THE EPIDEMIOLOGY OF TENOFOVIR-ASSOCIATED RENAL FAILURE IN PATIENTS ON ANTIRETROVIRAL THERAPY IN SWAZILAND

By

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DECLARATION OF AUTHORSHIP

"I declare that the dissertation, which I hereby submit for the degree Master of Science (Epidemiology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another University".

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ABSTRACT

Background: Tenofovir (TDF) forms part of the backbone of first and second line HIV infection management. There is limited data from resource-constrained settings on nephrotoxic adverse effects of Tenofovir based antiretroviral therapy (ART).

Objective: We investigated the incidence and risk factors of TDF-associated nephrotoxicity in a cohort of HIV-infected patients on either TDF or Zidovudine (AZT) based first line ART.

Methods: A retrospective analysis was conducted on a sample of participants initiated on first-line TDF- and Zidovudine (AZT)-based regimens between January 2010 and December 2015; from the national ART electronic database of Mbabane Government and Raleigh Fitkins Memorial hospitals in Swaziland. Time-to-onset of toxicity was assessed using Kaplan-Meier survival analysis and the log rank test was used to compare renal dysfunction by TDF or AZT first line regimen status. Toxicity incidence rate was calculated and multivariate Cox regression model was used to identify the potential risk factors.

Main Findings: A total of 1,119 participants with median age 36 years (IQR: 18 to 79 years), were included in the analysis 83% (n=929) on TDF and 17% on AZT (n=190). The median time to onset of toxicity was 6.72 months (IQR: 0.03 to 81.61). The overall incidence rate was 8.08 per 1000 person-months. TDF-associated nephrotoxicity was 9.44 per 1000 person-months (95% CI: 7.79 to 11.44) and 4.83 per 1000 person-months (95% CI: 3.18 to 7.33) for AZT. Participants on TDF-based regimen were more likely to develop toxicity in comparison to AZT, hazard ratio 2.08 (95% CI: 1.27 to 3.40, p=0.003). The mean CD4 count was 194 cells/µL (95% CI: 207.61 to 228.75), and mean eGFR was 123.56, (95% CI: 121.22 to 125.92). The co-variates: sex, age, WHO clinical staging, body mass index and CD4 cell count were not associated with developing nephrotoxicity (p>0.05).

Conclusion: Although TDF is effective in HIV management, it is associated with a two-fold increased risk of abnormal renal function during the follow-up period compared to those on AZT. This underlines the paramount importance of regular record-keeping for thorough pharmacovigilance and routine surveillance due to its wide usage.

Key words: Estimated Glomerular filtration rate (eGFR), Nephrotoxicity, Tenofovir (TDF), Zidovudine (AZT), Retrospective, Survival analysis, HIV cohort, Antiretroviral therapy (ART), Swaziland.

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ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immuno-deficiency syndrome
ART	Antiretroviral therapy
ARV	Anti-retroviral
ATI	Acute tubular injury
ATV/r	Atazanavir boosted with Ritonavir
AZT	Zidovudine
CKD	Chronic Kidney Disease
CrCl	Creatinine Clearance
D4T	Stavudine
DSD	Differentiated Service Delivery
EFV	Efavirenz
eGFR	Estimated Glomerular filtration rate
FDC	Fixed dose combination
HBV	Hepatitis B virus
HIV	Human Immunodeficiency Virus
LPV/r	Lopinavir boosted with Ritonavir
MDRD	Modification of Diet in Renal Disease
MGH	Mbabane Government Hospital
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NtRTI	Nucleotide analogue reverse-transcriptase inhibitor
NVP	Nevirapine
PLHIV	People Living with HIV
RFM	Raleigh Fitkins Memorial Hospital
RNA	Ribonucleic acid,
SD	Standard deviation
SDHS	Swaziland Demographic Health Survey
SNAP	Swaziland National AIDS program
SZL	Emalangeni

TAF	Tenofovir alafenamide
TDF	Tenofovir Disoproxil Fumarate
WHO	World Health Organization
ZAR	South African Rand

CHAPTER 1: INTRODUCTION

1. Background

1.1 HIV Epidemiology in Swaziland (Eswatini)

Swaziland (Eswatini) is a landlocked country in Southern Africa, bordered to the North, South and West by South Africa, and to the East by Mozambique. It is divided into four regions: Hhohho, Manzini, Shiselweni and Lubombo. Communicable diseases contribute the largest portion of the burden of disease in Swaziland.[1]

Swaziland has a generalised HIV epidemic, with the highest HIV prevalence in the world.[1] New HIV infections are declining and the incidence rate among adults aged 18-49 years is estimated at 1.4%, comprising 1.0% and 1.7% of men and women, respectively. New HIV infections have reduced by about 44% in five years since 2011. According to the Swaziland HIV Incidence Measurement Survey 2016/17, the HIV prevalence shows an increase with age for both sexes, reaching the peak earlier for women (35-39 years; 54.2%) than men (45-49 years; 48.8%).

The disproportion in prevalence by sex is most pronounced among adolescents and young people with 20-24 year old females having a five times higher prevalence (20.9%) than males (4.2%). [2] The UNAIDS estimated that in 2017, 210'000 adults and children in Swaziland were living with HIV and 3500 deaths were AIDS-related. [3]

1.2 The national HIV health sector response

In an effort to combat the high burden of HIV and AIDS, the government of Swaziland established the Swaziland National AIDS Program (SNAP) in 1987 to respond to the HIV epidemic.[4] The HIV programme is mandated to coordinate the health sector interventions. SNAP aims to ensure that all people living with HIV (PLHIV) are provided with appropriate health services which improve health outcomes, reducing the morbidity and mortality rates, through decentralisation.

The provision of decentralised HIV testing services (HTS) and antiretroviral therapy (ART) services, within the public and private sectors, has expanded rapidly over the last ten years to reach remotest of rural clinics in the country, using the primary health care approach. Decentralisation of ART services to lower level clinics has been a very important strategy in the HIV response in the country. This has ensured that the majority of the population living in the rural areas (approximately 70%) have access to the HIV treatment services, without suffering massive expenditure due to accessing essential health services.[4]

Currently more than 80% of the public health facilities are accredited to provide ART services and more than 60% of the ART initiations in the country are done by nurses who have been trained on ART initiation following the National HIV guidelines.[4] To further expand decentralised ART services, the country introduced the differentiated service delivery (DSD) model which extends ART services into the community.

This model is also aimed at addressing the barriers to HIV care linkage and poor retention rates over time. [5] At the end of 2017, the ART coverage in Swaziland was 85% among People Living with HIV (PLHIV), comprising 75% and 86% for children less than 15 years and adults older than 15 years, respectively.[6]

1.3 National HIV Guidelines: Treatment Regimens

The Swaziland Ministry of Health closely follows the World Health Organization (WHO) recommendations including guidelines for ART and has adopted a homogeneous first line therapy with Tenofovir disoproxil fumarate (hereafter referred to as "Tenofovir," and denoted as TDF) for adolescents and adults. Swaziland adopted the 2016 WHO HIV 'Test and Treat all' guidance, hence more people living with HIV were eligible and enrolled on a TDF-based regimen.[7]

At the time of this study, SNAP was using the 2010 WHO HIV guidelines recommendations for the management of ART eligible patients. The National HIV guidelines recommended the use of TDF (Tenofovir) + 3TC (Lamivudine) + EFV (Efavirenz) as a fixed-dose combination (FDC) to enable ease of administration, as well as to encourage and promote adherence. Since then, Tenofovir has been used as part of the first line and second line ART regimens in Swaziland for the management of HIV infection in adolescents and adults.[8]

The first line option was recommended for all HIV-infected eligible adolescents and adults who were treatment naïve patients, unless contraindicated, in which case the alternative regimens were offered as shown in Figure 1. In Swaziland, TDF is limited to the management of HIV. Patients co-infected with HIV and hepatitis B virus (HBV), benefit from the ART to manage and control the HBV infection. During the study period, the country did not have clinical guidelines for the management of HBV.

The 2015 Swaziland national guidelines indicate EFV as a contraindication in patients with moderate to severe EFV-induced skin reaction, severe mental illness (e.g. depression, psychosis) and bilateral gynaecomastia in males.[9]

	First Line Regimen	Second line regimen
1	TDF + 3TC + EFV	1. AZT + 3TC + LPV/r
2	TDF + 3TC + NVP	
3	AZT + 3TC + EFV	2. TDF + 3TC + LPV/r
4	AZT + 3TC + NVP	

Figure 1: Swaziland HIV Management Guidelines - ART Regimen: 2015

(SOURCE: Swaziland integrated Comprehensive HIV Management guidelines, 2015: page 86)

At the end of 2016, the ART coverage was 77% (149,160 of 193,715) of total number of adolescents and adults aged 15 years and older living with HIV and were eligible for ART based on guidelines at that time. The 2016 Ministry of Health annual HIV program report stated that at least 67% of adult and adolescent (above 15 years of age) patients who were active on first line ART were on a TDF-based ARV triple therapy and at least 29% of those were on a second line regimen. Table 1 shows the regimen breakdown. [10]

Table 1: Percentage of Adults (15+ years) on first line ART Regimen and second line ART regimen, 2016

FIRST LINE REGIMEN - Percent	SECOND LINE REGIMEN- Percent
TDF+3TC+EFV - 64%	ABC+3TC+LPV/r - 32%
AZT+3TC+NVP - 19%	TDF+3TC+LPV/r - 29%
D4T+3TC+NVP - 6%	AZT+3TC+LPV/r - 23%
AZT+3TC+EFV - 6%	D4T+3TC+LPV/r - 6%
TDF+3TC+NVP - 3%	ABC+DDI+LPV/r - 3%
Other - 2%	Other - 7%
TOTAL= 149 160 (100%)	TOTAL= 784 (100%)

Source: Annual HIV report, Source: HMIS 2016

1.4 The research problem

A significant number, although unquantified and undocumented, of patients in Swaziland who are initiated on a TDF-based triple regimen are at risk of developing TDF nephrotoxicity. Tenofovir is a nucleotide reverse transcriptase inhibitor (NRTI) and indicated as part of the first line antiretroviral therapy (ART) for the management of HIV infection. Despite normal renal function test results at baseline, some patients develop renal dysfunction with treatment progression. Those with renal failure have to undergo renal dialysis, and prompt substitution of TDF with another NRTI drug.

