ASSESSMENT OF TENOFOVIR-INDUCED NEPHROTOXICITY DEVELOPMENT AND RECOVERY IN HIV PATIENTS ON TDF BASED REGIMENS AT KENYATTA NATIONAL HOSPITAL COMPREHENSIVE CARE CLINIC

By

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DECLARATION

STUDENT

I declare that this research proposal is my original work and has not been presented for a degree or any award in any other University.

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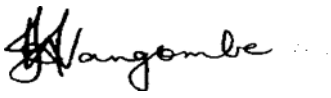
Signature  **Date** ……

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AKI- Acute kidney infection

ARV- Anti retroviral therapy

CKD- Chronic kidney disease

CCC-Comprehensive Care Center

GFR- Glomerular filtration rate

eGFR- Estimated glomerular filtration rate

HAART- Highly active antiretroviral therapy

TDF-Tenofovir Disoproxil Fumarate

HIV- Human Immunodeficiency Virus

NtRTI- Nucleotide Reverse Transcriptase Inhibitor

mtDNA- Mitochondrial deoxyribonucleic acid

SCr- Serum Creatinine

CHB- Chronic Hepatitis B

PK- Pharmacokinetic

KNH- Kenyatta National Hospital

AIDs- Acquired immunodeficiency syndrome

SCOLTA- Surveillance cohort long-term toxicity antivirals

FDA- Food and drug administration

CD4 – Cluster of differentiation 4

UON- University of Nairobi

ABSTRACT

Background: TDF containing HAART is currently the most approved global HIV first-line treatment. Despite its accessibility as a mixture single-tablet regimen for once daily dosing, favorable safety profile and resistance, and effective antiviral activity, it is associated with renal impairment among patients with concurrent use of protease inhibitors and those with advanced HIV virus. Several studies have been done investigating the risk factors for occurrence of kidney disease among HIV positive patients on TDF regimens but few have specifically investigated the time to recovery from this disease.

Objective: To assess Tenofovir-induced nephrotoxicity development and recovery among patients on TDF based regimen at KNH CCC between 2010 and 2015.

Study design and study population: The study was retrospective cohort that used HIV care follow-up data for patients (n≥528) started on TDF based regimens between 2009 and 2012. Information on the baseline distinctiveness of the patients at start of treatment and dates of change of regimen for patients that developed Tenofovir-induced nephrotoxicity was collected from the database using a structured data collection tool.

Data analysis: The two outcomes of interest were the time to development and time to recovery from Tenofovir-induced nephrotoxicity. Conditional Presmoothed Kaplan-Meier Weighted estimator was used to estimate the survival functions (time to development & recovery). Multivariate Log-rank test was utilized to evaluate the endurance functions based on the three TDF based regimens. Conditional risk set model was used to evaluate prognostic factors for time to recovery TDF-induced nephrotoxicity adjusted for time to diagnosis.

Results: Of the 534 patients were followed 324 were diagnosed with TDF-induced nephrotoxicity with only 88 reported to have been recovered. The median time to diagnosis was estimated at 43.7months after initiation of ART (IQR=24.3-59.9 months); three quarters had recovered by the 16th month upon withdrawal of TDF. Patient gender (Male-HR=0.68, P-value=0.013), age group (Adults-HR=0.66, P-value=0.029) and ALT/GPT levels (HR=1.01, P-value=0.044) were found to significantly affect the expected hazard of patient recovery.

Conclusion: Prolonged use of TDF in first line ART regimen is associated with nephrotoxicity which is reversible upon withdrawal. Males and older patients are at a high risk of taking longer to recover from the disease once diagnosed. Regular monitoring of creatinine authorization during follow-up with TDF uses is paramount to prevent nephrotoxicity especially in this high-risk group of patients.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Tenofovir disoproxil fumarate (TDF) became available in 2001 and it was the first nucleotide inhibitor of HIV reverse transcript. Since then, it has been expansively used globally and now it has become the most prescribed antiretroviral (ARV) drug. Its success has been attributed to its high antiviral activity and positive metabolic profile

Generally antiretroviral therapy works by inhibiting HIV replication stages. HAART which is the standard management of choice for HIV patients includes a combination of at least three antiviral drugs, usually from two different classes, Dybul *et al.*,(2002). The use of HAART has helped in reducing morbidity and mortality resulting from HIV. There are different types of HAART combination of available treatment that is determined by the therapeutic objectives, the cost and the tolerability, Ngondi *et al.,* (2006).

Tenofovir disoproxil fumarate (TDF) containing HAART regimen is the most preferred antiretrovirals (ARVs) among young people and adults due to its better pharmacokinetic (PK) profile and potency that allows daily dosage, Chapman*et al.,*(2003); Lyseng-Williamson *et al.,*(2005). Despite all the above, renal toxicity is associated with TDF containing antiretroviral regimen. The use of TDF in clinical practice is linked to proximal tubular dysfunction with or without decreased renal function causing acute renal failure, acute kidney injury and Fanconi's syndrome, Mouss *et al.,* (2005).

When they are detected early appearance of nephrotoxicity commonly improves due to discontinuation of the TDF drug. Herlitz et al, (2010) indicated that approximately 50% of infected persons improved renal function to baseline levels following 20+/- 26 months of tenofovir discontinuation after diagnosis of Acute Kidney Injury and others were reported to have partial recovery of renal function. Late detection of nephrotoxicity is reported to lead to irreversible tubule interstitial damage. There's no study that has assessed the dependency of the recovery time on the time to development of the disease. This information would be very useful for the clinicians when monitoring the patients put on TDF drugs, and would serve as an indicator for when to change the course of treatment so as to lessen the occurrence of TDF-induced nephrotoxicity.

1.2 Problem Statement

Chronic Kidney Disease (CKD) is still a stern snag in HIV-infected patients on ART; this is according to findings done in Sub-Saharan African Countries. A decreased anticipated glomerular filtration rate (eGFR) is seen in 25% of these patients when they were started on ART, while 72% have microalbuminuria, Msango *et al.*,(2013). Despite TDF being the most approved antiretroviral globally for the first–line treatment of HIV infection because they are available as a combination single-tablet regimen and can be taken once daily, favorable safety profile and resistance, and effective antiviral activity, it is associated with CKD among patients with coexisting use of protease inhibitors and those with complex HIV infection.

TDF is also associated with severe kidney injury and proximal tubular dysfunction in patients in developed countries, those with lower body mass index or those who have preexisting kidney disease, Scherzer *et al.*,(2012). However, despite all the above factors, there is no significant

work that has been done at KNH on patients on TDF based regimen to determine the time to diagnosis and recovery from kidney disease. This study will not only focus on the risk factors for development of and/or recovery from kidney disease but also capture the time to these events.

1.3 Justification

Several studies focus on connectivity between exposure to TDF and occurrence of kidney disease and few have specifically investigated the recovery from this disease. There's no study that has been done to evaluate the relationship between diagnosis of and recovery from nephrotoxicity among patients on TDF based regimen. The main endeavor of this study is to address this gap by assessing the dependency of time to recovery from TDF associated nephrotoxicity on the time to diagnosis using a multivariate failure time for ordered events. This information will be very useful for clinicians to monitor the duration of treatment for patients at risk of kidney disease based on their profile at the start of treatment.

