy were considered. This helped us study their effects on blood creatinine levels; a risk factor to HAART associated renal complications. The two line groups included; the TDF containing regimen (TDF+FTC) as first group and the non-TDF regimen as second group. The groups comprised participants on the drugs depicted in Table 2 below. Abbreviations as define earlier. From the SOP on ART provision in Zambia 2014 (Ministry Of Health, 2013)

First line regimen		**Second line regimen**		
	EFV		3TC	
TDF/FTC	OR	AZT	TDF/FTC	LPV/r
	NVP		D4T/3TC	

Figure 2: Zambia Ministry of Health Standard ART line regimen

Table 2: Different drug combination categorized into two groups

GROUP 1 (Tenofovir containing)	GROUP 2 (Non-Tenofovir containing)
TDF/FTC/NVP TDF/FTC/EFV, AZT/TDF/FTC/LPV/r TDF/FTC/LPV/r	AZT/3TC/LPV/r, AZT/D4T/3TC/LPV/r, ABC/3TC/EFV ABC/3TC/EFV/NVP AZT/3TC/NVP or EFV, D4T/3TC/NVP or EFV.

The sampling method involved listing all the study units (patient's files) of the study population into two groups according to treatment received. The minimum is as per sample size which was calculated.

3.5.2 ELIGIBILITY CRITERIA

Inclusion criteria

1. Aged 15 and above.
2. Current use of HAART regimen (NRTI and NNRTI based) with or without a PI.
3. Have no confirmed conditions which increase blood creatinine levels (impaired renal function, chronic nephritis, Urinary tract obstruction, HIV-associated nephropathy, muscle disease such as gigantism, acromegaly, myasthenia gravis nephropathy, muscle disease such as gigantism, acromegaly, myasthenia gravis and others such as congestive heart disease and shock).

Exclusion criteria

1. Renal abnormalities before treatment initiated e.g. impaired renal function, chronic nephritis
2. Patient on other antiretroviral-drug other than first/second line
3. Patients with no serum creatinine level results before treatment and after treatment.

3.5.3 PARTICIPANTS FOLLOW UP

Patient files were followed up according to their exposure status; HIV positive adults and adolescents exposed to Tenofovir, creatinine and creatinine clearance and those HIV positive patients who were not exposed to Tenofovir were followed up similarly as depicted in the figure 1. For example, those exposed to TDF, was creatinine levels done and how many had their creatinine clearance calculated. Follow up was for a period of 3 years following the original date of enrolment into the study. The period of recruitment into the study was from 2008-2009.

On recruitment all patients' files were selected and then exclude some who did met exclusion criteria. All patients who met the inclusion criteria were not excluded in the study. Follow up was done as recommended by World Health Organisation (WHO) ART guideline and Ministry Of Health (Ministry Of Health, 2013) at 6 Months, 12 months and 18 months. Absence of laboratory results on the day/month of review involved lead to estimation of results by the immediate next visit done by the patient. This was done to minimize loss to follow up.

Study participant flow:

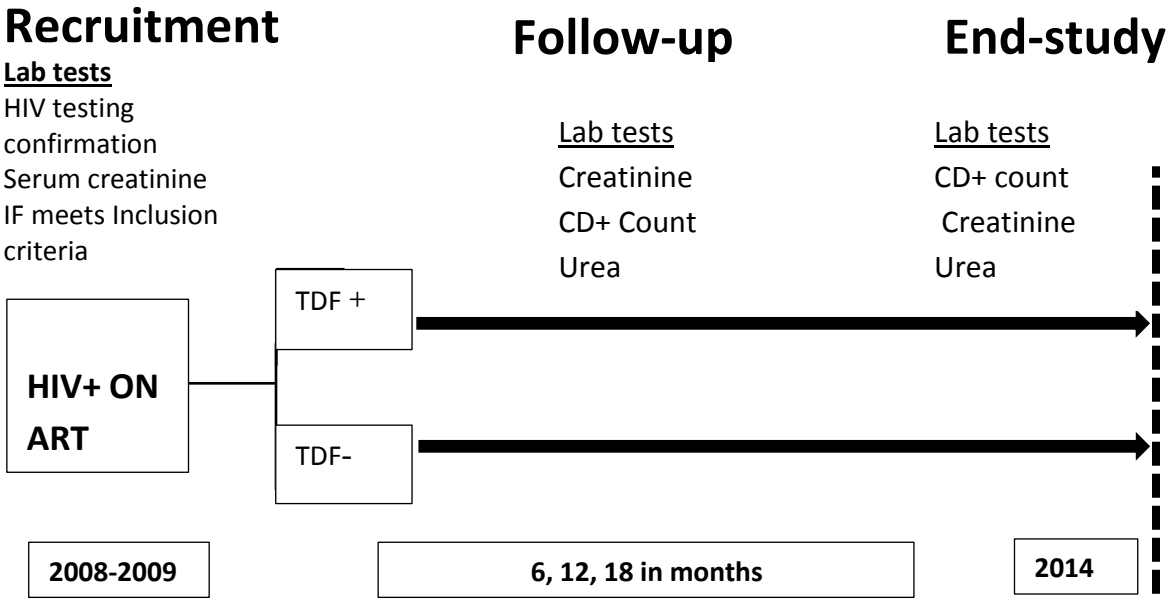

Figure 3: Study participant flow diagram

3.5.4 LABORATORY PROCEDURE

The assessment of renal dysfunction is as shown on appendices 8.1 on clinical staging of renal dysfunction. The Cockcroft Gault formula was preferred because it is relatively easy to use compared to the Modification of Diet in Renal Disease (MDRD), which has not been validated in acute renal failure. Since the formula does not adjust for body mass relative to the Cockcroft-Gault formula, it underestimates GFR for heavy people and overestimates it for underweight people. Creatinine clearance was calculated using the Cockcroft Gault formula as follows;

Calculation of Creatinine clearance (CrCl) in milliliter/minute;

In men,

CrCl = (140-age) (ideal body weight in kg)/72(serum creatinine in mg/dl)

In women,

CrCl = (140-age) (ideal body weight in kg) (0.85)/72(serum creatinine in mg/dl)

Renal dysfunction was classified by K/DOQI criterion, appendix 8.1. According to K/DOQI criterion; renal dysfunction calculated from Cockcroft- Gault equation was classified as follows: CrCl≥ 90mL/min was considered no renal dysfunction; CrCl of 60 - 89mL/min was mild renal dysfunction (K/DOQI stage 2); CrCl of 30 - 59mL/min as moderate dysfunction (K/DOQI stage 3); and CrCl lower than 30mL/min was severe dysfunction (K/DOQI stage 4 and 5).