In certain instances, the renal dysfunction or renal failure is reversible after commencing renal dialysis and TDF substitution. The onset, incidence and the economic impact of the TDF-associated toxicity are unknown in the Manzini and Hhohho regions. The risk factors are documented in literature as uncommon.[11]

In Swaziland, this information is undocumented, therefore, this remains a concern as nearly 70% of ART eligible HIV-infected patients were on a TDF-based first line triple regimen and about 30% of patients on second line were on a TDF-based regimen, in 2016. [10]

In 2017 Swaziland adopted and implemented the 2016 WHO "Test and Treat all" HIV treatment guidelines. The guidelines recommend the use of TDF in first and second line ART regimens.[7] It is therefore imperative to document the incidence of TDF-associated renal pathology as the implementation is at a national scale to enable comprehension of the magnitude of this health problem. The information on the incidence, the onset and risk factors of the TDF-associated renal failure is not available in Swaziland. Such information needs to be readily available since TDF forms part of the backbone of both first and second line therapy for the management of HIV infection.

1.5 Hypothesis

The null hypothesis (H_o): There is no difference in the risk of developing TDF-associated renal failure on first line ART regimen when comparing TDF+3TC+EFV/NVP and AZT+3TC+EFV/NVP.

The alternate hypothesis (H_A): There is a risk of developing TDF-associated renal failure on first line ART regimen when comparing TDF+3TC+EFV/NVP and AZT+3TC+EFV/NVP.

1.6 Aim and objectives

1.6.1 Aim

The aim of the study was to understand the epidemiology of TDF-associated renal failure of HIV-infected patients initiated on ART regimens at treatment centres: Raleigh Fitkin Memorial (RFM), a regional referral hospital and Mbabane government hospital (MGH), the national referral hospital; in Swaziland from January 2010 to December 2015.

1.6.2 Primary Objectives

1. To estimate the incidence of TDF-associated renal failure in patients on standard ART regimens.
2. To examine the association between ART regimen and renal failure among patients on standard first line and second line ART regimens.
3. To examine the risk factors for renal failure among patients using first and second line regimens.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The Swaziland national HIV management guidelines recommend that each patient must have baseline blood results of the following tests prior to commencing treatment; CD4 cell count, renal function, liver function and full blood count. Patients initiated on TDF are those assessed as having a normal range of baseline renal function tests including a calculated creatinine clearance for all patients prior to TDF initiation. The guidelines further state that patients with and/or at risk of renal disease (those with known underlying renal disease, older than 50 years, low BMI < 18.5kg/m^2, diabetes mellitus, hypertension and patients on nephrotoxic drugs) should be managed with caution.[12]

The recommended post-ART initiation clinical monitoring intervals are at two weeks, then at four to eight weeks.[9] Follow-up visits are increased to every two months if patients do not have complications and are stable on ART. The routine laboratory monitoring include renal function test, liver function test and full blood count as well as the CD4 cell count at least every six months and a viral load at month six of ART initiation, then annually thereafter.

In instances where the blood results are deranged, more intensive patient-specific follow-up and monitoring is instituted.[9] The clinic visits are documented, including any drug adverse events/toxicities experienced related to the ART regimen taken by the patient. The adverse events are recorded and reported as part of the pharmacovigilance activities.

2.2 HIV-associated Nephropathy

Nephropathy is documented as a relatively common complication in patients infected with HIV.[12] The HIV nephropathy could be a result of direct HIV infection of the kidney epithelial cells or from the adverse effects of antiretroviral drugs. The natural course of the HIV disease without ART (HIV-associated nephropathy), is characterised by rapid progression to renal failure and end-stage renal disease leading to the need for dialysis.[13,14]

The introduction of ART in HIV management has changed the natural course of HIV renal disease and has significantly slowed this disease progression. [11]

2.3 Tenofovir and its effects on the kidney

Tenofovir (TDF) is a nucleotide reverse transcriptase inhibitor (NtRTI) and indicated for the management of HIV and Hepatitis B virus infection. One of the documented side-effects is nephrotoxicity which could progress to renal failure. TDF is an orally bioavailable prodrug of Tenofovir, an acyclic nucleotide analogue NRTI. The proximal tubular cell is the main target for Tenofovir toxicity due to its complement of cell membrane transporters which favour its accumulation. [4]

TDF nephrotoxicity is characterised by proximal tubular cell dysfunction which may be associated with acute kidney injury or chronic kidney disease. [15] Studies have demonstrated that tubular dysfunction occurs in 17–22% of Tenofovir-treated patients, versus 6 and 12% of other ART-treated or naive HIV patients.[8,11]

TDF is eliminated unchanged in the urine by a combination of glomerular filtration and proximal tubular secretion.[14] Drug transporters expressed in renal proximal tubule cells are believed to influence Tenofovir plasma concentration and toxicity in the kidney.[16] About 20–30% of the drug is actively transported into renal proximal tubule cells by organic anion transporters in the basolateral membrane.[10,11]

Despite this, the general safety profile of TDF is good and the majority of patients tolerate the drug reasonably well.[13] The most effective treatment of TDF nephrotoxicity is stopping TDF and this often results in clinical improvement of kidney injury, which may be partial. The prevention of TDF-induced nephrotoxicity requires careful monitoring.[17][18]

It is expected that once TDF is stopped renal recovery should be quicker than the renal function decline.[19] However a study conducted in 2012 in the United States Veterans Health Administration, concluded that the exposure to TDF was independently associated with increased risk of kidney disease events, and did not appear to be reversible. [13]

2.4 Risk factors for TDF nephrotoxicity

Several risk factors which predispose patients to develop the TDF-induced nephrotoxicity are known and documented.[14] Post-marketing clinical data showed that advanced age, low body weight, higher serum creatinine levels before starting TDF treatment, comorbidities (diabetes, hypertension) concomitant nephrotoxic medications, advanced HIV infection (low CD4 cell count, AIDS), and, in some studies, male sex were risk factors for TDF-induced glomerular filtration rate (GFR) reduction.[13,14,17,20]

Some studies have reported the dominant contributors to the chronic kidney disease (CKD) risk score as the traditional kidney disease risk factors (age, glucose, systolic hypertension, triglycerides, and proteinuria). The overall 5-year event rate was 7.7% in TDF users. [12] [19]

The Swaziland Ministry of Health's 2016 first quarter pharmacovigilance newsletter stated that 81% of 398 reported adverse events were from patients on the TDF+3TC+EFV regimen and 150 of these adverse events were classified as severe to life-threatening or death, 5% of these serious adverse events were TDF-associated renal failure.[21] The report was based on 7 sentinel sites out of the 133 health facilities which offer ART services. It is estimated and projected that this complication develops in approximately 15% of patients on a TDF-based regimen, nationally in Swaziland. [21]

When renal failure is diagnosed at routine clinical monitoring, the patients are referred to one of the two dialysis units in Swaziland. The dialysis is commenced together with the substitution of TDF by another NRTI, Zidovudine (AZT) or Abacavir (ABC).

Renal dysfunction and/or failure develops despite normal renal function before ART initiation. In some cases the renal dysfunction/failure is reversible and irreversible in others. These outcomes are not documented in Swaziland. The development of renal failure poses a serious threat and a potentially life-threatening risk to the patient, and furthermore increases the demand and cost of dialysis on an already constrained and overstretched health system.

2.5 The public health implications of TDF use

The above-mentioned studies together with numerous other studies have demonstrated the advantages of the use of TDF as part of the ART regimen. The potential TDF-associated nephrotoxicity is not an impediment to its widespread use in the treatment of HIV infection, since the drug is well tolerated by the majority of patients. [22]The World Health Organization also continues to recommend TDF for the treatment of HIV, as well as for the prevention of HIV as pre-exposure prophylaxis. The Ministry of Health in Swaziland thus far is compelled by the available evidence to continue to use TDF in the ART regimen.

As the global price decreases annually it becomes even cheaper and more cost-effective to enrol patients on TDF-based regimen. However, renal toxicity poses higher risks in resource-limited settings, where routine laboratory monitoring is somewhat limited and death from acute renal failure may even approach 90%.[20]

The Swaziland National AIDS Program (SNAP) needs to continually ensure access to laboratory monitoring services for all patients; as well as assisting with the advocacy, forecasting and quantification of renal function tests laboratory reagents. This enables and ensures accurate patient monitoring and appropriate management.

CHAPTER 3: METHODS

3.1 Study Design

The study employed a retrospective cohort study design. The data were collected as secondary data from electronic medical records using a standardised tool (Annex 2).

3.2 Study Setting

The study setting was the ART clinics of Raleigh Fitkin Memorial hospital (RFM), a regional referral hospital, and Mbabane Government (MGH) hospital, a national referral hospital. MGH is located in Mbabane, the capital city of Swaziland, within the Hhohho region. RFM is located in the Manzini region, the largest city and economic hub of Swaziland. RFM is a mission hospital which receives funding through the Swaziland government subvention.