1.4 Broad Objective

To assess Tenofovir-induced nephrotoxicity development and recovery among patients on TDF based regimen at KNH CCC between 2009 and 2015.

1.4.1 Specific objectives

1. To describe the baseline profile of HIV patients at time of initiation into TDF based regimen at KNH-CCC
2. To compare the time to diagnosis of Tenofovir-induced nephrotoxicity among HIV patients onTDF+3TC+EFV, TDF+3TC+NVP, and TDF+3TC+LPV\r regimens at KNH- CCC

3. To compare the time to recovery from Tenofovir-induced nephrotoxicity among HIV patients on TDF+3TC+EFV, TDF+3TC+NVP, and TDF+3TC+LPV\r regimens at KNH- CCC
4. To determine the effect of factors associated with time to development and recovery from Tenofovir-induced nephrotoxicity among HIV patients on TDF based regimens at KNH-CCC

4.3 Research hypotheses

1. The time to diagnosis of Tenofovir-induced nephrotoxicity among HIV patients does not depend on the TDF-based ART regimen given.
2. The time to recovery from Tenofovir-induced nephrotoxicity among HIV patients does not depend on the TDF-based ART regimen given.
3. The time to development and recovery from Tenofovir-induced nephrotoxicity among HIV patients on TDF based regimens at KNH-CCC is completely random.

CHAPTER TWO

LITERATURE REVIEW

2.1 Kidney disease

According to Levey, *et al*., (2013), kidney disease is a mixed group of disorders that affects functions and structure of the kidney and it can be classified as acute kidney injury (AKI) or chronic kidney disease (CKD). Severe kidney injury is also referred to as severe renal failure, Bellomo, *et al.*, (2004). AKI is manifested by quick diminishing in renal excretory task that accumulates nitrogen metabolism products such as creatinine and urea. CKD is linked to age-related renal function decline accelerated in diabetes, obesity, hypertension and primary renal disorders, Gansevoort *et al.*, (2013). There is an intricate correlation between AKI and CKD; AKI can easily lead to CKD, and CKD is known to raise the risk of AKI, Bedford *et al.*, (2012).

2.1.1 Epidemiology of Kidney Disease

It is estimated that chronic kidney disease affects 10% of the world population and millions of people succumb to it due to lack of access to reasonable treatment, World Kidney Day, (2015). In 1990, chronic kidney disease was ranked 27th as a cause of many deaths globally but in 2010, it rose to position 18, (2010, Global Burden of Disease). The ranking was recorded the highest to that of AIDs, Jha*et al.*, (2013). It has also been reported that non-communicable diseases have overtaken communicable diseases by causing global untimely death. Over 80% of this load is reported in developing countries Couser*et al.*, (2011). According to WHO, CKD has become a global health problem, for instance in 2005, there were close to 58 million deaths reported in the

whole world, with 35 million accredited to persistent disease, Levy *et al.*,(2007. Early diagnosis and treatment can slow or stop the progression of the disease.

The total overall frequency of CKD in Sub-Saharan Africa was reported to be 13·9% (95% CI 12·2–15·7), this is according to Stanifer*et al.,* (2014). By 2030, it is assumed that over 70% of patients with end-stage renal disease will live in third world countries with a gross domestic product per person being less than US$1500 per year, Naicker, (2009). This is an alarming estimation in view of the fact that the world occurrence of maintenance dialysis has doubled since 1990, and that renal replacement therapy was accessed by 1·8 million people globally in 2004 with less than 5% coming from the South of the Sahara, Grassman*et al.*,(2004). There are many possible causes of CKD more especially in sub-Saharan Africa that has made this disease burdensome. It is also estimated that more than 22 million people in sub-Saharan Africa are HIV+, the impending for an irresistible burden of CKD is lofty, Stenifer*et al.*,(2014).

Since the incidence of a disease is directly correlated to its morbidity and mortality rate, the same is applicable to TDF-induced nephrotoxicity. According to Food and Drug Administration Events Reporting System conducted in 2001 – 2006, 164 subjects with Fanconi's syndrome were registered. 83% of these subjects received TDF combined with protease inhibitor, Gupta,(2008).

Surveillance Cohort Long-Term Toxicity of Antiretrovirals/Antivirals (SCOLTA) project evaluated 754 HIV infected subjects on TDF based regimen for a period of 19.5 months. They reported 2.5% occurrence of elevated creatinine level, which is 1.5-fold higher than the normal limit, Madeddu *et al.*,(2008).

In another study carried out in which a grade 1 increase in serum creatinine (SCr) was developed in seven (4%) of the patients, it was reported that fifteen (8.7%) patients recorded an addition in

SCr of greater than 1.5 times the baseline values. TDF was discontinued in Four (2.3%) who had a n increase in SCr and/or unusual urinalysis. Of 62 patients with abnormal urinalysis, Twenty-eight (16%) had grade 1 hypophosphatemia while Eleven (6%) had grade 2 hypophosphatemia, Antoniou*et al.*,(2005). A retrospective cohort analysis conducted on HIV-infected adults on TDF for 48 months reported outstanding incidence. Out of 890 patients initiated on TDF, the normal renal function was reported in 573 (64.4%), moderate renal dysfunction was reported in 46(5.2%), 2.4% had nephrotoxicity, 7.8% did not survive while 9.7% were left to follow up, Brennan *et al.*,(2013)

In terms of recovery, a study evaluated the advancement of renal damage after discontinuation of TDF in 183 exposed patients to the drug for 39 (22-63) months. The renal parameters went back to normal values 59% of the patients after 22 (13-49.5) months of TDF discontinuation, Bonjoch *et al.*,(2012). Reversibility of TDF is further supported by a cohort study that assessed 1286 HIV patients treated with TDF based regimen. When they were closely monitored for 48 weeks, an occurrence of 0.39 per 100/year was evident and this was reversed when the treatment was ceased, Santiago *et al.*,(2006). Therefore, the aim of this study was to establish the determinants of time to diagnosis and recovery from tenofovir-induced nephrotoxicity in HIV patients on TDF based regimen at the Comprehensive Care Clinic, Kenyatta National Hospital (KNH).

2.2 Tenofovir Disoproxil Fumarate (TDF)

According to Gallant *et al.*, (2003), TDF is an oral bio available drug of tenofovir; a nucleotide analogue reverse transcriptase inhibitor (NtRTI) with strong effectiveness against retroviruses and hepadnaviruses, Kearny *et al*, (2004). TDF was permitted by United States Food and Drug Administration to treat HIV virus in 2001 and chronic hepatitis B (CHB) infection in 2008. The

drug is extensively used as a constituent of antiretroviral regimens to treat both naive and experienced patients. It has a long intracellular half-life, allowing once-daily dosing and heartening management observance. Tenofovir is considered by US HIV treatment guideline as the ideal regimen for antiretroviral-naive grownups and young people in both low-to-middle–income and high income countries.