Since we extracted secondary data and serum or plasm could have been used to measure creatinine. We decided to use the word Creatinine to represent either of them. Creatinine (µmol/l, measured by Jaffe method) was used to calculate creatinine clearance as the primary measure of renal function. This measure of glomerular filtration rate has been validated in sub-Saharan Africa and is commonly used in describing renal outcomes for clinical practice and research.

3.5.5 CASE DEFINITION

1. **Severe renal dysfunction :** Kidney damage with GRF 15-29mL/min (GRF mL/min/1,74Am)
2. **Moderate renal dysfunction:** Kidney damage with 30-59 mL/min
3. **Mild renal dysfunction:** Kidney damage with 60-89 mL/min
4. **Baseline renal function** was further categorized into CrCl<50 mL/min and CrCl ≥50mL/min

3.6 SAMPLE SIZE

In this study two drug groups were compared, the TDF and non-TDF containing. The approximate number of HIV patients actively on HAART is over 5,121 at Ronald Ross General Hospital. The 5,212 (as of Dec 2014) included patients on TDF containing and non-TDF. The estimated percentage of these patients developing drug-related toxicity in the TDF containing group is 15% from study done in Tanzania, while in the non-TDF group it is 7% (Msango et al., 2011).

The required sample to show with 90% likelihood that the percentage of developing drug-related toxicity is different in these two groups would be;

(Formula for comparative study)

$$n = \frac{P_1(1-p_1) + p_2(1 - p_2)}{(P_1 - P_2)^2} (Z_\alpha + Z_\beta)^2$$

$$= \frac{(1.28 + 1.96)^2[(15(100-15) + 7(100-7))]}{(15-7)^2}$$

$$= \frac{10.4976 * (15*85 + 7*93)}{8^2}$$

$$= 343.912 \text{ (344 patients in each group)}$$

Adjusting for loss to follow up at 80%=430

Where

N= sample size

p= proportion (percent)

Z_β = one sided percentage point of normal distribution, corresponding to 100%-power (the power is the probability of finding a significant result) being 90%, Z_β =1.28

Z_α =percentage point of the normal distribution, corresponding to (two-sided) significant level. The significant level is 5% (for 95% CI), Z_α =1.96

Sample size of 860 HIV-1 patients was the target. Of the 860 patients, 50% was from the TDF containing (first group) and another 50% from the non-TDF (second group).

3.7 DATA COLLECTION

Demographic data was extract using a data collection form from the data software SMARTCARE. This software contains data about all HIV positive patients. It includes data on patient demographics, laboratory profiles, drugs and other variables. We verified data from software with patient files and doctors notes. The following demographic data were collected; age, sex, education, and employment status. Past medical history and laboratory was taken. The history was taken to identify other risk factors for renal dysfunction in these patients.

3.8 DATA MANAGEMENT AND ANALYSIS

When normality assumptions are met means and standard deviation were reported otherwise median and interquartile range was reported. Logistic regression was used for variables with binary outcomes. To measure the strength of the association, both unadjusted and adjusted risk ratio will be calculated. Crude rate ratios (RR), stratified RR and Mantel-Haenzel will be used to assess confounding and effect modification. We analyzed repeated measure corrections using repeated measure ANOVA and two-way ANOVA.

Cleaned data was analyzed using STATA for windows software (version 12.0). The p value was obtained via compared means-paired sample t-test. T-test for assessment of differences between baseline and subsequent values after treatment between and within groups was used.

Mean values at initial start and subsequent values was used to obtain an overview statistical correlation in creatinine clearance, CD4+ cell count, Urea and weight. Furthermore, frequency tables for age and sex will be obtained.

A summary is shown below;

VARIABLE	DATA ANALYSIS TOOL/TEST
Categorical variables	**Numbers, frequency distribution Proportions., Percentages and ratios**
Continuous variables (Descriptive)	**Means, Medians, SDs, Range, Background characteristics**
Associations/Difference	**Paired t tests P values to compare means Pearson correlation coefficient**
Independent associations	**Logistic regression**

3.9 ETHICAL CONSIDERATION

The study was approved by the University of Zambia Biomedical Research Ethics Committee. In this study, sampling of the study files involved the use of reference numbers that Ronald Ross General Hospital uses. These reference numbers were used by both the laboratory, pharmacy and the HMIS for easy follow up of patients. It involved going into Ronald Ross General Hospital database to select the subjects on HAART that were routinely on blood creatinine tests.

The use of reference numbers protected the privacy, confidentiality of patient's files and ensured the integrity of the data. Access to the data files and data extraction tools was highly restricted and reference numbers have not been used in this report. We obtained permission to analyse secondary data from Hospital authorities and collected data to be destroyed after 4 years.

CHAPTER 5: RESULTS

A total of 1,342 adults and adolescents on antiretroviral drugs were enrolled in this study from February 11, 2008 through September 15, 2014. After exclusion of some participants due to renal abnormalities, absence of creatinine results before initiation, 834 patients meet the inclusion criteria. Of the 834, 447 (53.6%) were on a regimen containing TDF and 387 (46.4%) were on a non-TDF containing regimen. A total of 153/447 participants on TDF and 123/387 on non-TDF actually completed the follow up of 18 months. This was as a result of non-availability of results. However, this was considered in the design stage and analysis as stated in the methods. At baseline, the TDF exposed group was older 36 years (IQR: 30, 42) than the non TDF group 35 years old (IQR: 29, 45), though not clinically significant (p-value=0.21). There were more females than males in this study (Table 3).

Table 3: Baseline demographic characteristics

Characteristic		Tenofovir (n=447)	Non-Tenofovir (n=387)
		n (%)	**n (%)**
Gender	Male	136 (50.56)	133 (49.44)
	Female	310 (55.06)	253 (44.94)
Age (years)	15-34	219 (52.64)	197 (47.36)
	35-49	182 (56.70)	139 (43.30)
	50-80	46 (47.42)	51 (52.58)
Education	Primary	130 (48.51)	138 (51.49)
	Secondary	179 (54.74)	148 (45.26)
	Tertiary	67 (58.77)	47 (41.23)
Employment Status			
	Employed	137 (54.37)	115 (45.63)
	Not employed	209 (53.18)	184 (46.82)

Most of the participants were females aged 15-34 years old, unemployed and had a secondary level education. Renal function defined by creatinine clearance at baseline before ART initiation was similar in both groups (Figure 3 and 4). There were 14 failure events observed during a total of 1399.4 person-years. The longest follow-up time for an individual was 12.7 years. The incidence rate (attack rate) in those exposed to TDF was 15.3 per 1000 per person-year