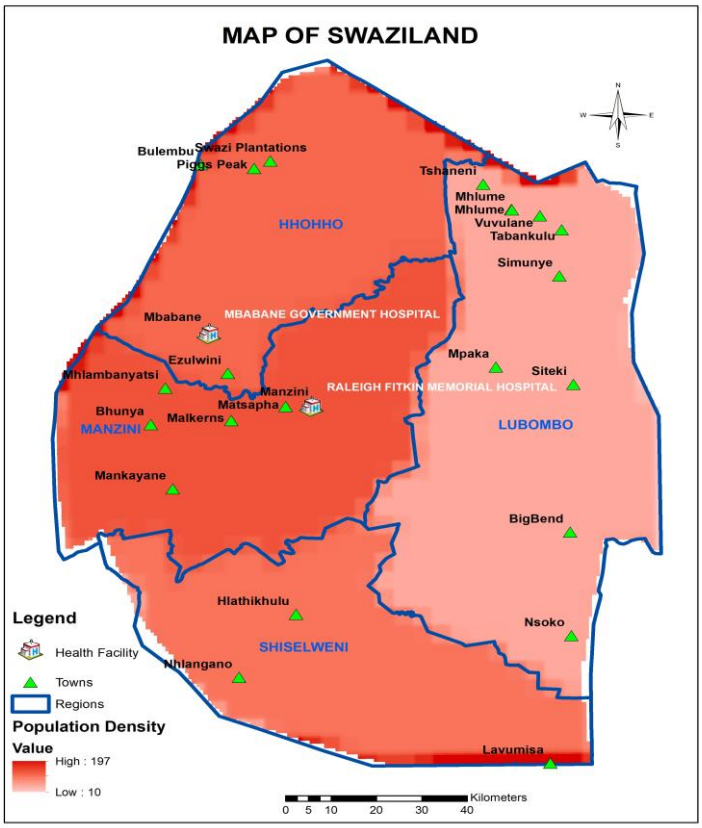

Figure 2: Map of Swaziland

Source: Makhoselive Dlamini, WHO Geographic Information System Analyst - 12 February 2018, Mbabane, Swaziland

These hospitals are both public sector hospitals and located in the two largest and most populated urban cities and regions of Swaziland. MGH and RFM serve primary health clinics and smaller hospitals called health centres, which in turn refer patients to the two hospitals in the respective regions. Additionally, MGH also serves as the national referral hospital for all regional hospitals, including RFM.

The two hospitals are serviced by specialists, medical officers and nurses; and both have renal units which offer dialysis services in the respective regions for all the referred patients. The MGH ART clinic is serviced by medical officers, nurses and lay cadres while the renal unit is serviced by general medical officers, internal medicine physicians and a nephrologist.
The RFM ART clinic is serviced by general medical officers, nurses and lay cadres and the renal unit is serviced by medical officers, an anesthesiologist and a visiting nephrologist, who reviews complicated cases on a weekly basis.

The two hospitals are financially supported by the Swaziland government through the Ministry of Health. The HIV commodities and antiretroviral medicines are all procured and distributed by the central medical stores of the Ministry of Health. In both hospitals, HIV services including HIV treatment are offered at no cost to the patient.

3.3 Patient selection

3.3.1 Study Population

The study population comprised all the HIV-infected patients initiated on a TDF-based standard regimen betweeen January 2010 and December 2015. TDF was first available and used in Swaziland in 2010, for patients aged 18 years and older at the two hospitals.

3.3.2 Patient selection/recruitment

The study participants' data were extracted from the national ART electronic database for Mbabane Government Hospital and Raleigh Fitkin Memorial Hospital. The sampling frame comprised all HIV-infected patients initiated on ART from January 2010 to December 2015, listed on the national electronic ART database for the two hospitals.
The data were then filtered according to the inclusion and exclusion criteria as listed below:

3.3.3 Inclusion Criteria:

- HIV-infected patients, aged 18 years and above
- HIV-infected patients to be on a TDF or AZT-based regimen (between 01 January 2010 and 31 December 2015)
- HIV-infected patients with all baseline and follow-up creatinine blood results

3.3.4 Exclusion Criteria

- HIV-infected patients aged less than 18 years
- Patients taking TDF for viral Hepatitis B infection only
- Patient medical records with no baseline and follow-up creatinine blood results

The study used secondary data from the national data base captured from the ART medical clinical records. A standard data collection form (Annex 2) which included all the variables of interest was used to abstract the data. The demographic and clinical variables at baseline and follow-up were recorded. The pregnancy status of the female participants was disregarded.

The following variables were considered and measured. Demographic variables: age in years at last birthday during ART initiation, sex, health facility, date of ART initiation and date of current ART regimen. Clinical variables: body mass index (BMI=weight (kg)/height $(m)^2$), WHO clinical (Annex 5) stage at initiation, last WHO clinical stage while on ART, CD4 cell count (cells/µL) at initiation of ART, the last CD4 cell count, the last serum creatinine taken before the close of the study period, and the estimated glomerular filtration rate (eGFR) calculated for each participant using serum creatinine result.

3.4 Definition of the Outcome

The outcome variable of interest was TDF-associated renal dysfunction (including failure). In this study, it was measured as a proxy by looking at the study participants who had their start ART regimen modified from a TDF-based ART regimen to either an AZT- or ABC-based regimen. Renal dysfunction was defined as per the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1, July 2017 (Table 2); using the parameters: Creatinine, High and Creatinine Clearance or eGFR, Low.[19]

Table 2: Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1, July 2017

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Calcium, High (mg/dL; *mmol/L*)				
≥ 7 days of age	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	≥ 13.5 *≥ 3.38*
< 7 days of age	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 *≥ 3.38*
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 *≥ 1.8*
Calcium, Low (mg/dL; *mmol/L*)				
≥ 7 days of age	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 *< 1.53*
< 7 days of age	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High **Report only one*	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase to 1.3 to < 1.5 x participant's baseline	> 1.8 to < 3.5 x ULN OR Increase to 1.5 to < 2.0 x participant's baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x participant's baseline
Creatinine Clearance[14] or eGFR, Low **Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m² OR 10 to < 30% decrease from participant's baseline	< 60 to 30 ml/min or ml/min/1.73 m² OR 30 to < 50% decrease from participant's baseline	< 30 ml/min or ml/min/1.73 m² OR ≥ 50% decrease from participant's baseline or dialysis needed
Glucose (mg/dL; *mmol/L*)				
Fasting, High	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	≥ 500 *≥ 27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	≥ 500 *≥ 27.75*

[14] Use the applicable formula (i.e., Cockcroft-Gault in mL/min or Schwartz, MDRD, CKD-Epi in mL/min/1.73m2). Sites should choose the method defined in their study and when not specified, use the method most relevant to the study population.

*Reminder: Choose the method that selects for the higher grade.

DAIDS AE Grading Table Corrected Version 2.1- July 2017
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The eGFR or creatinine clearance was estimated using the glomerular filtration rate (GFR) for each study participant using the Modification of Diet in Renal Disease (MDRD) formula;

GFR (mL/min/1.73m2)= 175 × (Scr)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 black ethnicity)

The required variables: creatinine, age, ethnicity and sex were available from the medical records database. Swaziland population is predominantly of black ethnicity. This formula has been validated extensively and has shown good performance for patients with all common causes of kidney disease.[23] A study conducted in South Africa also validated the formula.[24] Renal dysfunction was defined as an abnormal GFR or creatinine clearance of at least moderate severity (i.e. moderate, severe or potentially life-threating severity).[19]

3.5 The main exposure variable

The exposure variable is TDF, which is the drug of choice for ART naïve patients and patients who fail on first line AZT based regimen and have to be switched to second line ART regimen. The national HIV treatment guidelines indicate that if patients experience TDF-induced nephrotoxicity on routine clinical monitoring, they must be substituted to an alternative drug, either AZT or ABC, provided there is no clinical evidence of treatment failure.

In this study renal dysfunction and renal failure was graded according to the chronic kidney disease classification of renal dysfunction as shown below:

Table 3: Chronic Kidney Disease Classification

Stage	**GFR (mL/min/1.73m^2)**
1 (nil or ↑GFR)	≥90
2 (mild ↓GFR)	60 - 89
3 (moderate ↓GFR)	30 - 59
4 (severe ↓GFR)	15 - 29
5 (kidney failure)	<15 or dialysis

Source: http://www.scymed.com/en/smnxps/psdgq277.htm

3.6 Measurements

The data were extracted from the national database of the two hospitals (MGH and RFM) and transcribed onto Microsoft Excel spreadsheet. They were secured with a password for restricted access, and backed up on a safely-store external hard drive. Table 4 below shows the measured of variables.

Table 4: Definitions of the Demographic and Clinical variables

Demographic variable	**Variable definition**
Age	Age in years during ART initiation at last birthday
Sex	Male or Female
Health Facility Cluster	Mbabane Government Hospital/Raleigh Fitkin Memorial Hospital
Clinical variable	
BMI	Body Mass Index (weight/height^2) at ART initiation Underweight < 18.5 kg/m^2 Normal- 18,5 – 24.9 kg/m^2 Overweight 25-30 kg/m^2 Obese- >30 kg/m^2
WHO Clinical Stage[25]	Clinical stage of HIV clinical disease according to the World Health Organization classification at ART initiation (WHO clinical stage I; WHO clinical Stage II; WHO clinical Stage III; WHO clinical Stage IV)
Baseline CD4 cell count	CD4 cell count at initiation of ART and the last CD4 result before 31st December 2015 <100 cells/µL; 100-199 cells/ µL 200-349 cells/ µL; ≥ 350 cells/ µL
Date of ART Initiation	dd/mm/yyyy
Start ART regimen	The ARV combination (regimen) at ART initiation
Date of current ART regimen	Date of last ART regimen change
Current ART Regimen	The ARV combination (regimen) at the end of December 2015
Serum Creatinine (µmol/L)	The baseline and last recorded serum creatinine in the electronic file
estimated glomerular filtration rate(eGFR)	eGFR (mL/min/1.73m^2): measure of the renal function, calculated using serum creatinine, and graded as follows: Stage 1 (nil)≥90; Stage 2 mild)=60-89; Stage 3 (moderate)=30-59; Stage 4 (severe)=15-29; Stage 5 (kidney failure)≤15

3.7 Sampling Method

A total of 65,178 ART patients who were on ART between January 2010 and December 2015, aged 18 years and older and listed on the national electronic ART database, comprised the sampling frame. Of these, 65% were from Manzini and Hhohho regions.[4] Systematic random sampling was used to eliminate subjectivity and minimize bias.