TDF is linked to dose-dependent renal toxicity in animal studies in which the first case of TDF-induced nephrotoxicity in an HIV+ patient was recorded in 2002, Verhelst et *al.,* (2002). Many case reports of TDF-induced nephrotoxicity in HIV+ patients have been published since then; TDF has a danger of tubular toxicity for HIV-infected patients.

2.3 Tenofovir-induced Nephrotoxicity

Tenofovir nephrotoxicity clinically presents mainly as proximal tubular dysfunction that has a sealed renal function and proximal tubular dysfunction linked to a decline renal function categorized as AKI, CKD, or GFR compared to baseline values, albeit within normal confines.

2.3.1 Glomerular Filtration Rate (GFR)

Cases of tenofovir-connected nephropathy recognized incomplete or complete as Fanconi syndrome, Izzedine *et al.,* (2010), related or not with a reduction in GFR, Cooper *et al.,* (2010). Tubular dysfunction may pave the way for the turn down of renal function. Tubular proteinuria indicates existence of higher amounts of urine in small-sized proteins liberally filtered in the glomerulus but re-absorbed by proximal tubules.β2-microglobulinuriaGFRPapaleo *et al.,* (2007). Other manifestations of proximal tubulopathy are osteomalacia and reduced bone mass, Perrot *et al.,* (2009).

2.3.2 Acute Kidney Disease (AKI)

Tenofovir is related to a tiny, but enlarged threat of AKI, Cooper *et al.,* (2010). Tenofovir-induced AKI is both non-oliguric and oliguric and may need dialysis, Herlitz *et al.,* (2010). Proof of proximal tubular dysfunction after use of drugs is discontinued could lead to partial renal function recovery. However, CKD that needs dialysis following AKI is observable in tenofovir and cidofovir treated patients, Ortiz *et al.,* (2005).

The proximal tubular cell is used on tenofovir toxicity as a result of cell membrane transporters that supports tenofovir growth. Proximal tubular cells are responsible for tubular movement of molecules, reabsorbing over 200g NaCl, and 1kg glucose. The cells are affluent in cell membrane movement (Sons, 2008).Proximal tubule mitochondria trigger 25 dihydroxycholecalciferol by 1αhydroxylation; this yields the dynamic metabolite of vitamin D, Calcitriol. They also discharge required ammonia by distal segments to emit protons into the urine.

2.3.3 Nephrotoxicity and TDF exposure

Previous studies conducted prior to FDA endorsement of TDF had reported no or only partial nephrotoxicity; but these did not include participants with pre-existing renal injury, Squires *et al.,*(2003). According to Campbell *et al.,* (2009), an increased threat of tenofovir-induced toxicity is linked to older age and lower CD4 count. Cooper et al., (2010), concluded that patients treated with TDF experienced a tiny but major loss of kidney function while receiving treatment in comparison with controls (mean difference in eGFRs, 3.9 mL/min; 95% confidence interval, [2.1-5.7]). The statistical heterogeneity for these results was however hefty due to the design of the study, previous ART exposure or due to industrial sponsored studies. Several case

reports and case series provides rigorous cases of renal tubular toxicity linked to TDF contact, de la Prada *et al.,* (2006).

2.4 Risk factors and management of Tenofovir-induced Nephrotoxicity

The general outline of tenofovir is positive hence forecast on the person who is risky of nephrotoxicity is needed to deal with patients. The probability of a major renal function reduction was 3.7 times higher for patients put on tenofovir plus ritonavir-boosted protease inhibitor regimes than those on tenofovir plus nonnucleoside reverse transcriptase inhibitor-based therapy after adjusting for HIV load, Goicoechea *et al.,* (2008). The fundamental renal ailment with low GFR brings about the danger of tenofovir toxicity by reducing tenofovir renal clearance and increase tenofovir in the flow and proximal tubular cells, Rodriguez-Novoa *et al.,* (2010). Reducing the dosage can be done if GFR is not high but this is difficult to execute when one pill has many anti-retroviral.

Patients receiving TDF and are meeting any one (1) of four (4) criterion must measure their kidney function (eGFR) no less regularly than every 6 months, Gupta *et al.,* (2005). The commonly used criterions based on recognized risk factors are: GFR <90 mL/min/1.73 m2, patients should be checked every 3 months during the first year of treatment and then afterward twice every year because presently it is not probable to correctly envisage which patients will develop complications.

Hypophosphatemia can be a snag in TDF toxicity; it is therefore advisable to measure fractional discharge of phosphate rather than serum phosphate alone. There is also need to reduce TDF dose in patients with pre-existing reduced kidney function. In some cases, TDF-induced

nephrotoxicity is reversible and management must immediately be discontinued if complications are observed.

The most successful therapy of tenofovir nephrotoxicity is to stop tenofovir when features of nephrotoxicity habitually get better. In a follow-up of 20 ±26 months after tenofovir was stopped, about 50% of patients fully recovery from renal function to baseline levels that included the patient in need of dialysis for 4 months, Herlitz et al., (2010).

Other patients were reported with fractional improvement of renal function from a mean peak of sCr 5.6 ±3.8 to sCr 1.5 ± 0.3 mg/dL. Early revelation of nephrotoxicity and tenofovir withdrawals is essential to shun irreparable tubule interstitial harm.

CHAPTER THREE

METHODOLOGY

3.1 Study design

The study was aimed at assessing the effect of baseline characteristics (e.g. presence of co morbidities like hypertension, creatinine levels) of the patient at the time when TDF based ART treatment was introduced during development of TDF-induced nephrotoxicity. In achieving this, a retrospective cohort study design was adopted to investigate the characteristics associated with time to diagnosis of TDF-induced nephrotoxicity and recovery among HIV patients on TDF based regimen. Patients put on TDF based ART between 1st January 2009 and 31st December 2012 were sampled from the KNH-CCC database; Information on their baseline characteristics was retrieved from the screening records of the patients before initiation of treatment, and dates when diagnosis of TDF-induced nephrotoxicity was done and when the patients were declared to have recovered from the nephrotoxicity.

3.2 Study site

This study was done at Kenyatta National Hospital Comprehensive Care Centre Clinic in Upper hill Nairobi which is an ongoing HIV clinic supported by CDC and working with collaboration of University of Nairobi running from Monday to Friday serving all patients who are able to follow set national guideline for HIV clinic services. This site started prescribing TDF based regimens in January, 2009. Patients registered in this sight do benefit from services such as nursing care, clinical care and psychosocial support, nutritional and physiotherapy services.

3.3 Study population

The population for this study consisted of HIV positive patients initiated into TDF based regimens between 1st January, 2009 and 31st December, 2012 at KNH-CC. The TDF based regimens prescribed at the facility are; TDF+3TC+EFV, TDF+3TC+NVP, and TDF+3TC+LPV\r. A sum of 1852 patients was put on TDF based regimen between 2009 and 2012. By 2015, 177 patients had developed TDF-induced nephrotoxicity.