Table 4: Clinical characteristics of study population

Variable	Tenofovir (n=447)	Non-Tenofovir (n=387)	P value
Baseline laboratory values			
CD4 cell count (Count. /uL)			
Before ART initiation†	211 (109, 338)	240 (125, 340)	0.13
6 months†	334.5 (227, 465)	395 (280, 525)	**<0.001**
12 months†	375 (262, 516)	398 (309, 555.5)	**<0.007**
18 months†	388 (272, 540)	458 (360, 639)	**<0.001**
Creatinine (µmol/l)			
Before ART initiation*	64 (22.6)	64 (23.7)	0.86
6 months*	65 (21.5)	63 (21.5)	0.23
12 months*	66 (23.2)	63 (25.2)	0.08
18 months*	67 (20.8)	63 (18.7)	**0.04**
Creatinine Clearance (mL/min)			
Before ART initiation*	106.2 (38.7)	105.8 (35.1)	0.86
6 months†	104.2 (37.2)	111(40.8)	0.08
12 months†	105.4 (38.8)	114 (41.3)	**0.01**
18 months*	104.9 (35.4)	109.7 (39.5)	0.16
Urea			
Before ART initiation†	3 (2.2,3.5)	2.7 (2.1,3.5)	0.07
6 months (first review) †	2.6 (2.1, 3.6)	2.9 (2.2, 3.8)	0.22
12 months†	2.9 (2.4, 3.7)	3.2 (2.5, 4.1)	**0.01**

18 months†	2.8 (2.3,3.5)	3.1 (2.5,3.7)	0.07
Weight (kg)			
Before ART initiation †	55 (49,60.5)	56 (49,62.5)	0.23
6 months†	55.8 (50,62)	55.5 (49,63)	0.66
12 months†	57 (51,62)	57 (49.3,65)	0.57
18 months†	57 (52, 62.5)	55 (51, 63)	0.36
Medication use			
Anti-Tb medication, n (%)			
YES	156 (56.7)	119 (43.3)	0.27
NO	257 (52.6)	232 (47.4)	
Co-trimoxazole use, n (%)			
YES	220 (60.8)	142 (39.2)	**<0.001**
NO	94 (42.7)	126 (57.3)	
Renal Dysfunction stage, n (%) **			
I= Normal or increased GFR	193 (53.5)	168 (46.5)	0.51
II=Slightly decreased GFR	83 (49.7)	84 (50.3)	
III= Moderately decreased GFR	17 (60.7)	11 (39.3)	
IV= severely decreased GFR	1 (100)	0	

276 participants had missing creatinine results at 18 months and were excluded from this table as creatinine clearance could not be calculated.

* Mean (SD) and chi-square test for association

† Median (IQR): Two-sample Wilcoxon rank signed test (Mann–Whitney) performed

** 18 months Creatinine clearance after ART initiation

(8.5-27.7) compared to those not exposed to TDF 4.4 per 1000 per person-year (1.4-13.6), 627 records included in analysis. The point prevalence of mild and severe renal dysfunction among HIV-positive adults on TDF-based therapy was 18.4% and 0.2% respectively at 18 months. Relative risk of TDF exposure and non TDF was 3.7 per 100 and 1.5 per 100 respectively. The risk ratio was 2.5 between the exposed and unexposed. The odds ratio between those exposed to TDF and those not exposed was 2.9.In addition, 59.3% of renal dysfunction in this study was attributed to TDF exposure of these participants. The median CD4 cell count for TDF-exposed group and non-TDF expose respectively before initiation of therapy was 211 cells/uL and 240 cells/uL.

There was statistically significant difference in CD4 cell count (at 3 time points) between groups, in creatinine (at 18 months), creatinine clearance (at 6 months) and co-trimoxazole use between the two groups (Table 4). Maximum likelihood estimate of the rate ratio (RR) between those exposed to TDF and those not was 0.29. 95% CI 0.08-1.02, p-value=0.04 and log-rank test for equality of survivor function had p-value=0.04.Creatinine clearance calculation was performed in 423 participants (423/447, 95%) on TDF and 358 (358/387, 93%) for non-TDF group at baseline. At 18 months during treatment creatinine clearance was performed on 294/447 (65.8%) patients on TDF and 264/387, (68.2%) on non-TDF regimen.

This actually agreed with the highly criticized assumption that HIV patients renal functions are primarily monitored by creatinine changes, which is against World Health Organization (WHO) recommendations and Zambia Ministry of Health guidelines (Ministry Of Health, 2013). There was no significant difference in baseline weight, renal dysfunction stage and whether the patient was once on TB medication or not between groups.

In repeated measure ANOVA analysis, the null hypothesis that all subjects (n=734) had the same creatinine clearance at baseline and 18 months after exposure to tenofovir was firmly rejected (p-value=0.7853). A correction factor (Box's conservative≤Greenhouse–Geisser ≤Huynh–Feldt) was 1 which is the same as no correction. The purpose of this analysis was to investigate variation in creatinine clearance (within-group factor) over the 18 months follow up when exposed/unexposed to TDF (between-group).

In a two-way ANOVA performed on 1380 participants to examine the effect of gender and TDF exposure on renal dysfunction development in HIV positive patients. There was no significant effect of tenofovir, interaction between gender and tenofovir exposure on renal dysfunction, F (1,55)=0.60, P=0.4400.No simple main effects analysis was performed as interaction effect was insignificant (p-value=0.440). However there exist a significant difference between males and females on renal dysfunction (p<0.0001). Females were more at risk. Further, exposure/non exposure to TDF and the time creatinine clearance was performed were insignificant (p-value=0.3359 and 0.4901 respectively). In other words, we found no evidence that the time-scores of creatinine clearances performed were different by treatment type (p-value=0.3328).

Within the TDF exposed group at baseline, 288 presented with normal renal function, 28.5% (125) presented with mild renal dysfunction, 24 (5.5%) had moderate renal dysfunction and 2 (0.5%) had severe renal dysfunction. Those on TDF who developed severe renal dysfunction had a baseline CD4 cell count <200 cells/uL by initiation date. At 18 months, 83 (49.7%) patients on TDF had developed mild renal dysfunction (Stage III) compared to 84 (50.3%) on non-TDF combination (Table 4). Two patients on TDF developed severe renal dysfunction (stage IV) compared to three on non-TDF regimen after 18 months therapy.

TDF exposed group who had GFR < 50 ml/min at baseline were 15 (3.4%) which represented participants wrongly initiated on TDF according to Zambia guidelines. When compared to patients who were started correctly on TDF, this group comprised of more females, older patients (50-77 years) which was consistent with the risk factors for low creatinine clearance at 18 months identified in multivariate analysis. Among those who remained on TDF regimen, 5 were discontinued after 12 months of therapy and 2 developed severe renal dysfunction (GFR <30ml/min).