A computer-generated random number, 4, was selected using Microsoft Excel 2013. Every 4th patient file was selected from the Mbabane Government Hospital and Raleigh Fitkin Memorial Hospital database until there were 593 patient files from each hospital as per the calculated sample size. This gave the total study a sample size of 1186 study participants, of whom 82.9% (n=983) were on TDF and 17.1% (n=203) were on AZT. The distribution by gender; 61.8% (n=733) were female and 38.3% (n=453) were male.

3.7.1 Sample size justification

The sample size was estimated for the main study objective – To examine the impact of TDF versus AZT regimen on the risk of renal failure among patients on standard first line and second line ART regimens. In reference to the data of Scherzera, Estrellab, Lia et al. [13] , the survival analysis sample size estimation involving comparisons between groups was done using the log-rank test which requires estimates of hazards of the two groups of primary comparisons.

The majority (80%) of patients in the Swaziland ART database are on a TDF based regimen. The hazard ratio for progression to renal derangement among those on TDF compared to those on AZT ranged from 1.25 to 2.50. [13][26]

Using the statistical package, STATA version 13, the sample size calculation for the log-rank test; assuming a 5% level of significance, the use of a two-sided test in the analysis and 20% prevalence of those on AZT based regimen and hazards ratio of renal derangement of between 1.25 and 2.50; Table 5 presents estimated minimum power for the different scenarios using the estimated sample size of 1120; and thus a robust enough power (at least 80%) to detect HR of at least between 1.25 and 2.50.

Table 5: Sample size estimation for varying hazard ratio

Parameter			
Hazard ratio	1.25	1.30	2.5
Sample size	1120	1120	1120
Level of significance	5%	5%	5%
Power	80%	89%	>99%

3.8 Data analysis

The data were transcribed on the Microsoft Excel 2013 spreadsheet, and then checked for accuracy, completeness, consistency and cleaned through inspection and correction of errors and extreme outliers prior to analysis and then exported to STATA version 13 (STATA Corp, 2014, College Station, Texas, USA) for analysis.

3.8.1 Descriptive Analysis

Descriptive statistics were used to summarise the data. For categorical data, frequency distribution tables were used to summarise data using numbers and percentages. The analysis of numerical and continuous data depended on the distribution of data, which were assessed using the histogram and normality tests. Numerical data which assumed normal distribution were summarised using the mean as the measure of central tendency and the standard deviation (SD) as the measure of variation. For skewed data, the median was used as a measure for central tendency and the inter-quartile range (IQR) as a measure of variation.

3.8.2 Analysis by Objective

Further analysis was done using Kaplan-Meier survival curves and the log-rank test to compare renal dysfunction by TDF or AZT first line regimen status.

The person-time (follow-up time) was accrued from date of ART initiation until the earliest of 1) the outcome – renal dysfunction/failure; 2) last documented visit by the health facility; or 3) close of the dataset at the end of December 2015.

For objective 1, to estimate the incidence of TDF-associated renal failure in patients on first line and second line ART regimens, the cumulative incidence of renal failure was calculated and expressed as the total number of persons developing renal failure per 1000 person-months of follow-up. This was then stratified by ART regimen.
The outcome was defined using the DAIDS parameters [23], the abnormal renal and the eGFR low. An increase of ≥ 1.3 in creatinine change was considered to be abnormal whereas an increase <1.3 was considered normal. The eGFR decrease of ≥ 10% was considered abnormal, whereas the eGFR decrease of <10% was normal.[19]

For objective 2, to examine the association of ART regimen and renal failure among patients on standard first line and second line ART regimens, the Kaplan-Meier curves and the log-rank test were used to compare survival probabilities by ART regimen.

For objective 3, to examine the risk factors for renal failure among patients using regimens, bivariate and multivariate Cox-proportional hazards regression models were applied to identify risk factors for renal failure. Specifically renal dysfunction and renal failure (event of interest) was regressed against the following covariates: age, gender, WHO clinical staging, CD4 cell count.

The following socio-demographic and clinical variables were screened for inclusion into multivariate models: biological sex, age, type of first line ART regimen, body mass index, CD4 count, and WHO clinical stage at enrolment.

The variables which were identified *a priori* and had a p-value <0.20 in bivariate analyses, were included in the multivariate regression models as potential confounders. The proportional hazard assumption was checked using graphical methods. To evaluate the robustness of these results, the univariate and multivariate analysis was repeated by applying a second definition of renal dysfunction which utilises the creatinine, high row of the DAIDS Toxicity Tables. [23]

The proportionality assumption was checked using graphical methods. Variables that were identified as a *priori* or significant at the p = 20% level were considered automatically for inclusion in the final multivariate model. All p-values <0.05 were considered statistically significant.

3.9 Ethical Considerations

The Helsinki Declaration principles were observed throughout the life span of the research project. The ethical clearance was granted by the Swaziland Health Ethics Committee and the University of Pretoria's Faculty of Health Sciences Research Ethics committee. Permission to access the national database was granted by the health management and information system (HMIS) department, as well as the hospital management of the RFM and MGH.

Other ethical principles, which were applied, include; that the study findings would be used for the greater benefit of the patients, the study avoided unnecessary suffering and did not cause harm (death/disability) to the study participants. The study benefits outweigh the risks of not conducting the study.

No patient identifiers were used and confidentiality of patient information and medical records was ensured at all times. The study protocol, data and all other information generated was held in strict confidence. No information concerning the study or the data was released to any unauthorized third party.

CHAPTER 4: RESULTS

The results are presented according to the objectives of the study. Between January 2010 and December 2015; 65,178 HIV-infected subjects were initiated ART in the Hhohho and Manzini regions. [4][27] Among these, 1186 met the study inclusion criteria, and 67 were excluded. This left 1,119 patients for analysis.

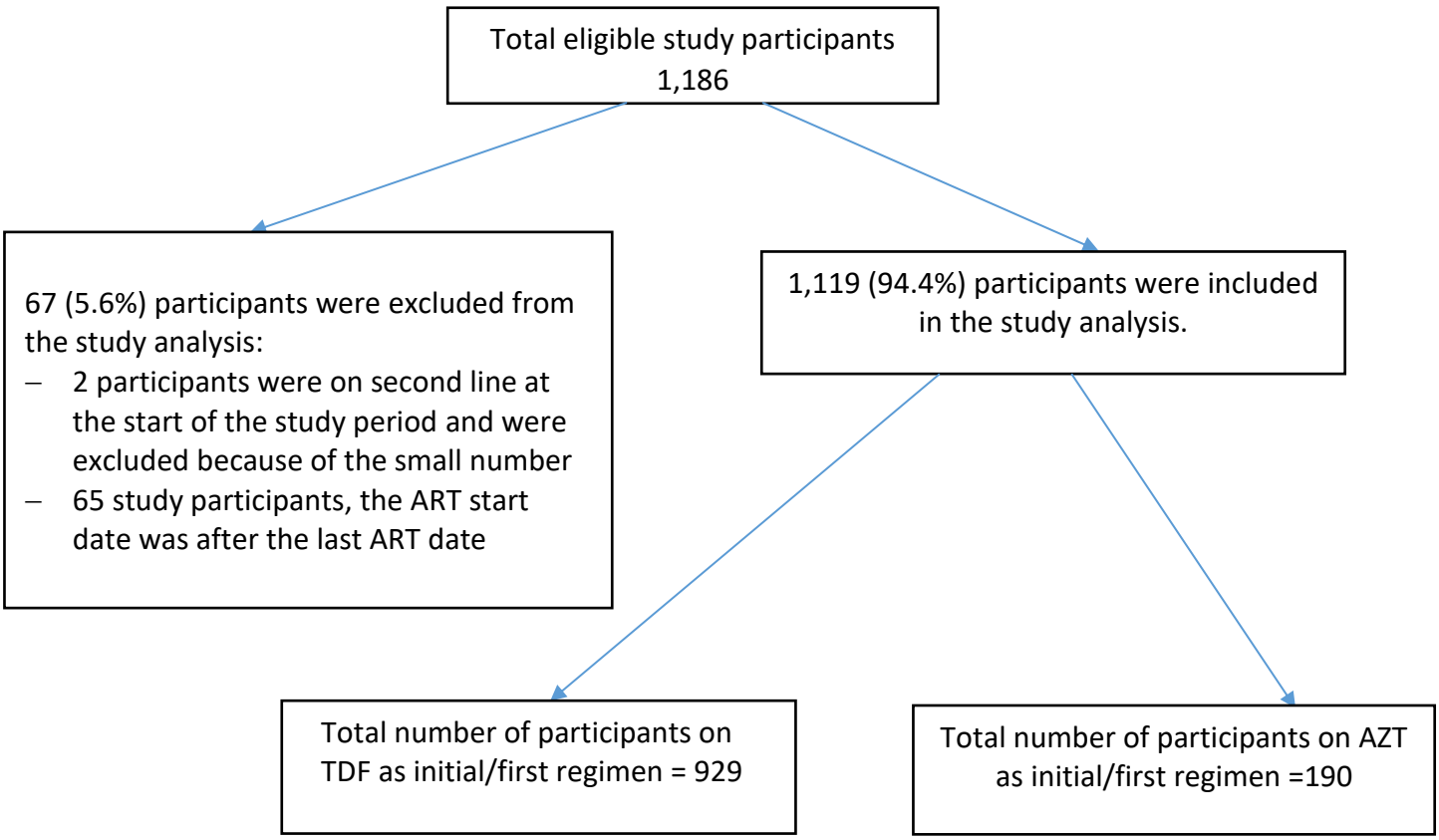

Figure 3: Flow diagram of study participants at MGH and RFM hospital, 2010-2015

Of the patients that were included in the study analysis, 83% (n=929) were on TDF and 17% (n=190) were on AZT. The median age was 36 years (IQR: 18 to 79 years), mean CD4 count was 194 cells/µL (95% CI: 207.61 to 228.75) and the mean GFR was 123.56, (95% CI: 121.22 to 125.92). The eGFR was normal at baseline for 81.41% (911) of the study participants.