3.3.1 Inclusion criteria

- Must be a HIV patient who was put on TDF based ART at KNH-CCC between 15st January, 2009 and 31st December, 2012.
- Must have complete patient records' screening results at the commencement of TDF based on ART treatment

3.3.2 Exclusion criteria

- Patient that developed TDF induced nephrotoxicity but whose dates of change of ART regimen are not available in the database.

3.4 Sampling method

Stratification was done based on type of TDF based regimen to have a representative sample for each type of TDF regimen (TDF+3TC+EFV, TDF+3TC+NVP, and TDF+3TC+LPV\r). TDF+3TC+EFV is a fixed combined dose type of regimen and is therefore preferred regimen to TDF+3TC+NVP. TDF+3TC+LPV\r is a second line ART regimen, therefore the number of patients initiated on this regimen depends on the number of patients failing on first line. At any given point in time there are more patients on TDF+3TC+EFV compared to TDF+3TC+NVP, and TDF+3TC+LPV\r. A list of patients in each regimen was obtained from the records. N-

random numbers were generated using Microsoft Excel random function based on the number of patients in each list. Simple random sampling with proportional size allocation was used to select patients in each stratum (regimen type).

3.5 Sample size determination

Multivariate log-rank (Wei-Lachin) test was used to compare the effect of different TDF based regimens on time to diagnosis and recovery from TDF-induced nephrotoxicity. Sample size was determined based on this test using the formula, Lachin, (2014);

$$n \geq \left[\frac{(Z_{1-\alpha} + Z_{1-\beta})\theta_k}{\delta_a + \delta_b}\right]^2$$

Where; n represents the minimum sample size required

$Z_{1-\alpha}$Represents the standard normal distribution critical value at α-level of significance for one sided test (α=0.05; $Z_{1-\alpha}$=?)

$Z_{1-\beta}$Represents the standard normal distribution critical value at β-type II error (β =0.2; $Z_{1-\alpha}$=?)

δ_aRepresents a vector of coefficients of covariates for event ***a*** (diagnosis of TDF-induced nephrotoxicity)

δ_bRepresents a vector of coefficients of covariates for event ***b*** (recovery from TDF-induced nephrotoxicity)

θ_kRepresents variance of$\delta_a + \delta_b$

Let S_a=SD (δ_a); S_b=SD (δ_b) and $Corr_{ab}$=Corr ($\delta_a \delta_b$)

$\theta^2{}_k$=$S^2{}_a$+ $S^2{}_b$+ 2*var($\delta_a + \delta_b$)

S_a=k*S_b where **k** is a constant

var ($\delta_a + \delta_b$)=$Corr_{ab}$*S_a*S_b =$Corr_{ab}$*kS_a

Let **r** represent $Corr_{ab}$;

$\theta^2{}_k = S^2{}_a + k^2 S^2{}_a + krS^2{}_a$

Assuming sample size allocation ratio is one with no missing observations, then;

$\theta^2{}_k = 4\ (S^2{}_a + k^2 S^2{}_a + krS^2{}_a)$

With expected difference in the coefficients of 0.25SD in each event, then

$$n = \left[\frac{(Z_{1-\propto} + Z_{1-\beta})2\sqrt{(S^2{}_a + k^2 S^2{}_a + krS^2{}_a)}}{0.25S_a + 0.25kS_b}\right]^2$$

For a one-sided test at 0.05, the sample size required to give a power of at least 0.9;

$$n = \left[\frac{(Z_{1-\propto} + Z_{1-\beta})2\sqrt{(S^2{}_a + k^2 S^2{}_a + 2k)}}{0.25S_a + 0.25kS_b}\right]^2$$

Because we are interested in the minimum sample size;

$$n \geq \left[\frac{2.927*2\sqrt{(1+k^2+(2kS^2{}_a))}}{0.25(1+k)}\right]^2 = \left[\frac{2.927*2}{0.25}\right]^2$$

Using the formula and defined parameters, the estimated minimum sample size is 550 patients.

3.6 Data collection and analysis

Data was retrieved from KNH-CCC electronic records for patients initiated into TDF based regimens between 2009 and 2011 and stored in MS Access database. Data cleaning, coding and analysis will be done using STATA version 13 SE.

Exploratory data analysis was done to summarize the data. Histograms were plotted to show the distribution of quantitative variables and measures of central tendency (mean/median) and dispersion (standard deviation/inter-quartile range) reported in tables. For categorical variables bar/pie charts were plotted to show the distribution and frequencies and proportions reported in tables.

Conditional Kaplan-Meier Weighted estimator was used to estimate the survival functions;

Let (**T1,T2**) represent a pair of successive event times corresponding to two ordered consecutive events (such as diagnosis, and recovery from Kidney nephrotoxicity) measured from the start of the follow-up ($\mathbf{T_1 < T_2 = T}$).

The conditional survival probabilities can be estimated as;

$$\mathbf{P(T2>y|T1>x) \text{ or } P(T2>y|T1\leq x)}$$

Let **S1** and **S** represent the marginal survival functions of **T1** and **T**, that is;

S1(y)=P(T1>y) and **S(y)=P(T>y)** with conditional probabilities **P(T>y|T1>x)** and **P(T>y|T1≤x)**

Since **S(y|x)** can be expressed as;

$$S(y|x) = P(T>y|T1>x) = 1-P(T\leq y|T1>x) =$$

$$\frac{1 - P(T_1 > x, T \leq y)}{(1 - P(T_1 \leq x))}$$

then the conditional survival function is estimated as;

$$\widehat{S}(y|x) = \left(1 - \frac{\sum_{i=1}^{n} W_i I(\widetilde{T}_{1[i]} > x, \widetilde{T}_{(i)} \leq y}{\widehat{S}_1(x)}\right)$$

where $\widetilde{T}_{1[i]} \leq ... \leq \widetilde{T}_{(n)}$ denotes the ordered $\widetilde{T}$-sample and W_i is the Kaplan-Meier weight attached to $\widetilde{T}_{(i)}$.

Median times to development of kidney disease and median time to recovery were reported.

Multivariate Log-rank (Wei-Lachin) test was applied in comparing the continued existence functions based on the different TDF based regimens. Chi-square omnibus statistic and corresponding p-value was reported.

Consider the case where each subject can experience one or both of two events ***A*** and ***B***, with log hazard ratios β_a and β_b, respectively, for groups x and y

$$Z_S = \frac{J'\widehat{\beta}}{\sqrt{J'\widehat{\Sigma}J}} = \frac{\widehat{\beta}_a + \widehat{\beta}_b}{\widehat{\sigma}_S}$$

Where $\mathbf{J} = (1\ 1)'$ and $Z_S \sim N(0,1)$ under the null hypothesis ($H_0: \beta_a = 0$ and $\beta_b = 0$) from Slutsky's theorem. $\widehat{\sigma}_S$ is the is the Wei Lachin variance computed as $\mathbf{Var}(\widehat{\beta}_a + \widehat{\beta}_b)$.

The test rejects the test rejects H_0 in favor of H_1 when $Z_S \leq Z_\alpha$ at level α one-sided.