In both unadjusted and adjusted analysis for mean changes in GFR from baseline to 18 months, TDF exposed patients were 2.5 times more likely to have decline in GFR through 6 and 12 months compared with the non-TDF containing group though it was not statistically significant (Table 5).The decline in GFR was more pronounced among TDF-exposed group if the baseline creatinine clearance levels was greater than 80mL/min/1.73m^2, but also if GFR was between 50 and 79mL/min/1.73m^2.

The only statistically significant predictors of greater than 50% decline in GFR or as a continuous outcome measure were baseline age and CD4 cell count at 18 months follow up (Table 5).The sensitivity analysis performed reviewed an Receiver Operating Characteristic Curve (ROC) value of 0.76 which was close to 1 than 0.5 suggesting that classification was not due to chance.

Increase in creatinine was a statistically significant predictor of renal dysfunction unadjusted for other variables (OR: 1.00, 0.98-1.02, P-value=0.056). Exposure to TDF after 18 months of follow up had no statistically significant effect (AOR:2.28 95% CI 0.98-7.36, P-value=0.529) on renal function in both univariate and multivariate logistic analysis (Table 5). Using the p value <0.05 and 95% CI to define statistically significant potential predictors of renal dysfunction, we found baseline creatinine, age and CD4 cell count at 18 months therapy to be significant predictors of renal dysfunction.

In univariate analysis age per year increase <49, baseline creatinine and higher CD4 cell count >500cells/uL were factors less likely associated with renal dysfunction. When we compared the baseline and 18 months creatinine level changes between those on TDF and those on non TDF regimen, we found it was insignificant (p-value=0.86 and p-value=0.08 respectively). Similarly, we compared the baseline and 18 months creatinine clearance changes between those on TDF and those on non TDF regimen. There were both not significantly different (Figure 3 and 4).

Table 5: Univariate and Multivariate Analysis of renal function as a binary outcome

	Univariate Analysis			Multivariate Analysis		
Variable	**OR**	**95% CI**	**Unadjusted P value**	**OR**	**95% CI**	**Adjusted P value**
Baseline creatinine	1.00	0.98-1.02	0.056	1.00	0.99-1.00	0.118
TDF exposure						
Non TDF	1		1	1		1
TDF	2.52	0.79-8.00	0.118	1.01	0.98-1.04	0.529
Baseline urea	1.13	0.74-1.73	0.568	1.00	0.98-1.01	0.990
Gender						
Female	1		1	1		1
Male	0.61	0.17-2.20	0.453	0.98	0.95-1.02	0.365
Age						
15-34	1		1	1		1
35-49	1.81	0.12-1.57	0.983	0.98	0.95-1.02	0.302
>50	6.35	**2.18-18.4**	**0.001**	1.78	**1.03-1.92**	**0.001**
Baseline CD4 cell count						
<350	1		1	1		1
350-500	3.03	**0.98-9.28**	**0.053**	1.02	**0.97-1.07**	**0.051**
>500	0.69	0.08-5.52	0.726	1.00	0.98-1.05	0.974
Baseline weight						
30-49	1		1	1		1
50-69	0.52	0.18-1.46	0.218	0.96	0.93-1.00	0.089
70-80	1.63	0.12-2.85	0.992	0.95	0.89-1.01	0.133
TB medication						
Yes	1		1	1		1
No	1.66	0.55-5.02	0.367	1.02	0.98-1.06	0.221

Predictor variables included in full model: demographics, baseline creatinine, weight, age, TB medication and baseline CD4 cell count. Final model developed by backward selection until all covariance were at P < 0.05 level. OR = Odds ratio, CI = Confidence interval

In the adjusted logistic regression (Table 5), all other crude odd ratios were not statistically significant except age and CD4 cell count. Patients aged <49 years than ≥59 (OR: 1.78 (1.03-1.92), p=0.001) were less likely associated with renal dysfunction controlling for baseline creatinine, exposure to TDF, gender, age, baseline CD4+ cell count, weight and urea. In addition, patients with a high CD4+ cell count >500 cells/uL (AOR: 1.24 (0.97-1.07), P=0.051) were less likely associated with renal dysfunction adjusting for weight, age, gender, urea, baseline creatinine and TDF exposure. Baseline urea, medications (TB medication, Cotrimoxazole), baseline weight, age, whether employed or not and education level had no significant effect on renal function in this study.