4.1 Descriptive analysis

The baseline demographic and clinical characteristics of the 1119 study participants initiated on ART between January 2010 and December 2015 at the two hospitals are shown in Table 6.

Table 6: Descriptive analysis of participants on ART initiation of TDF-based and AZT-based regimen at MGH and RFM hospitals, 2010-2015

	TDF-based		AZT-based		Total	
	n=929	%	n=190	%	N=1119	100%
Gender						
F	544	58.56%	147	77.37%	691	61.75%
M	385	41.44%	43	22.63%	428	38.25%
Facility Cluster						
MGH	458	49.30%	91	47.89%	549	49.06%
RFM	471	50.70%	99	52.11%	570	50.94%
Age group						
<40 years	565	60.82%	126	66.32%	691	61.75%
>40 years	364	39.18%	64	33.68%	428	38.25%
Median age 36						
WHO clinical stage						
I and II	628	67.60%	133	70.00%	761	68.01%
III and IV	301	32.40%	57	30.00%	358	31.99%
BMI						
Under weight (<18.5)	95	10.23%	20	10.53%	115	10.28%
Normal (18.5 - 24.9)	424	45.64%	76	40.00%	500	44.68%
Over weight (25 - 30)	410	44.13%	94	49.47%	504	45.04%
Median=24.45						
CD4 count						
<100	251	27.02%	44	23.16%	295	26.36%
100-199	232	24.97%	49	25.79%	281	25.11%
200-349	309	33.26%	72	37.89%	281	34.05%
350+	137	14.75%	25	13.16%	162	14.48%
Median CD4 count = 194						
GFR						
Normal	757	81.49%	154	81.05%	911	81.41%
Mild	151	16.25%	30	15.79%	181	16.18%
Moderate	21	2.26%	6	3.16%	27	2.41%
Mean GRF= 123.57						

TDF: Tenofovir
AZT: Zidovudine
F: Female
M: Male
MGH: Mbabane Government Hospital
RFM: Raleigh Fitkin Memorial Hospital
BMI: Body Mass index
GFR: Glomerular filtration rate

Of the study participants enrolled, 691 (61.75%) were female, 428 (38.25%) were male. Those aged less than 40 years were 691 (61.75%). Participants who had early disease (WHO clinical stage I and II) were 761 (68.01%) and those with advanced HIV disease (WHO stage III and IV) were 358 (31.99%). At enrollment 500 (44.68%) participants had a normal BMI whereas 115 (10.28%) and 504 (45.04%) were underweight and overweight, respectively.

The GFR was normal in 911 (81.41%) of the study participants and it was mild in 181 (16.18%) and moderate in 27 (2.41%) of the participants. Of note, is that none of the participants had severe GFR nor were in renal failure at ART initiation (that is, failure due to sole HIV disease).

4.2 Outcome variable definition

The outcome variable, renal failure and dysfunction, was measured as a proxy by inferring a regimen modification from a TDF-based regimen to either ABC or AZT based regimen and where either in first line or second line ART regimen.

The outcome was measured by the change in the eGFR from the baseline, which is an abnormal GFR. The change had to be $\geq$ 10% decrease from each participant's baseline GFR, based on the division of AIDS (DAIDS) table for grading the severity of adults and pediatric adverse events. An abnormal GFR was graded as moderate, severe or potentially life-threating. [28]

The study participants with an abnormal eGFR or a $\geq$ 10% decrease from the baseline comprised 11.26% (126) of the entire cohort.

4.3 Overall follow-up time

The overall median follow-up time was 6.72 months (IQR: 0.03 to 81.61) and total person-time was 15,579 person-months (95% CI: 6.79 to 9.63).

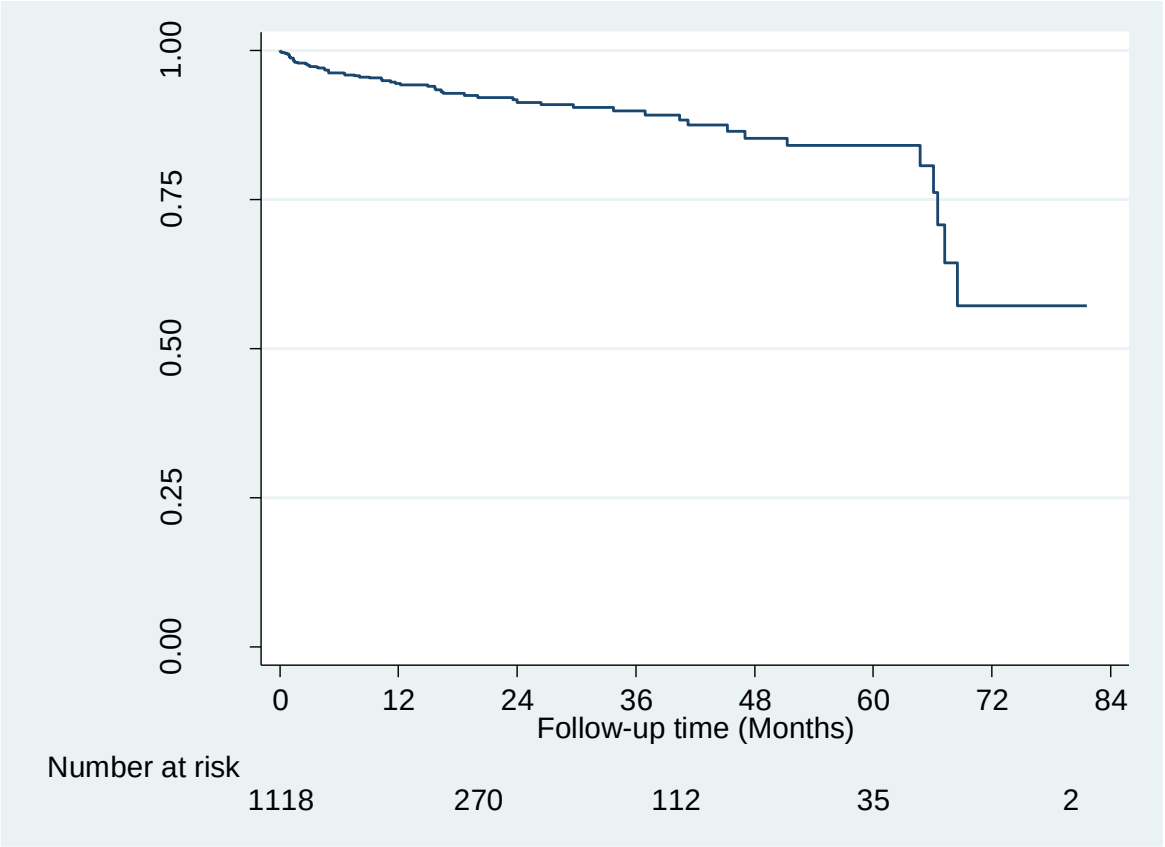

Figure 4: Kaplan-Meier curve: overall follow-up time (months)

The overall incidence rate for abnormal GFR was 8.1 per 1000 person-months and the total 15579 person-months. The larger proportion of participants (848) developed TDF-associated nephrotoxicity during the first 18-month follow-up period.

4.4 Incidence rate

The outcome was defined using the DAIDS parameters, the abnormal renal and the eGFR low.[28]

4.4.1 Incidence by outcome using abnormal renal function

The overall incidence rate of failure was 4.4 per 1000 person-months and the total person-months was 14,853 (95%CI: 3.43 to 5.58) during the follow-up period.

The incidence rate of TDF-associated nephrotoxicity was 5.1 per 1000 person-months (95%CI: 3.94 to 6.72) and 10499 total person-months. Whereas the incidence rate of abnormal renal function due to non-use of TDF (AZT) was 2.5 per 1000 person-months (95%CI: 1.40 to 4.56) and 4,355 total person-months.

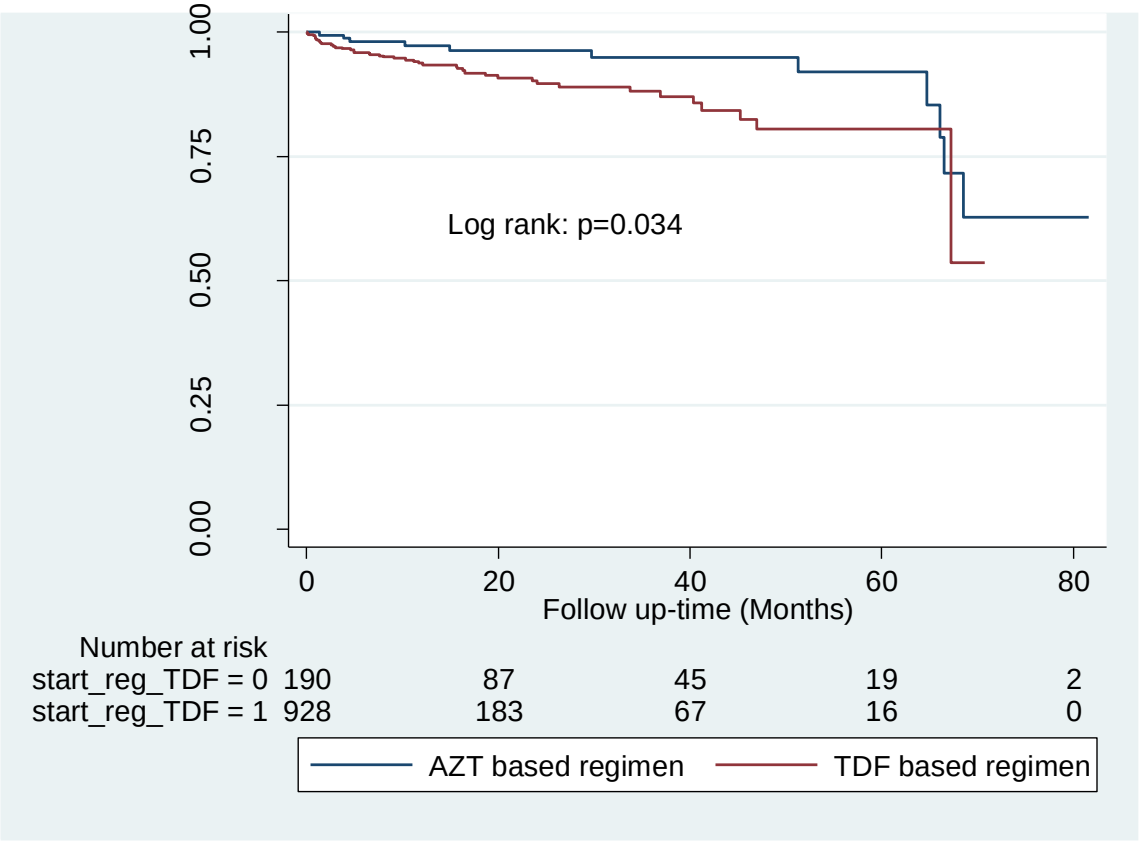

Figure 5: Kaplan-Meier curve and Log-rank test for main exposure (TDF versus AZT), by abnormal renal function (creatinine) of study Participants at MGH and RFM hospital, 2010-2015

The incidence rate of abnormal renal function among those on TDF was about two times higher than those on the non-TDF regimens.