According to Prentice et al, (1981), provisional risk set model was used to evaluate prognostic factors for expansion of kidney disease and recovery. Development of kidney disease and recovery were considered as ordered events in the sense that recovery cannot occur before diagnosis of the disease. In this model, the provisional risk set at time t for event k is made up of all subjects under inspection at time t that have had event k − 1. Hazard ratios and corresponding confidence intervals were reported.

Conditional risk set model

$$\lambda_{ik}(t|Z) = e^{\beta' Z_k \lambda_{k(t)}}, t \geq 0$$

Where; λ_{ik} represents the hazard role for the $\mathbf{k}^{th}$ event of the $\boldsymbol{i}^{\mathbf{th}}$ subject at time **t**

$\boldsymbol{\beta'}$ represents a vector p-dimensional regressions coefficients for the p-covariates

$\lambda_k\ (t)$represents the baseline hazard function for the $\boldsymbol{k}^{th}$ event

$\boldsymbol{Z_k}$ represents a matrix of ***p***-covariates dim (**1**, ***p***) for the $\boldsymbol{k}^{th}$ event

Under this model, the patient enters the process upon initiation of TDF-based ART regimen and is at risk of diagnosis with TDF-induced nephrotoxicity (first event). The patient enters the second risk set upon diagnosis and treatment changed. The effect of covariate **p** on the risk of recovery of a patient at time ***t*** is $\boldsymbol{e^{\beta}}$ conditional on the history of diagnosis at time ***t-1***

3.7 Ethical considerations

Ethical consent was required from the University of Nairobi/Kenyatta National Hospital Research and Ethics Committee. Further approvals were sought from the KNH departmental head Health Information Services and also from head of unit at Kenyatta national hospital comprehensive care center to use the electronic medical records servers was granted.

CHAPTER FOUR

RESULTS

4.1 Baseline characteristics of the patients

We retrieved a total 534 complete patient records for this study. Close to three quarters were female (73.0%). Their age ranged between 10 years and 85.3 years with a median of 39.6 years (IQR=47.3-32.3). The age distribution was bimodal with peaks at 17 and 39 years (Figure 1). The patients were classified as pediatric (≤15 years) or adults (>15 years) depending on the age at the time of initiation into ART.

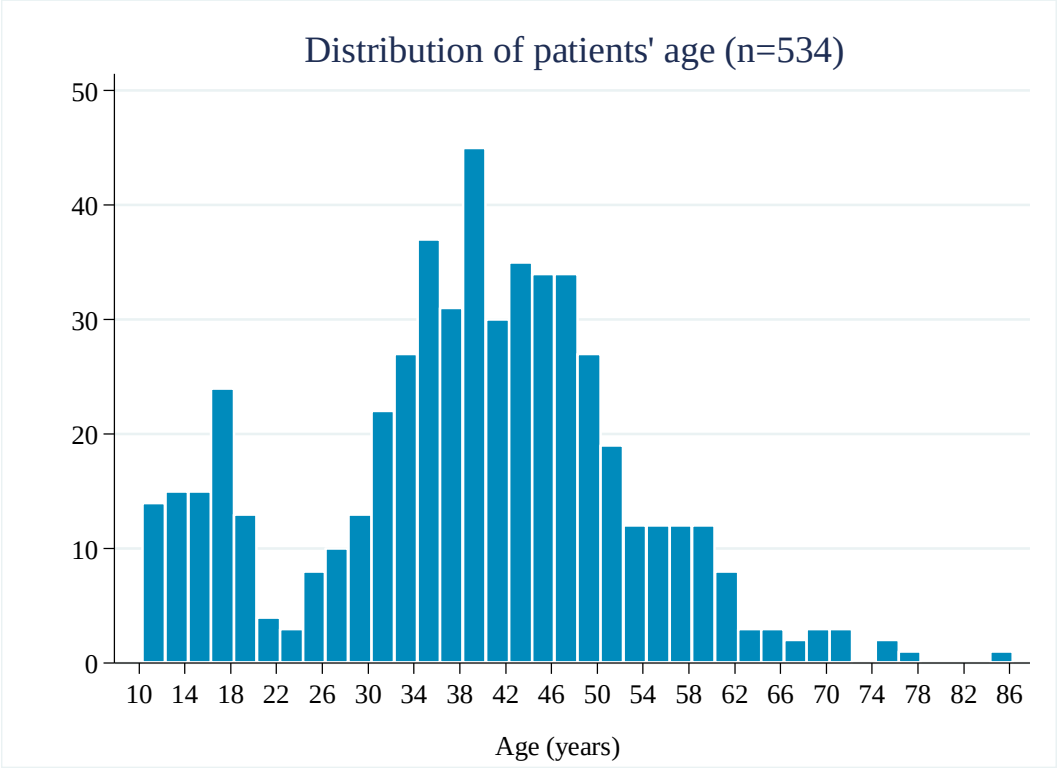

Figure 1: Graph showing patients' age distribution

Majority (70.4%) of the patients were started on TDF+3TC+EFV regimen and about a quarter (24.4%) were taking TDF+3TC+NVP regimen. The hemoglobin level was discretized at normal for patients with at least 10g/dl (male) and 12g/dl (female) below which the patient was regarded as having low hemoglobin level. One-third (33.7%) of the patients were found to have low hemoglobin levels. With regards to CD4 count, more than half (58.6%) had less than 500cells/l of blood. Majority (77.7%), had undetectable viral load by the second visit after initiation of ART.

Table 1: Baseline characteristics of the patients

Factor	Category	Count (percent)
Gender	Female	390 (73.0)
	Male	144 (27.0)
Age group	Pediatric	37 (6.9)
	Adult	497 (93.1)
Start regimen	TDF+3TC+EFV	375 (70.4)
	TDF+3TC+LVP\r	29 (5.3)
	TDF+3TC+NVP	130 (24.4)
Hemoglobin level	Normal (≥10g/dl for males & ≥12g/dl for female)	354 (66.3)
	Low	180 (33.7)
CD4 count	Normal (≥500 cells/l)	221 (41.4)
	Low (<500 cells/l)	313 (58.6)
Viral load	Undetectable (<50copies/l)	415 (77.7)
	≤1000 copies/l	41 (7.7)
	>1000 copies/l	78 (14.6)

4.2 Diagnosis and recovery from TDF-induced nephrotoxicity

A patient was considered to have TDF-induced nephrotoxicity following a creatinine clearance rate of less 50mL/min during the clinic follow-up visits after initiation into TDF-based ART regimen. Out of the 534 patients, 324 were diagnosed with TDF-induced nephrotoxicity and only

88 had recovered by the end of the study follow-up period. The overall incidence rate for TDF-induced nephrotoxicity was estimated at 2 cases per 100 person-years (534 patients/18079.3 time at risk).