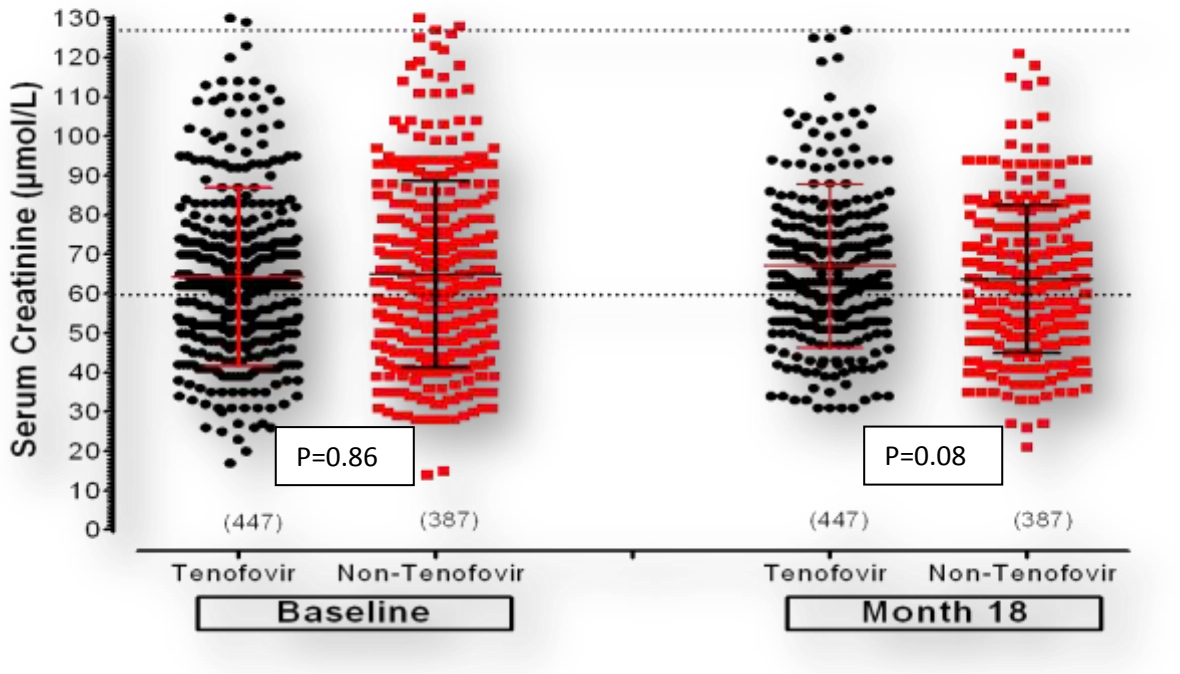

Figure 4: Serum Creatinine at Baseline and Month 18

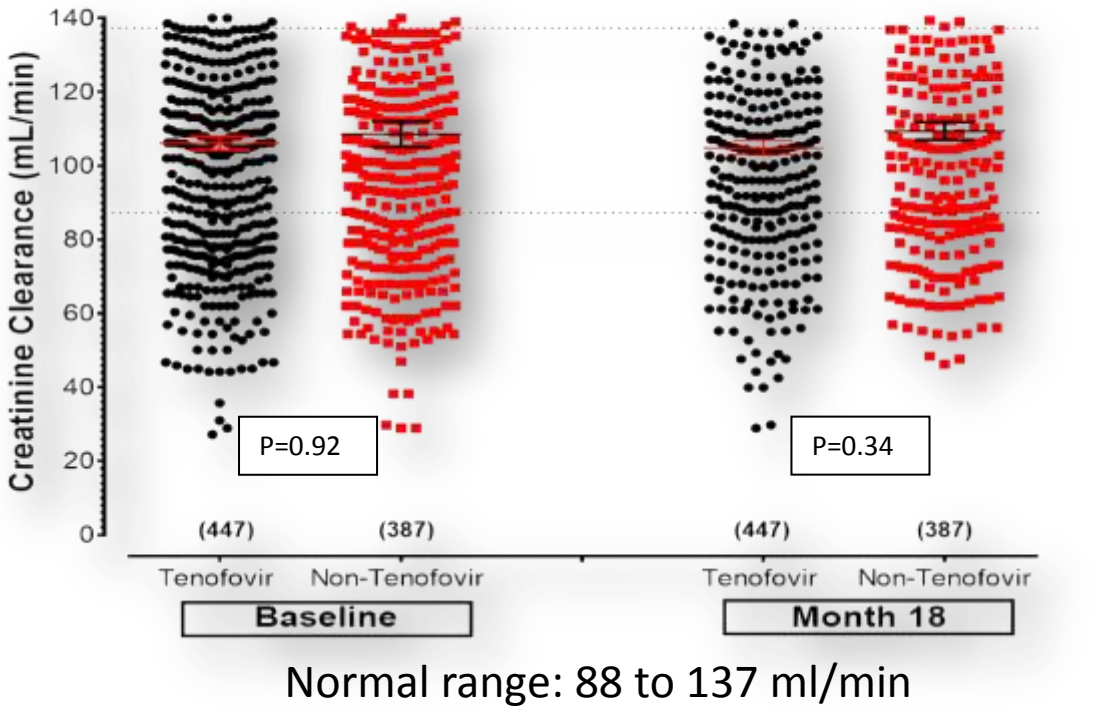

Figure 4: Creatinine clearance at Baseline and Month 18

Among the TDF exposed group statistically significant predictors of creatinine rise were increase in age, increased weight and lower CD4 cell count. Among patients with baseline CD4 counts less than 50 cells/uL (74), there was a statistically greater risk of developing increased creatinine with TDF exposed compared with non-TDF exposed patients (AHR: 8.1, 95% CI 1.72, 67.4, p-value=0.03).Comparing the two groups, we observed a steady percentage rise in creatinine and decrease in creatinine clearance on those exposed to TDF compared to the non TDF exposed patients. Although this was not statistically different between the groups (figure 3 and 4).

CHAPTER 6: DISCUSSION

6.1 DISCUSSION

Renal dysfunction deaths in Zambia stood at 16.37 per 100,000 (WHO., 2014).Despite been one of the countries affected with high HIV burden there is limited data on renal disease and the risk factors predisposing the population to it. Our primary goal in this study was to evaluate whether Tenofovir Disoproxil Fumerate (TDF) treatment of HIV positive patients alter creatinine clearance (CrCl) and to compared the incidence of renal dysfunction among those exposed to TDF and those not.

We found that TDF exposure places HIV positive patients at relative risk of 15.3 per 1000 per person-year to 4.4 per 1000 per person-year to develop renal dysfunction after 12.7 months follow up relative to those not exposed to it. This is an indication of the disease distribution in the population affecting the therapeutic measures currently available. The high incidences can further be explained by the long duration of the disease because renal dysfunction is been missed at follow up. The high number of creatinine clearance attest to this error.

The attributable risk due to TDF exposure (59.3%) represents the expected reduction in disease in the population if the exposure could be removed. Considering that these patients are on these drugs for such a long duration, screening at every follow up visit could be intensified. This was further supported with evidence that those patients exposed to TDF were 2.7 more likely to develop renal dysfunction with 2.9 odds than those not exposed to it. This was quite very interesting because the baseline renal function as estimated by the creatinine clearance was not different between groups at baseline even at 18 months during follow up.

Other studies have found renal dysfunction among those exposed to TDF and had a baseline CD4 cell count <200cells/uL (Mulenga et al., 2008, Micheal et al., 2009). In this present study, the median CD4 cell count was 243.9cells/uL for both groups eliminating the assumption that it could have led to renal dysfunction. However, on univariate and multivariate analysis, 3.02 odds and 2.45 existed among those who had a CD4 count between 350 to 500 indicating that their more likely to develop renal dysfunction. This suggests that CD4 cell count could be attributed to and was an important factor predisposing patients to renal dysfunction in this study. This is vital because low CD4 cell count has been shown to lower GFR of patients, especially in advanced HIV disease (WHO stage 2 and 4).

The only explanation which could explain the variation in incidence rate could be the prevalence, TDF exposure and maybe the duration of the disease in the population. Differences in diet and environment conditions may also have contributed to the results. Despite this our results clearly evidence that our analysis are not due to chance.

We found no association between treatment with TDF-based regimen and development of abnormal creatinine clearance at 18 months during treating (P>0.05) on both univariate and multivariate analysis. Indeed the risk exists when exposed to TDF but the effect size (strength of association) was not statistically significant. It however does indicate that renal dysfunction was more likely to occur in the exposed group. Further, the prevalence of mild renal dysfunction in this study was found to be 28.5% which agrees with similar studies done ranging from 27.5% to 42% (Mulenga et al., 2008, Freeman et al., 2015, Micheal et al., 2009)

The similarity of the baseline creatinine and creatinine clearance in both our patient population as depicted in figure 3 and 4 points out an important factor usually ignored by clinicians. On close observation, their existed a steady decline in creatinine clearance at all times points during treatment on those exposed to TDF though the creatinine seemed indifferent between the group. Most of the clinicians in heath institutions would monitor renal function primarily based on creatinine and urea levels.