4.4.2 Incidence by outcome using eGFR

The overall incidence rate for abnormal eGFR was 8.1 per 1000 person-months and the total person-months 15579 (95% CI: 6.79 to 9.63) during the follow-up period.

The incidence rate of TDF associated nephrotoxicity was 9.44 per 1000 person-months (95%CI: 7.79 to 11.44) and 11022 total person-months. The incidence rate of abnormal renal function due to non-use of TDF (AZT) was 4.83 per 1000 person-months (95%CI: 3.18 to 7.33) and 4557 total person-months.

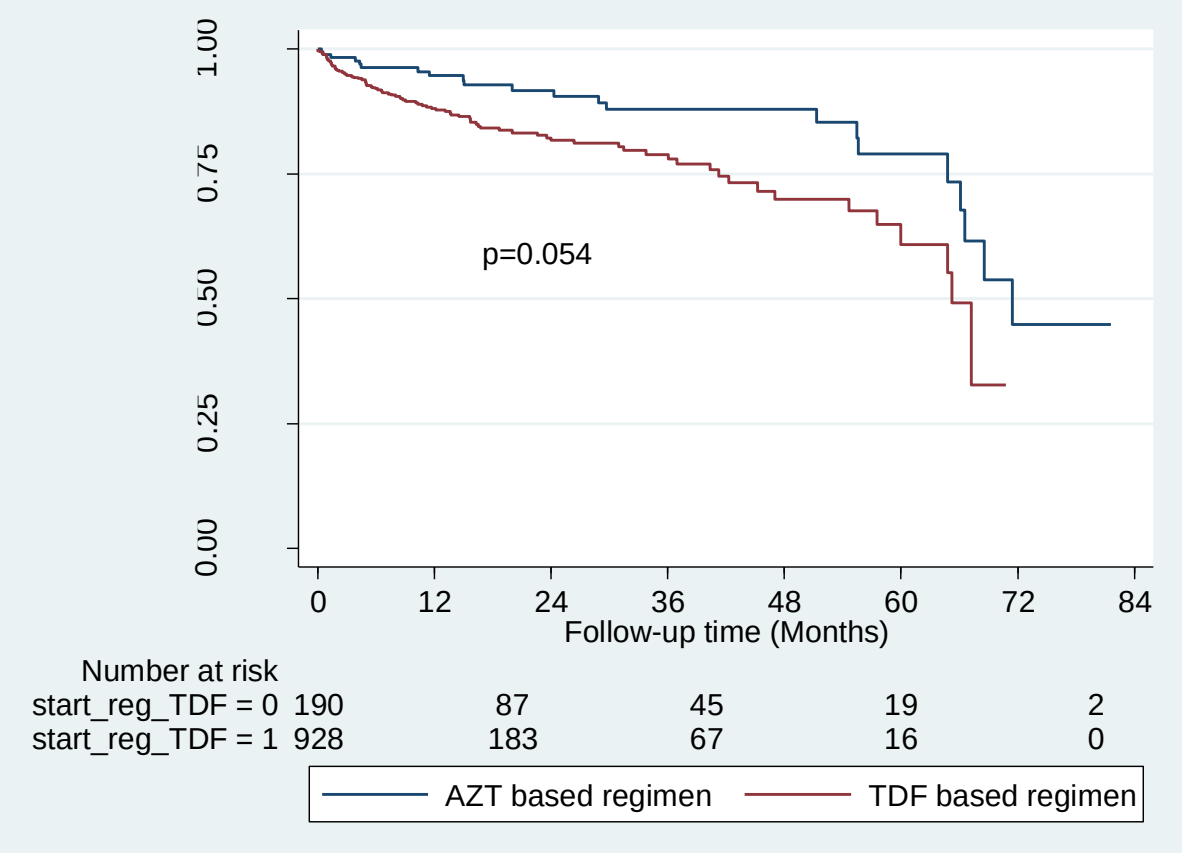

Figure 6: Kaplan-Meier curves and Log-rank test for main exposure (TDF versus AZT) by abnormal eGFR of study Participants at MGH and RFM hospital, 2010-2015

The incidence rate of abnormal renal function (low eGFR) among those on TDF was about two times higher than that of TDF non-use.

4.5 Univariate analysis

The covariates: TDF versus AZT, sex, age, WHO stage, BMI, gender and CD4 cell count were analysed. Age, BMI and CD4 cell count were analysed both as categorical variables and as continuous variables to explore the effect they would have on renal dysfunction using the eGFR.

Table 7: The univariate analysis by covariates: sex, age, WHO stage, BMI, CD4 cell count

Covariate	Hazard Ratio	95%CI	p-value
AZT	1		
TDF	2.08	1.27 - 3.40	0.003*
Sex:			
Female	1		
Male	1.35	0.94 - 1.93	0.099
Age:			
<40 years	1		
>40years	1.45	0.81 - 1.63	0.450
All ages	1.00	0.99 - 1.02	0.664
WHO clinical stage :			
I and II	1		
lll and IV	0.96	0.66 - 1.39	0.823
BMI:			
Underweight	1		
Normal	0.84	0.46 - 1.56	0.597
Obese	1.07	0.59 - 1.94	0.833
All BMI	1.03	0.99 - 1.06	0.103
CD4:			
<100	1		
100 -199	1.19	0.74 - 1.90	0.476
200-349	0.90	0.56 - 1.45	0.671
350+	0.84	0.47 - 1.50	0.551
All CD4 cell counts	1.00	0.99 - 1.00	0.171

*p-value is <0.05 (statistically significant)

TDF: Tenofovir

AZT: Zidovudine

BMI: Body Mass index

The use of a TDF-based regimen compared to that of an AZT-based regimen was associated with 2-fold increased hazard of failure during the follow-up period (HR: 2.08, 95%CI 1.27 to 3.40, p=0.003).

Similarly, being male compared to female sex (HR 1.35, 95%CI 0.94 to 1.93), p=0.099) and being older (HR 1.45, 95%CI 0.81 to 1.63) showed a trend towards increased hazard of renal dysfunction (eGFR) during the follow-up period. Although these estimates were imprecise, they did not reach statistical significance.

4.6 Multivariate Analysis

As seen from Table 7 above, the use of TDF was significant in the univariate analysis, although the other listed covariates were not statistically significant. All covariates were analysed in order to explore the effect they would have on the renal dysfunction using the eGFR.

Table 8: The multivariate analysis by covariates: sex, age, WHO stage, BMI, CD4 cell count

Covariate	Hazard Ratio	95% CI	P-value
TDF use	2.04	1.24 - 3.35	0.005*
Sex	1.28	0.87 -1.88	0.203
Age	1.07	0.75 - 1.53	0.725
WHO clinical stage	0.89	0.60 - 1.33	0.573
BMI			
Normal	0.80	0.43 - 1.48	0.473
Obese	1.08	0.58 - 2.02	0.809
CD4			
100-199	1.20	0.74 - 1.93	0.462
200-349	0.90	0.55 - 1.47	0.672
350+	0.85	0.46 - 1.55	0.594

***p-value<0.05 (statistically significant)**

The multivariate analysis (Table 8) showed that the exposure to TDF had a 2 fold (times) increased risk of an abnormal renal function (eGFR) during the follow-up period (p-value=0.005), after adjusting for sex, age, WHO clinical staging, BMI and CD4 cell count. The listed covariates had p-values >0.05 and were therefore, not statistically significant.

CHAPTER 5: DISCUSSION

This retrospective data analysis explored the incidence and risk factors of nephropathy associated with TDF use among HIV-infected adults exposed to TDF and were active on antiretroviral therapy in Swaziland, during the period 2010-2015.

5.1 Descriptive Analysis

A total of 1,186 participants met the study inclusion criteria and 67 participants were excluded from the analysis. The 67 participants were excluded because only 2 participants were on second line at the start of the study period which is a small number, and 65 study participants had an ART start date that was after the last ART date.

In the study, 83% (n=929) were on TDF and 17% were on AZT (n=190). The median age was 36 years (IQR: 18 to 79 years), mean CD4 count 194 cells/µL (95% CI: 207.61 to 228.75) and the mean baseline eGFR was 123.56, (95% CI: 121.22 to 125.92). The eGFR was normal at baseline for 81.41% (911) of the study participants and it was mild in 181 (16.18%) and moderate in 27 (2.41%) of the participants.