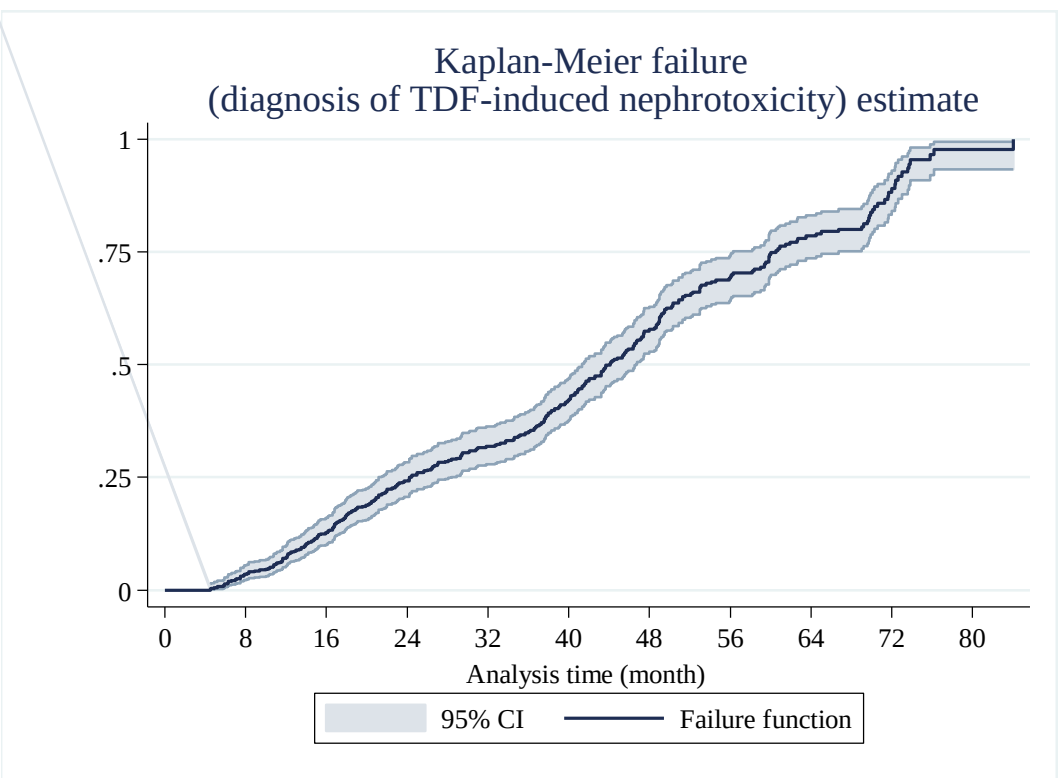

Figure 2: Kaplan Meier Failure function estimate (Risk of being diagnosed with TDF-induced nephrotoxicity)

The risk of a patient being diagnosed with TDF-induced nephrotoxicity increased gradually with change in time/continuous exposure to the drug. The median time to diagnosis of TDF-induced nephrotoxicity was 43.7 months (IQR=59.9-24.3 months). After the 70th month the risk of being diagnosed with the disease rose drastically up to about the 80th month (Figure 2).

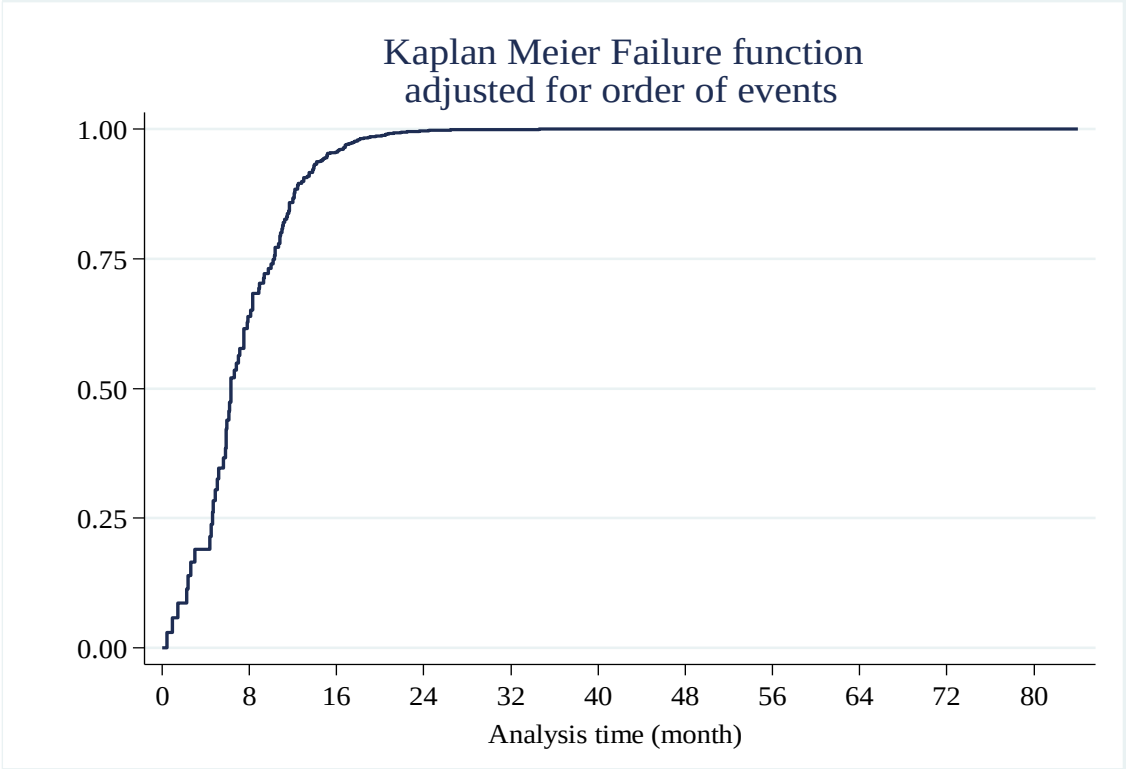

Figure 3: Kaplan Meier Failure function (recovery from TDF-induced nephrotoxicity)

Adjusting for the time to development of TDF-induced nephrotoxicity, the patients were found to recover fast with majority recovering within 16 months (Figure 3) after withdrawal of TDF as a part of the ART regimen.

3.3 Comparison of survival functions based on ART start regimen

Figures 4 and 5, show the failure functions as estimated using Kaplan Meier method corresponding to the risk of a patient being diagnosed with TDF-induced nephrotoxicity and recovery, respectively. Within the first 24 months, the risk of being diagnosed with TDF-induced nephrotoxicity at any given point in time among those started on LVP\r regimen was equivalent to the risk among those on EFV regimen. The risk of being diagnosed with TDF-induced nephrotoxicity among patients on NVP appeared to increase gradually after the first 8 months of initiation up-to the 28th month. The slope of the NVP failure curve was steeper after the 34th month up until the 50th month. After the 48th month the risk of being diagnosed with the disease was based on the start regimen was equivalent.