However, as it has been shown, quite a number of ineligible patients who should not begin on TDF are been missed at the initiation period, What come out quite surprising also was the insignificant variables such as; gender, baseline creatinine clearance and exposure to TDF in both univariate and multivariate analysis. These variables have been showing by other studies to affect this outcome. We decided not to retain them in the nested model because there were insignificant. Age and weight were confounders and effected modifier and as such the reported rate ratio (RR) was the Mental Hanzal RR and the age-specific rate ratios.

The definition of renal dysfunction also differed in our analysis with other studies such as Freeman et al and Szczech et al who used creatinine as a continuous predictor variable while we used categorical definitions of estimated creatinine clearance. Furthermore and probably many some of these studies that demonstrated a mild or severe renal dysfunction due to TDF had more covariant (Diabetes, hepatitis c co-infection) than we did.

It can be said that TDF does indeed affect the GRF by decreasing it. However this decrease was not statistically significant between those who were exposed to TDF and those who were

not. Prevalence of mild renal insufficiency ranges from 24% in Zambia to 41.2% in Malawi, moderate insufficiency ranges from 7.6% in Zambia to 21.8% in Malawi, and severe insufficiency was under 2% in all studies (Bygrave et al., 2011b, Mulenga et al., 2008). We find a closely similar prevalence.

The design of our study, long follow up and the choice of covariates which were used due to recommendations from other similar studies done gives our results significance to our patient population, emphasis on already existing knowledge highlighting the gaps exist in the management of such patients. Other studies could have the ability to control for a variety of many confounders potentially associated with renal dysfunction.

We defined renal dysfunction according to the Kidney Disease Outcome Quality Initiative Classification by GFR (K/DOQI) and further categorized it as CrCl<50 ml/min and CrCl>50ml/min. Our results agree with the other studies done in Zambia that TDF leads to renal dysfunction at 6 and 12 months of follow up (Mulenga et al., 2008, Freeman et al., 2015). We have found that at 18 months during TDF and non TDF therapy, there was no clinical difference in creatinine and creatinine clearance between groups (Figure 3 and 4).Even though 2 patients exposed to TDF developed renal dysfunction it was partly due to the low baseline CD4 cell count (84 and 80 respectively) at Initiation.

TDF is currently a recommended first-line agent in combination with other antiretroviral for HIV management in Zambia, because of its favorable pharmacokinetic profile, good antiviral potency and high tolerability (Patel et al., 2010, Ministry Of Health, 2013).Zambia adopted the use of TDF in first line regimen for management of HIV in 2007. This meant all newly eligible HIV positive patients had to start with this drug combination

TDF has been considered safe and associated with fewer side-effects in many clinical trials (Swartz et al., 2015, Sax et al., 2015, Kabbara and Ramadan, 2015). However, there have been many case reports and cohort studies such as this one describing TDF-associated nephrotoxicity (Mulenga et al., 2008, Freeman et al., 2015, Wools-Kaloustian et al., 2007, Mitra et al., 2014). In many studies recommendations and guidelines have made that monitoring of renal function by done use of creatinine clearance rather than measurement of serum creatinine only. In this study interest was to determine incidence of renal dysfunction on patients on ART exposed to TDF or not, then evaluate the significance of performing creatinine clearance in intervals in such patients.

Similarly, the median weight for TDF-exposed was 55.2 kg and 55 kg for non TDF before initiation of ART indicated that less weight may not be an important factor to renal dysfunction in this study. Increases or decrease in weight of patients can affect the creatinine and hence affecting the output in renal clearance. Although it may appear that the decrease in GFR or rise in creatinine is small on the absolute scale, exposure to such therapy for a long period of time is worrisome if no renal monitoring is done.

Given the current commitment to long-term ART, small incremental decline in kidney function could eventually lead to kidney failure. Other studies did not found an association of TDF exposure and decline in GFR and some attributed renal effects to other causes. In our study, high risk patients appeared to have increased age and lower CD4 cell count at baseline. It is not surprising that such small incremental decline in renal function could be noticed especially considering that most clinicians do not follow GFR but, rather creatinine. In addition, the gradual increase in CD4 cell count is reassuring because lower CD4 cell count is associated with accelerated kidney function. In this cohort, creatinine clearance monitoring was not consistent and the accuracy of calculation if available was poor. Implementation of TDF in health facilities in which lesser-trained health cadres will be responsible for routine care provisions thus need to be accompanied with assessment of tools and protocols to ensure that calculations is performed electronically.

The strength of our study lies in the fact that we used data from a routine ART programme setting in an established programme using SMART CARE and verified with patients files, which provides a useful complaint to data derived from more controlled and better resource research settings. However, like all clinical cohort studies, not all patients received tests at every point, loss to follow up and thus had missing data. This was considered at design stage, when collecting data and in the analysis. At design we had a similar population between the exposed and unexposed. To minimize loss to follow up, we collected the immediate next values/results done on patients if missing at exact day/month. We also had a limited number of covariates for analysis. Our study was further limited by the lack of indecision regarding which formula to use to calculate the creatinine clearance (Cockcroft Gault vs MDRD) and compared with the gold Standard. All patients had enough measured criteria to be eligible for analysis.

6.2 CONCLUSION AND RECOMMENDATIONS

Our study leads further evidence to previous reports from Africa that TDF-associated renal toxicity is rare and if it does occur usually transient. We found no evidence to suggest that TDF had significant impact on renal function in our patient population at 18 months during therapy. Although efficacious, isolated incidences occurred and the long term adverse effects on renal function may limit the use of TDF for patients at higher risk of renal dysfunction. Long-term monitoring of renal function by creatinine clearance in intervals is vital and not just measurement of creatinine.

Given the low levels of toxicity observed in this and other similar clinical studies, and considering the burden and cost of laboratory monitoring in limited-resource sites, high HIV burden settings, the possibility of TDF implementation should balance the long term renal monitoring that is useful. The prevalence of mild and severe renal dysfunction among HIV-positive adults on Tenofovir-based therapy was 18.4% and 0.2% respectively at 18 months during therapy. Those patients on TDF who were older than 50 years and presented with CrCl <50 ml/min and a CD4 cell count below 500 cells/uL at baseline were more at risk of developing renal dysfunction

7.0 REFERENCES

BANDA, J., MWEEMBA, A., SIZIYA, S., MWEENE, M., ANDREWS, B. & LAKHI, S. 2010. Prevalence and Factors Associated with Renal Dysfunction in HIV Positive and Negative Adults at the University Teaching Hospital, in Lusaka. *Med J Zambia,* 37**,** 136-142.