Of note, is that none of the participants had severe GFR nor were in renal failure at during ART initiation (that is, failure due to sole HIV disease). This is similar and represents the demographics of the population on ART in Swaziland and is comparable to other African countries and the sub-Sahara region.[29][30][31]

5.2 Overall follow-up time

The median follow-up time was 6.72 months (IQR: 0.03 to 81.61) and total person-time was 15,579 person-months (95% CI: 6.79 to 9.63), which was within the mean follow-up range found in other studies (range: 0.3-9.7 years). [32],[20],[26]

5.3 Incidence rate of abnormal eGFR

This retrospective analysis showed that the overall incidence rate for abnormal eGFR (eGFR, Low) was 8.1 per 1000 person-months and the 15,579 total person-months for the study participants had a >10% change from the baseline eGFR. Conversely, this was noted when using the variable, change in creatinine. The eGFR is the more robust variable than creatinine. Both the eGFR and creatinine changes are suggestive of the documented nephrotoxic effects of TDF during follow-up time.[15][16][34]

The frequency of the occurrence of the outcome of interest (TDF-associated nephrotoxic adverse effect) was in 11.26% of the study participants. The frequency in this study is higher than the 10.8%, 8.16%, 6.3% and 2.4%, observed in other studies.[11][33][35][36] However, it still remains lower than what is documented is some studies which have demonstrated that tubular dysfunction with TDF occurs in 17–22% of Tenofovir-treated patients; as opposed to 6% and 12% of ART-treated or naive HIV patients, respectively.[37][34]

A Zambian study found that the point prevalence of renal dysfunction among HIV-positive adults exposed to TDF was 18.6% at 18 months follow-up. [29] However, a Zimbabwean study, noted the adverse events frequency of TDF at 39%.[36] In these studies, none of the study participants developed end-stage renal failure.

Our study further analysed the incidence rate by the exposure status, TDF and AZT. Notably, the TDF-associated nephrotoxicity was 9.44 per 1000 person-months (95% CI: 7.79 to 11.44) and 11022 total person-months. The incidence rate of abnormal renal function due to non-use of TDF (AZT) was 4.83 per 1000 person-months (95% CI: 3.18 to 7.33) and 4557 total person-month.

Our findings concur with the pool of evidence from other studies, that exposure to TDF is nephrotoxic, since the participants exposed to TDF showed a two-fold increased risk of an abnormal renal function during the follow-up period.[38],[39] A published editorial commentary affirms that TDF users with a baseline normal eGFR or mild kidney disease were 2-3 times more likely to progress to advanced kidney disease than their non-TDF counterparts.[30]

5.4 Risk factors associated with Tenofovir associated renal dysfunction

In this cohort the exposure to TDF had a significant association with the development of renal dysfunction, although there was no renal failure. This study also set out to determine the associated risk factors for TDF associated renal dysfunction in Swaziland, however there was no confirmed association between the assessed covariates of sex, age, WHO clinical staging, BMI and CD4 cell count and developing renal dysfunction (p-value>0.05).

Some studies have confirmed that in addition to the above listed covariates/risk factors, other predisposing factors include elevated baseline serum creatinine, pre-existing decrease in kidney function, concomitant nephrotoxic medications, gender, presence of hypertension, high BMI, low body weight, advanced age, use of TDF with microalbuminuria, as well as the duration of TDF treatment.[36][40][41]

The only factor associated with renal dysfunction in this study was TDF use which is similar to the one study conducted in Ghana.[42] This was evident in both the univariate and multivariate analyses.

5.5 Economic estimation of the cost of reversing the TDF associated renal dysfunction

The TDF-associated renal dysfunction or nephrotoxicity has a dire economic effect since patients under routine clinical monitoring need to undergo renal dialysis to recover from the toxic effects of TDF. [17][37][43] It is routinely documented in the patient registers in the dialysis units of the two hospitals (MGH and RFM), that patients can take from 3 days to 12 weeks to recover from the TDF nephrotoxic effects at a cost of Emalangeni (SZL) 1,500.00 or US Dollar (USD) 125.00 per dialysis session.

This cost is borne by the government whereas SZL20.00 or USD 1.60 is borne as an out-of-pocket expense by the patients towards hospital stay. Other dire financial consequences include the loss of household income while the patient recovers.

5.6 HIV and HBV co-infection

Although TDF is an effective and widely used treatment for Hepatitis B virus (HBV) infection, most cases of TDF-associated nephrotoxicity have been described in patients co-infected with HIV rather than sole HBV.[41] TDF is the drug of choice for the management of HBV and its use is expected to increase so will the cases of the associated renal dysfunction. New drugs indicated for the management of HIV and/or HBV with a less side-effect profile have been introduced in the market.

5.6.1 Tenofovir Alafenamide

Tenofovir Alafenamide (TAF) is a nucleotide reverse transcriptase inhibitor and the next-generation Tenofovir prodrug, with a distinctive metabolism intended to maximize antiviral potency and clinical safety. It is indicated for use in the treatment of HIV infection and chronic hepatitis B. TAF is more stable in plasma which results in higher intracellular levels leading to increased distribution to tissues of lymphatic origin when compared with Tenofovir.
The Tenofovir in TAF is actively transported from the blood into renal proximal tubule cells by anion transporters OAT1 and OAT3. The reduction in plasma exposures results in lower concentrations in proximal tubular cells and less nephrotoxicity. [44][45][46]

TAF has been demonstrated to have improved clinical efficacy, long-term greater antiviral efficacy, a higher barrier to resistance and an improved safety profile relative to TDF. Unlike TDF, which should be avoided or dose-adjusted in patients with renal dysfunction or estimated creatinine clearance (CrCl) < 80 mL/min, TAF-containing regimens appear to be safe and are FDA-approved for use in patients with estimated CrCl as low as 30 mL/min.[44][45][46]

However the evidence on safety and efficacy in pregnancy and TB-co-infection of TAF has prevented its inclusion into the WHO guidelines and the WHO Essential Medicines List. [45][47]
In a large randomized trial, tests done on bone and renal function, significantly favoured the switch to TAF. The trial showed that TAF has a better side-effect profile and proved to be more superior than TDF in maintaining virologic suppression in patients who switch to TAF. Hence, TAF was proved to be safe and well-tolerated.[46][48] On the contrary, a systematic

review did not confirm any difference in efficacy between the two formulations on HIV RNA suppression, clinical adverse events and discontinuation due to renal or bone side effects.[49]

CHAPTER 6: STRENGTHS AND WEAKNESSES (LIMITATIONS)

6.1 Strengths

6.1.1 To our knowledge, this is the first study to be conducted which has examined TDF-associated nephrotoxicity in Swaziland.

6.1.2 The availability of the electronic data medical clinical records from the national data base made it possible to have study participants with follow-up results over the study period of 2010-2015.

6.2.3 The study was conducted in two of the densely populated regions and two of the high volume, public serving hospitals in Swaziland using the nationally defined standard of care, therefore the results of the study are generalizable to the HIV-infected population on first line ART.

6.2 Limitations

6.2.1 The secondary data obtained from the National ART data base for MGH and RFM were incomplete. Hence, not all the key variables were completed e.g. co-morbidities, nor was the data base designed for operative research purposes.

6.2.2 HIV infection in itself causes renal pathology and differentiation of the aetiology was not done. The study excluded those patients who may have died before the study which led survivorship bias.

6.2.3 Despite the effort to minimise selection bias through the systemic sampling method, it can not be entirely excluded. Possible misclassification bias was minimised by blinding from exposure/outcome status.

6.2.4 Potential confounders include age, HIV disease and co-morbid chronic conditions (such as hypertension and diabetes mellitus), which can independently cause renal dysfunction.

6.2.5 Since this study was not a randomized clinical trial there were concerns with attribution. While the study was able to associate TDF with reduced renal function; it cannot, however, attribute that to TDF in isolation because there are other likely but unmeasured confounders (for example, pharmacokinetics, pharmacogenetics, host immune response differences).

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

Although TDF is safe and effective in HIV management, it is associated with 2 fold (times) increased risk of an abnormal renal function during the follow-up period compared to those who initiated on AZT. This is low compared to documented literature. Other variables (sex, age, WHO stage, BMI, CD4 cell count) showed no associated risk in the study participants.

Most worthwhile is for the Ministry of Health in Swaziland to start to intensify renal function monitoring of patients on TDF and start to consider and plan for the introduction of Tenofovir alafenamide (TAF) for the benefit of patients who experience the adverse-events which are associated with TDF. The high-resource countries in Asia have recommended that TDF should be discontinued early for patients at risk of developing TDF-associated nephrotoxicity and in patients with chronic kidney disease and replace it with its prodrug TAF.[40]

7.1 Tenofovir Alafenamide (TAF)

Tenofovir alafenamide (TAF), an oral prodrug of TDF, is now included as a component of several recommended first-line antiretroviral therapy regimens. These recommendations are based on data from comparative trials demonstrating that TAF-containing regimens are as effective in achieving or maintaining virologic suppression as TDF-containing regimens and is effective for HBV treatment but with more favorable effects on markers of renal and bone health. Patients being treated for HIV and or HBV would benefit from TAF and experience less or no side effects compared to TDF. However, the cost-effectiveness of replacing TDF with TAF containing regimen in resource limited settings remains unclear.

7.2 Database Redesign

The current Ministry of Health's HMIS national database has to be designed, be tailored for research purposes and be monitored for quality. This can be achieved by the engagement and involvement of the departments that have an interest, a mandate and vast experience with the management of national datasets and databases.

These include, but are not limited to the Strategic Information Unit, the Ministry of Information Communication and Technology as well the Royal Science and Technology Park.

7.3 Review of HIV Management Guidelines

Now that the burden of TDF-associated nephropathy has been revealed and quantified by this study and it supports the Ministry of Health's decision to continue to use TDF the HIV management. The SNAP working closely with the National Health Laboratory Services (NHLS) will need to actively collaborate, advocate and ensure nationwide access to laboratory monitoring services for all patients, particularly those on TDF, as well as to ensure that the forecasting and quantification of renal function tests laboratory reagents is done. This will assist and ensure accurate patient monitoring and appropriate management in all health care facilities in the country. It is imperative that routine monitoring of all patients using TDF should be performed.[41]

7.4 Further Research

There is a need to also conduct further studies using longitudinal data from clinical monitoring visits in order to examine the renal function over time while patients are on TDF, and to conduct a more comprehensive economic evaluation on the effects TDF associated renal dysfunction or nephrotoxicity. It would be beneficial to also conduct studies on the use of TDF both in HBV-infected non-HIV patients and in HIV/HBV co-infected patients, so as to add to the body of evidence both nationally and internationally.