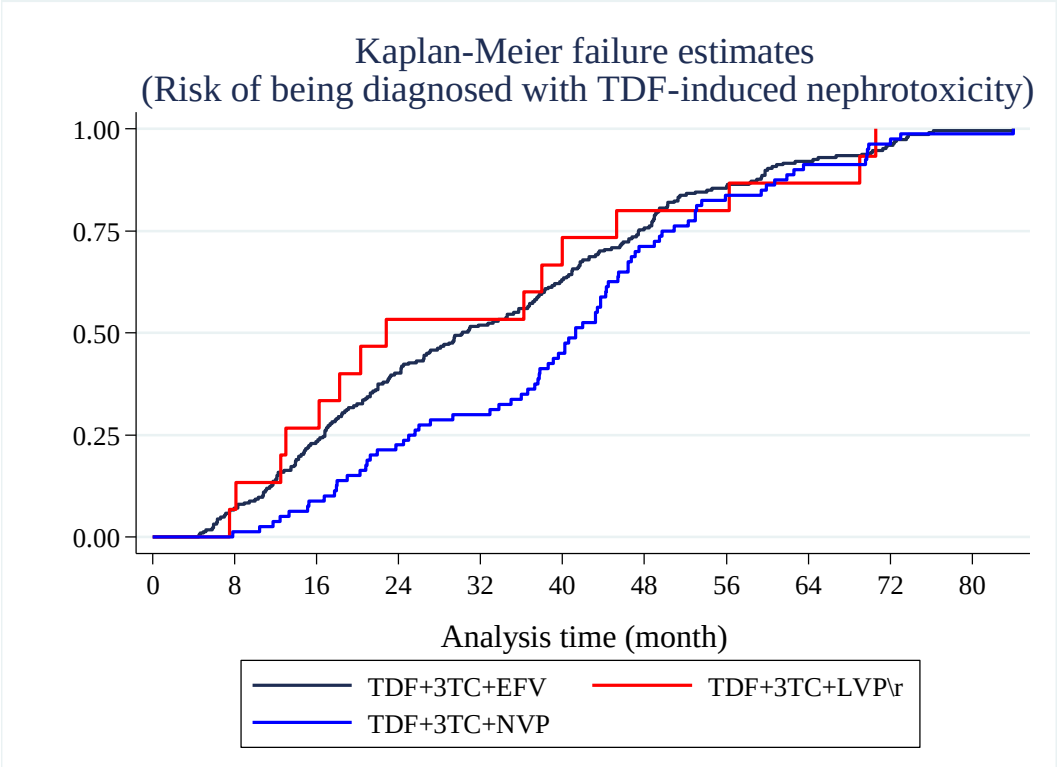

Figure 4: Comparison of KM-failure functions for diagnosis of TDF-induced nephrotoxicity by ART start regimen

Following the withdrawal of TDF drug, the risk of a patient recovering from the disease increased very gradually with respect to time. The patients previously started on LVP\r portrayed a better recovery experience relative to the patients started on EFV and NVP (Figure 5).

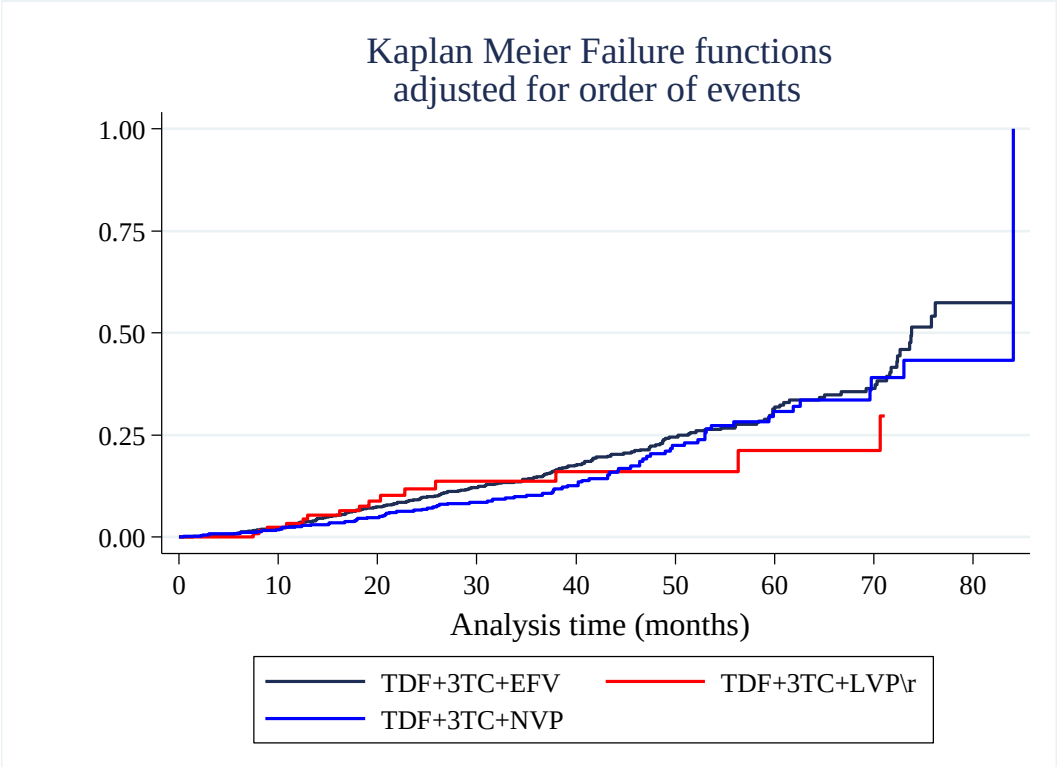

Figure 5: Comparison of KM-failure functions for recovery from TDF-induced nephrotoxicity by ART start regimen

Log rank tests were done to evaluate the overall survival functions of patients following the diagnosis of TDF-induced nephrotoxicity with respect to the patient characteristics and treatment regimen. There was significant difference in the survival functions with respect to patient's gender (p-value=0.006), age group (p-value=0.003) and CD4 count (p-value=0.028). In terms of the ART start regimen, there was no significant difference (P-value= 0.915) in the overall recovery experience between the three treatment regimens conditional on the time to diagnosis of the disease (Table 2).

Table 2: Comparison of survival functions with respect to patient characteristics

Factor	Category	Chi2	P-value
Gender	Female Male	7.57	0.006
Age group	Pediatric Adult	8.97	0.003
Start regimen	TDF+3TC+EFV TDF+3TC+LVP\r TDF+3TC+NVP	0.18	0.915
Hemoglobin level	Normal Low	2.54	0.111
CD4 count	Normal (≥500 cells/l) Low (<500 cells/l)	4.86	0.028
Viral load	Undetectable ≤1000 copies/l >1000 copies/l	0.45	0.799

3.4 Factors associated with time to development and recovery from Tenofovir-induced nephrotoxicity

The conditional risk set model was used to explore the effect of ART start regimen, CD4 count, hemoglobin level, ALT/GPT level, viral load, gender and age group of the patient on the time to recovery from the disease. The time to recovery was measured from the time of a patient's diagnosis with TDF-induced nephrotoxicity and not from the time of entry into the study.

A change in the patient's ALT/GPT level was found to be positively associated (HR=1.01, P-value=0.044) with the time taken for a patient to recover from TDF-induced nephrotoxicity. The male patients were found to take longer to recover compared to female patients (HR=0.68, P-value=0.013), adjusting for the effect of other covariates in the model. An adult patient had 34%

(HR=0.66, P-value=0.029) decline in the expected hazard relative to a pediatric patient, holding other factors constant. The ART start regimen, CD4 count, hemoglobin level and viral load of the patient did not have a significant effect on the expected hazard.