BRENNAN, A., EVANS, D., MASKEW, M., NAICKER, S., IVE, P., SANNE, I., MAOTOE, T. & FOX, M. 2011. Relationship between renal dysfunction, nephrotoxicity and death among HIV adults on tenofovir. *AIDS,* 25**,** 1603-9.

BYGRAVE, H., FORD, N., VAN CUTSEM, G., HILDERBRAND, K., JOUQUET, G., GOEMAERE, E., VLAHAKIS, N., TRIVINO, L., MAKAKOLE, L. & KRANZER, K. 2011a. Implementing a tenofovir-based first-line regimen in rural Lesotho: clinical outcomes and toxicities after two years. *J Acquir Immune Defic Syndr,* 56**,** e75-8.

BYGRAVE, H., KRANZER, K., HILDERBRAND, K., JOUQUET, G., GOEMAERE, E., VLAHAKIS, N., TRIVINO, L., MAKAKOLE, L. & FORD, N. 2011b. Renal safety of a tenofovir-containing first line regimen: experience from an antiretroviral cohort in rural Lesotho. *PLoS One,* 6**,** e17609.

CENTRAL STATISTICAL OFFICE, C. Z., MINISTRY OF HEALTH, M. Z., INTERNATIONAL., I., UNIVERSITY TEACHING HOSPITAL, U. & TROPICAL DISEASE RESEARCH CENTRE, T. 2014. Zambia Demographic and Health Survey 2013-14. *Key Findings* Rockville, Maryland, USA: Central Statistical Office, Ministry of Health, ICF International.

DELLA NEGRA, M., DE CARVALHO, A. P., DE AQUINO, M. Z., PINTO, J. A., DA SILVA, M. T., ANDREATTA, K. N., GRAHAM, B., LIU, Y. P. & QUIRK, E. K. 2015. Long-term efficacy and safety of tenofovir disoproxil fumarate in HIV-1-infected adolescents failing antiretroviral therapy: the final results of study GS-US-104-0321. *Pediatr Infect Dis J,* 34**,** 398-405.

FABIAN, J. & NAICKER, S. 2008. HIV infection and the Kidney *Southern African Journal HIV medicine* 9-12.

FREEMAN, C., GIBSON, S., AGGREY , M., MWENYA , K., KASONDE, M., NJELEKA, B. & SOKA, N. 2015. Comparison of creatinine clearance in HIV/AIDS Patients on Tenofovir and two non-Tenofovir- based NRTIs after one year of therapy. *journal of Medical Sciences and Technology* 25.

GANONG, W. 2003. *Review of medical physiology,* New York Chicago San Francisco Lisbon London Madrid Mexico City Milan New Delhi San Juan Seoul Singapore Sydney Toronto Lange Medical Books/McGraw-Hill Medical Publishing Division

GUPTA, S. K., ONG'OR, W. O., SHEN, C., MUSICK, B., GOLDMAN, M. & WOOLS-KALOUSTIAN, K. 2011. Reduced renal function is associated with progression to AIDS but not with overall mortality in HIV-infected Kenyan adults not initially requiring combination antiretroviral therapy. *J Int AIDS Soc,* 14**,** 31.

IZZEDINE, H., HARRIS, M. & PERAZELLA, M. A. 2009. The nephrotoxic effects of HAART. *Nat Rev Nephrol,* 5**,** 563-73.

IZZEDINE, H., ISNARD-BAGNIS, C., HULOT, J. S., VITTECOQ, D., CHENG, A., JAIS, C. K., LAUNAY-VACHER, V. & DERAY, G. 2004. Renal safety of tenofovir in HIV treatment-experienced patients. *AIDS,* 18**,** 1074-6.

JONES, R., STEBBING, J., NELSON, M., MOYLE, G., BOWER, M., MANDALIA, S. & GAZZARD, B. 2004. Renal dysfunction with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy regimens is not observed more frequently: a cohort and case-control study. *J Acquir Immune Defic Syndr,* 37**,** 1489-95.

KABBARA, W. K. & RAMADAN, W. H. 2015. Emtricitabine/rilpivirine/tenofovir disoproxil fumarate for the treatment of HIV-1 infection in adults. *J Infect Public Health*.

KINAI, E. & HANABUSA, H. 2009. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses,* 25**,** 387-94.

MARRAZZO, J. M., RAMJEE, G., RICHARDSON, B. A., GOMEZ, K., MGODI, N., NAIR, G., PALANEE, T., NAKABIITO, C., VAN DER STRATEN, A., NOGUCHI, L., HENDRIX, C. W., DAI, J. Y., GANESH, S., MKHIZE, B., TALJAARD, M., PARIKH, U. M., PIPER, J., MASSE, B., GROSSMAN, C., ROONEY, J., SCHWARTZ, J. L., WATTS, H., MARZINKE, M. A., HILLIER, S. L., MCGOWAN, I. M., CHIRENJE, Z. M. & TEAM, V. S. 2015. Tenofovir-based preexposure prophylaxis for HIV infection among African women. *N Engl J Med,* 372**,** 509-18.

MAUSS, S., BERGER, F. & SCHMUTZ, G. 2005. Antiretroviral therapy with tenofovir is associated with mild renal dysfunction. *AIDS,* 19**,** 93-5.

MICHEAL, H., BETH, T., WILLIAM, T., MICHEAL, S., SUSAN, B., LEO, H., JOSEPH, C. & JACKIE, B. 2009. Impact of tenofovir on Renal Function in HIV -infected ,Antiretroviral-Naive patients. *Acquir Immune Defic Syndr,* 53**,** 53:62-69.

MINISTRY OF HEALTH, M. 2013. Antirereoviral Therapy treatment Standard Operating Procedure For adults and adolescents. *Antirtroviral Therapy Guiderline.* 2013: Minustry Of Health.

MITRA, S., PRISCILLA, R., RAJEEV, K., SAURADEEP, S., RAJKUMAR, S. & CHERIAN, A. O. 2014. Renal tubular dysfunction associated with tenofovir therapy. *J Assoc Physicians India,* 62**,** 580-2.

MIZUSHIMA, D., TANUMA, J., DUNG, N. T., DUNG, N. H., TRUNG, N. V., LAM, N. T., GATANAGA, H., KIKUCHI, Y., VAN KINH, N. & OKA, S. 2014. Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study. *J Infect Chemother,* 20**,** 784-8.