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ANNEXES

Annex 1: Permission to access records approval

Permission to access Records / Files / Data base at the Mbabane Government Hospital

To: Chief Executive Officer/Information Officer
Mbabane Government Hospital
Dr P.S. Mahaliyana.

From: The Investigator
World Health Organization-Swaziland
Dr Sithembile Dlamini-Ngeketo

Re: **Permission to do research at Mbabane Government Hospital**

Dr Sithembile Dlamini-Ngeketo is a researcher working at the World Health Organization- Swaziland, TB/HIV/HEPATITIS Unit. I am requesting permission to conduct a study on the Mbabane Government Hospital grounds that involves access to patient records.

The request is lodged with you in terms of the requirements of the Promotion of Access to Information Act. No. 2 of 2000.

The title of the study is: The epidemiology of Tenofovir-associated renal failure in patients on antiretroviral triple regimen in Swaziland.
The researcher requests access to the following information:

Access to the clinical files, record book and the data base.

We intend to publish the findings of the study in a professional journal and/ or at professional meeting like symposia, congresses, or other meetings of such a nature.

We intend to protect the personal identity of the patients by assigning each patient a random code number.

We undertake not to proceed with the study until we have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Yours sincerely

Signature of the Principle Investigator

Permission to do the research study at this hospital and to access the information as requested is hereby approved.

Chief Executive Officer

Mbabane Government Hospital

Dr P.S. Mahaliyana.

Signature of the CEO

MBABANE GOVERNMENT HOSPITAL
SMO
12 OCT 2016
P.O. BOX 8
MBABANE
SWAZILAND

Hospital Official Stamp

Permission to access Records / Files / Data base at the Raleigh Fitkins Memorial (RFM)Hospital

To: Chief Executive Officer/Information Officer
Raleigh Fitkins Memorial Hospital
Dr RAYMOND BITCHONG

From: The Investigator
World Health Organization-Swaziland
Dr Sithembile Dlamini-Nqeketo

Re: **Permission to do research at Raleigh Fitkins Memorial (RFM) Hospital**

Dr Sithembile Dlamini-Nqeketo is a researcher working at the World Health Organization- Swaziland, TB/HIV/HEPATITIS Unit. I am requesting permission to conduct a study on the Raleigh Fitkins Memorial Hospital grounds that involves access to patient records.

The request is lodged with you in terms of the requirements of the Promotion of Access to Information Act. No. 2 of 2000.

The title of the study is: The epidemiology of Tenofovir-associated renal failure in patients on antiretroviral triple regimen in Swaziland.
The researcher requests access to the following information:

Access to the clinical files, record book and the data base.

We intend to publish the findings of the study in a professional journal and/ or at professional meeting like symposia, congresses, or other meetings of such a nature.

We intend to protect the personal identity of the patients by assigning each patient a random code number.

We undertake not to proceed with the study until we have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Yours sincerely

Signature of the Principle Investigator

Permission to do the research study at this hospital and to access the information as requested is hereby approved.

Chief Executive Officer

Raleigh Fitkins Memorial Hospital

Dr RAYMOND BITCHONG

Signature of the CEO

RALEIGH FITKIN MEMORIAL HOSPITAL
P.O. BOX 14 MANZINI SWAZILAND
SOUTHERN AFRICA

Hospital Official Stamp

Annex 2: Data collection form: TDF associated nephrotoxicity and risk factors

Study Site: …… ART Clinic/hospital: ……..

1. **Demographic variables**

1.1. Date of birth: ……..

1.2. Sex (X): Male…… Female……

2. **ART Eligibility criteria and Baseline Clinical Information**

2.1. Date of initiation of ART:……../……/……

2.2 **Clinical parameters**	**Result**
Patient BMI (Kg/Ht^2)	
CD4 cell count (cells/mm^3)	
WHO clinical staging(25) (at initiation) WHO clinical staging (Last visit)	
Listed Co-morbidities	
Baseline serum creatinine Date of baseline serum creatinine	
Follow-up serum creatinine Date of follow-up serum creatinine	
Baseline Renal status (Normal/ abnormal)	
Final Renal status (Normal/ abnormal)	
Date of last clinic visit	

2.3 Initial 1st line ART Regimen: ……

2.4 Second line ART Regimen …….

3. Management of TDF-associated nephrotoxicity:

3.1 Date of ARV substitution: ……..

3.2 Reason for ARV substitution: ……..……

3.3 Current ART Regimen ……

3.4 Last $CD4^{+}$ count (cells/mm^3)……..

Annex 3: Ethical approval reference no: 428/2016

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 28 August 2018.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

24/11/2016

Approval Certificate
New Application

Ethics Reference No.: 428/2016

Title: THE EPIDEMIOLOGY OF TENOFOVIR-ASSOCIATED RENAL FAILURE IN PATIENTS ON ANTIRETROVIRAL THERAPY IN SWAZILAND

Dear Dr Sithembile Dlamini-Nqeketo

The **New Application** as supported by documents specified in your cover letter dated 14/11/2016 for your research received on the 14/11/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 23/11/2016.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**428/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Level 4-60*

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

012 356 3084 deepeka.behari@up.ac.za / fhsethics@up.ac.za http://www.up.ac.za/healthethics
Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

Annex 4: Ethical approval reference no: 428/2016 for amendment of protocol

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

31/05/2018

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No: 428/2016

Title: THE EPIDEMIOLOGY OF TENOFOVIR-ASSOCIATED RENAL FAILURE IN PATIENTS ON ANTIRETROVIRAL THERAPY IN SWAZILAND

Dear Dr Sithembile Dlamini-Nqeketo

The **Amendment** as described in your documents specified in your cover letter dated 28/02/2018 received on 3/05/2018 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 30/05/2018.

Please note the following about your ethics amendment:
- Please remember to use your protocol number (**428/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committe may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics amendment is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers, MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60 / 61, 31 Bophelo Road, Gezina, Pretoria

Annex 5: WHO clinical staging of HIV disease in adults, adolescents and children

Annex 5. WHO clinical staging of HIV disease in adults, adolescents and children

Source: Adapted from *WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children.* Geneva, World Health Organization, 2007 (www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf).

Adults and adolescents[a]	Children
Clinical stage 1	
Asymptomatic Persistent generalized lymphadenopathy	Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2	
Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruption Fungal nail infections Seborrhoeic dermatitis	Unexplained persistent hepatosplenomegaly Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, sinusitis, tonsillitis) Herpes zoster Lineal gingival erythema Recurrent oral ulceration Papular pruritic eruption Fungal nail infections Extensive wart virus infection Extensive molluscum contagiosum Unexplained persistent parotid enlargement
Clinical stage 3	
Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhoea for longer than 1 month Unexplained persistent fever (intermittent or constant for longer than 1 month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 x 10^9/l) and/or chronic thrombocytopaenia (<50 x 10^9/l)	Unexplained moderate malnutrition[b] not adequately responding to standard therapy Unexplained persistent diarrhoea (14 days or more) Unexplained persistent fever (above 37.5°C, intermittent or constant, for longer than one 1 month) Persistent oral candidiasis (after first 6 weeks of life) Oral hairy leukoplakia Lymph node tuberculosis Pulmonary tuberculosis Severe recurrent bacterial pneumonia Acute necrotizing ulcerative gingivitis or periodontitis Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 x 10^9/l) or chronic thrombocytopaenia (<50 x 10^9/l)

Adults and adolescents[a]	Children
Clinical stage 3	
	Symptomatic lymphoid interstitial pneumonitis Chronic HIV-associated lung disease, including bronchiectasis
Clinical stage 4[c]	
HIV wasting syndrome	Unexplained severe wasting, stunting or severe malnutrition[d] not responding to standard therapy
Pneumocystis (jirovecii) pneumonia	*Pneumocystis (jirovecii) pneumonia*
Recurrent severe bacterial pneumonia	Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)
Chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)	Chronic herpes simplex infection (orolabial or cutaneous of more than 1 month's duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)	Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis	Extrapulmonary tuberculosis
Kaposi sarcoma	Kaposi sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)	Cytomegalovirus infection (retinitis or infection of other organs with onset at age more than 1 month)
Central nervous system toxoplasmosis	Central nervous system toxoplasmosis (after the neonatal period)
HIV encephalopathy	HIV encephalopathy
Extrapulmonary cryptococcosis, including meningitis	Extrapulmonary cryptococcosis, including meningitis
Disseminated nontuberculous mycobacterial infection	Disseminated nontuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy	Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis	Chronic cryptosporidiosis (with diarrhoea)
Chronic isosporiasis	Chronic isosporiasis
Disseminated mycosis (extrapulmonary histoplasmosis, coccidioidomycosis)	Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis)
Lymphoma (cerebral or B-cell non-Hodgkin)	Cerebral or B-cell non-Hodgkin lymphoma
Symptomatic HIV-associated nephropathy or cardiomyopathy	HIV-associated nephropathy or cardiomyopathy
Recurrent septicaemia (including nontyphoidal *Salmonella*)	
Invasive cervical carcinoma	
Atypical disseminated leishmaniasis	

[a] In the development of this table, adolescents were defined as 15 years or older. For those aged less than 15 years, the clinical staging for children should be used.

[b] For children younger than 5 years, moderate malnutrition is defined as weight-for-height <–2 z-score or mid-upper arm circumference ≥115 mm to <125 mm.

[c] Some additional specific conditions can be included in regional classifications, such as penicilliosis in Asia, HIV-associated rectovaginal fistula in southern Africa and reactivation of trypanosomiasis in Latin America.

[d] For children younger than 5 years of age, severe wasting is defined as weight-for-height <–3 z-score; stunting is defined as length-for-age/height-for-age <–2 z-score; and severe acute malnutrition is either weight for height <–3 z-score or mid-upper arm circumference <115 mm or the presence of oedema.