Table 3: Conditional risk model evaluating the effect of covariates on time to recovery

Patient characteristics	Hazard Ratio	P-value	[95% Confidence Interval]	
Start regimen				
TDF+3TC+EFV	Ref			
TDF+3TC+LVP\r	0.90	0.719	0.52	1.58
TDF+3TC+NVP	0.89	0.343	0.70	1.13
Gender				
Female	Ref			
Male	0.68	0.013	0.51	0.92
Age group				
Pediatric	Ref			
Adult	0.66	0.029	0.46	0.96
CD4 count				
Low	Ref			
Normal	0.91	0.421	0.72	1.14
Hemoglobin level				
Low	Ref			
Normal	0.78	0.054	0.61	1.00
ALT/GPT levels	1.01	0.044	1.00	1.01
Viral Load				
Undetectable VL	Ref			
<1000 copies	0.91	0.654	0.60	1.37
>1000 copies	1.08	0.601	0.81	1.42

CHAPTER FIVE

DISCUSSION

The use of TDF in first-line ART among HIV-infected patients has been widely documented (Soler-Palacin *et al*, 2011; Calza *et al*, 2013, Boswell et al, 2017; Venter *et al*, 2018) to have nephrotoxic potential resulting in Kidney injury, CKD or Fanconi Syndrome. The current study finding supports these reports, considering the high number of patients reported to have decreased creatinine clearance rate (below 50mL/min) following initiation TDF-based ART regimens. Considering the undisputed benefits of TDF in HIV care, safe-regular monitoring of its use is essential to prolong the patient's life.

In estimating the probability of a patient on TDF-based regimen to be diagnosed with nephrotoxicity, we noted that the risk increased positively with time; that is the longer a patient stays on TDF based ART, the higher the chances of that patient presenting signs of nephrotoxicity. Beyond two years of TDF-based regimen use, patients had more than 25% chance of developing the disease and more than 50% risk after three and a half years. This result is useful for clinicians managing newly diagnosed HIV patients and started on first-line ART, in determining the appropriate time to consider a change of regimen to prevent the adverse events associated with renal failure as a consequence of using TDF.

With regards to recovery from TDF-induced nephrotoxicity, creatinine clearance rate was found to resolve fast, with 75% of the patients reported to have recovered within the first 16months after withdrawal of TDF. This finding is consistent with the report by Bonjoch *et al*, (2012) and Herlitz *et al*, (2010) which reported that half of the patients recovered from TDF-induced renal

impairment to baseline creatinine levels within 13-49.5 months and 20+/-26 months, respectively.

The study further sought to explore factors affecting the expected hazard of a patient recovering from TDF-induced nephrotoxicity. ALT/GPT was found to be positively associated with the time to recovery from TDF-induced nephrotoxicity. This has not been reported in the previous studies evaluating the risk factors for TDF- induced renal impairment. ALT/GPT being a marker for the liver function would be quite useful in informing the clinicians in case of drug- induced liver damage which would further complicate the recovery of a patient from TDF-induced renal failure.

In this study, the CD4 cell count had no effect on the expected hazard, of patient recovering from TDF-induced nephrotoxicity. This finding agrees with the results by Ojeh *et al*, (2018) in a study conducted in Nigeria evaluating the incidence and predictors of TDF-induced renal injury in HIV infected patients in Nigerian. Previous studies by Tourret *et al*, (2013) and Wantakisha *et al*, (2017) however, indicate baseline CD4 cell count being an important risk factor for the disease, with patients having >350 CD4 cell count having a higher threat of TDF-induced renal dysfunction among HIV patients. It is possible that the CD4 cell count at the time of diagnosis of the disease among the cases with reduced creatinine clearance rate was comparable to patients with normal rate.

A male HIV-infected patient diagnosed with TDF-induced nephrotoxicity was found to have a significant decrease in the expected hazard relative to a female patient, after adjusting for the effect of other covariates. Consequently, male patients are likely to suffer from the disease for a longer period despite the withdrawal of TDF compared to female patients. Considering previous reports indicating male HIV-infected patients being at a higher threat of renal impairment

(Nelson et al, 2007; Madeddu *et al*, 2008), special attention should be accorded to those receiving TDF-based ART regimen to determine when to change the course of treatment as a precaution to prevent this adverse event.

Older patients, usually above 50 year are considered to have increased risk of TDF-induced nephrotoxicity (Fernandez *et al*, 2011; Wantakisha *et al*, 2017). In this study, adult patients had reduced expected hazard of recovery from TDF-induced nephrotoxicity, relative to pediatric patients, implying that they take longer to recover. The sole purpose of ART is to improve the quality of life of a HIV-infected patient, and to achieve this, patients at risk of drug induced adverse events that would further complicate the management need to be prevented through intensified safety monitoring.

CONCLUSION

Kenya is one of the countries with higher incidence of HIV infections; the use of TDF in first line ART regimen is invaluable. This study has however, highlighted the differential effect of age group, gender and ALT/GPT levels on the time to diagnosis and recovery from the TDF induced nephrotoxicity. Whereas factors such as gender and age group cannot be changed, the findings of this study inform on the high risk group of patients that require special attention in terms of safety monitoring to ensure sustained improved health.

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65. **World Kidney Day (2015)**: Chronic Kidney Disease.

WORK PLAN

Weeks	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Proposal writing																
Seek ethical approval																
Data collection																
Data analysis																
Report writing and presentation																
Compilation of final report/ dissemination																

BUDGET

Item	**Unit cost**	**Quantity**	**Cost**	**Total cost**
PROPOSAL AND THESIS				
Proposal typing and printing (65pages)	3	3*65	195	
Photocopying final report (3copies)	2	2*610	1220	
Proposal paper binding	4	4*65	260	
Ethics committee fee	1	1*2000	2000	
Data analysis and presentation	1	1*5000	5000	
Data processing and analysis	30,000	1	30,000	
Research book binding	15000	1	15,000	
Sub-total				53,000
TOTAL				**150, 000**

DATA EXTRACTION TOOL

A. IDENTIFICATION

1. Patient ID…….

B. SOCIO-DEMOGRAPHIC CHARACTERISTICS (BASELINE)

2. Patient's age in years……..
3. Gender ☐ Female ☐ Male

C. LABORATORY TEST RESULTS (BASELINE)

4. Hemoglobin level…….
5. CD4 Count……..
6. Viral load……..
7. Urea, Electrolytes and Creatinine (UEC)…….
8. Alanine aminotransferase (ALT)…….

D. MEDICATION HISTORY

9. TDF regimen prescribed……..
10. Date of treatment initiation……
11. Date of tenofovir-induced nephrotoxicity diagnosis…….
    a. UEC levels in the diagnosis……
    b. Type of renal toxicity diagnosed…….
12. Date of withdrawal of TDF from the regimen…….
13. Date of initiation of new regimen……..

a. New regimen initiated……

14. Type of management for the diagnosed renal disease…….

a. Date of initiation of renal disease management……

15. Date of recovery from renal toxicity……..