MSANGO, L., DOWNS, J. A., KALLUVYA, S. E., KIDENYA, B. R., KABANGILA, R., JOHNSON, W. D., JR., FITZGERALD, D. W. & PECK, R. N. 2011. Renal dysfunction among HIV-infected patients starting antiretroviral therapy. *AIDS,* 25**,** 1421-5.

MULENGA, L. B., KRUSE, G., LAKHI, S., CANTRELL, R. A., REID, S. E., ZULU, I., STRINGER, E. M., KRISHNASAMI, Z., MWINGA, A., SAAG, M. S., STRINGER, J. S. & CHI, B. H. 2008. Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. *AIDS,* 22**,** 1821-7.

PATEL, K. K., PATEL, A. K., RANJAN, R. R., PATEL, A. R. & PATEL, J. K. 2010. Tenofovir-associated renal dysfunction in clinical practice: An observational cohort from western India. *Indian J Sex Transm Dis,* 31**,** 30-4.

PEDROL, E., CARO-MURILLO, A. M., CASTANO, M. A., RIERA, M., OLALLA, J., DOMINGO, P., ARAZO, P., GOMEZ-SIRVENT, J. L., PULIDO, F., ROMERO-PALACIOS, A., AGUIRREBENGOA, K., VERA, F., FERRER, P. & BLANCO RAMOS, J. R. 2015. Renal safety of coformulated tenofovir/emtricitabine vs other nucleoside analogues in combination therapy in antiretroviral-naive patients aged 50 years or older in Spain: The TRIP study. *HIV Clin Trials,* 16**,** 43-8.

PICHIT, D. & SOMNUE, S. 2010. Use of Tenofovir Disoproxil Fumarate and the Monitoring of Renal Function among HIV-1 Infected Patients in a Resource-limited Setting. *J Infect Dis Antimicrob Agents 2010,* 27 number 2**,** 77 - 84.

QUESADA, P. R., ESTEBAN, L. L., GARCIA, J. R., SANCHEZ, R. V., GARCIA, T. M., ALONSO-VEGA, G. G. & FERRANDEZ, J. S. 2015. Incidence and risk factors for tenofovir-associated renal toxicity in HIV-infected patients. *Int J Clin Pharm*.

QUINN, K. J., EMERSON, C. R., DINSMORE, W. W. & DONNELLY, C. M. 2010. Incidence of proximal renal tubular dysfunction in patients on tenofovir disoproxil fumarate. *Int J STD AIDS,* 21**,** 150-1.

SAX, P. E., WOHL, D., YIN, M. T., POST, F., DEJESUS, E., SAAG, M., POZNIAK, A., THOMPSON, M., PODZAMCZER, D., MOLINA, J. M., OKA, S., KOENIG, E., TROTTIER, B., ANDRADE-VILLANUEVA, J., CROFOOT, G., CUSTODIO, J. M., PLUMMER, A., ZHONG, L., CAO, H., MARTIN, H., CALLEBAUT, C., CHENG, A. K., FORDYCE, M. W., MCCALLISTER, S. & TEAM, G.-U.-S. 2015. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non-inferiority trials. *Lancet*.

STRUIK, G. M., DEN EXTER, R. A., MUNTHALI, C., CHIPETA, D., VAN OOSTERHOUT, J. J., NOUWEN, J. L. & ALLAIN, T. J. 2011. The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi. *Int J STD AIDS,* 22**,** 457-62.

SWARTZ, J. E., VANDEKERCKHOVE, L., AMMERLAAN, H., DE VRIES, A. C., BEGOVAC, J., BIERMAN, W. F., BOUCHER, C. A., VAN DER ENDE, M. E., GROSSMAN, Z., KAISER, R., LEVY, I., MUDRIKOVA, T., PAREDES, R., PEREZ-BERCOFF, D., PRONK, M., RICHTER, C., SCHMIT, J. C., VERCAUTEREN, J., ZAZZI, M., ZIDOVEC LEPEJ, S., DE LUCA, A., WENSING, A. M. & EUROPEAN SOCIETY FOR TRANSLATIONAL ANTIVIRAL, R. 2015. Efficacy of tenofovir and efavirenz in combination with lamivudine or emtricitabine in antiretroviral-naive patients in Europe. *J Antimicrob Chemother,* 70**,** 1850-7.

SZCZECH, L. A. 2008. Renal dysfunction and tenofovir toxicity in HIV-infected patients. *Top HIV Med,* 16**,** 122-6.

THUPPAL, S. V., WANKE, C. A., NOUBARY, F., COHEN, J. T., MWAMBURI, M., OORIAPDICKAL, A. C., MULIYIL, J., KANG, G., VARGHESE, G. M., RUPALI, P., KARTHIK, R., SATHASIVAM, R., CLARANCE, P., PULIMOOD, S. A., PETER, D. & GEORGE, L. 2015. Toxicity and clinical outcomes in patients with HIV on zidovudine and tenofovir based regimens: a retrospective cohort study. *Trans R Soc Trop Med Hyg,* 109**,** 379-85.

UNAIDS 2009. On this day Worlds AIDS Day in 2008/20 years after the first One/Several milestone have been reached. *In:* DAY, W. A. (ed.). Worlds AIDS Day: Worlds AIDS Day.

UNAIDS 2011. 2011 report special edition. special edition ed.: UNAIDS.

WHO. 2014. *Kidney Disease Death Rate Per 100,000 Age Standardized* [Online]. World Health Organization.

WOOLS-KALOUSTIAN, K., GUPTA, S. K., MULOMA, E., OWINO-ONG'OR, W., SIDLE, J., AUBREY, R. W., SHEN, J., KIPRUTO, K., ZWICKL, B. E. & GOLDMAN, M. 2007. Renal disease in an antiretroviral-naive HIV-infected outpatient population in Western Kenya. *Nephrol Dial Transplant,* 22**,** 2208-12.

8.0 APPENDICES

8.1 Stages of Chronic Kidney Diseases According to the Kidney Disease Outcomes Quality Initiative Classification by Glomerular Filtration Rate (GFR) Value

Stage	Description	GFR (mL/min/1.73A m)
I	Kidney damage with normal or increased GFR	=90 (with other evidence of chronic kidney damage*)
II	Kidney damage with slightly decreased GFR	60-89 (with other evidence of chronic kidney damage*)
III	Moderately decreased GFR	30-59
IV	Severely decreased GFR	15-29
V	Kidney failure	<15

*Other evidence of chronic kidney damage may be one of the following: persistent albuminuria; persistent proteinuria; persistent haematuria; structural abnormalities of the kidneys at ultrasound scanning or other imaging tests; biopsy-proven chronic glomerulonephritis. (Wool et al, 2007).

8.2 Approval letter from Ethics