

Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir- containing regimens in Lesotho

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ABSTRACT

Title: Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing regimens in Lesotho

Tenofovir (TDF)-containing highly active antiretroviral therapy (HAART) regimens are the most preferred first-line regimens in the treatment of Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome (HIV/AIDS) in Lesotho. Although these regimens have proven efficacious, their use is associated with progressive deterioration of renal function. The benefits and risks associated with the use TDF-containing HAART regimens varies with determining factors such as sex, body weight, treatment duration and age at antiretroviral therapy initiation. The aim of this study was to evaluate the clinical outcomes [body weight and Cluster of differentiation-4 (CD4) cell count] and renal safety (serum creatinine concentration, creatinine clearance and estimated glomerular filtration rate (eGFR)) in HIV/AIDS patients taking TDF-containing HAART regimens in Lesotho.

An observational, descriptive, retrospective, longitudinal study design was implemented in Paballong HIV/AIDS care centre located in Berea district, Lesotho. Two hundred fifty-five participants were enrolled in the study (56.10% females, n=143); with the mean age of 39.76 ± 11.93 years and 59.00 ± 12.87 kg mean baseline body weight. Most participants were initiated antiretroviral treatment at World Health Organization (WHO) clinical stage II (69.80%, n=178). Most patients were initiated with TDF/Lamivudine (3TC)/Efavirenz (EFV) (84.70%, n=216) HAART regimen. The baseline mean CD4 cell count and serum creatinine concentration were 328.16 ± 167.25 cells/mm³ and 83.39 ± 37.50 µmol/l respectively. Seventy-three participants (28.60%) were on antiretroviral therapy for > 42 months.

The results for clinical outcomes analysis revealed that upon treatment initiation at any age, patients gained an estimated body weight of to 0.10 kg from baseline over one year of treatment period ($p < 0.05$). The female sex had greater weight gain, estimated at 2.49 kg over the treatment period, than males ($p < 0.05$). The results for CD4 cell count unveiled an estimated increase in CD4 cell count of 0.02 cells/mm³ in patients initiated on treatment at any age over one year treatment duration. In contrast to males, being female was associated with an estimated additional increase in CD4 cell count of 69.13 cells/mm³ over the treatment duration ($p = 0.02$). The results entail that clinical outcomes improve following treatment and females were at more advantage to experience better clinical improvements over treatment period than males.

The results for renal safety analysis from baseline levels over treatment duration showed an increase in serum creatinine concentration, estimated at 0.23 µmol/l at any age ($p = 0.004$). Females also had more elevations in serum creatinine concentration over males, estimated at

12.14 $\mu\text{mol/l}$ over treatment duration ($p<0.05$). Creatinine clearance results portrayed significant decline in glomerular filtration rate by 1.10 ml/min per day over treatment duration at any age ($p<0.05$). Although not statistically significant, females had an additional decline in glomerular filtration rate by 1.86 ml/min when compared to males ($p=0.30$). However, the body weight predicted statistically significant increase in glomerular filtration rate estimated at 1.49 ml/min over the treatment duration ($p<0.05$). The estimated glomerular filtration rate results contended that sex, age at antiretroviral therapy initiation and body weight are risk factors to developing renal toxicity over treatment duration. The estimated glomerular filtration rate declined significantly by 0.78 ml/min/ 1.73m^2 per day at any age ($p<0.05$) while females were significantly more compromised in renal function by 13.05 ml/min/ 1.73m^2 daily when compared to males ($p<0.05$). Although not statistically significant, body weight predicted a daily decline of 0.02 ml/min/ 1.73m^2 ($p=0.80$) over treatment duration. The results reveal an underlying renal insufficiency due to treatment and females appeared to be more at risk to developing kidney disease than males over treatment period.

In conclusion, clinical outcomes manifesting by weight gain and CD4 cell count elevation improve upon initiation of antiretroviral therapy at any age. Females are far more at advantage to experience better clinical outcomes than males over the treatment duration. The renal function is progressively deteriorated following initiation of antiretroviral therapy at any age. Females experience more renal compromise than males.

Keywords: Tenofovir-containing HAART regimens, clinical outcomes, CD4 cell count, body weight, renal safety, creatinine clearance, eGFR, Pabalong HIV/AIDS care centre, Lesotho

OPSOMMING

Titel: Kliniese uitkomst en nierveiligheid in MIV/Vigs-pasiënte op regimens wat tenofovir bevat in Lesotho

HAART-regimens wat tenofovir (TDF) bevat is die voorkeurregimens vir eerste gebruik in die behandeling van MIV/Vigs in Lesotho. Alhoewel hierdie regimens effektief bewys is, word hulle gebruik geassosieer met progressiewe agteruitgang van nierfunksie. Die voordele en risiko's geassosieer met die gebruik van HAART-regimens wat TDF bevat, wissel afhangend van bepalende faktore soos geslag, liggaamsgewig, duur van behandeling en ouderdom tydens aanvang van antiretrovirale terapie. Die doel van hierdie studie was om die kliniese uitkomst (liggaamsgewig en CD4-seltelling) en nierveiligheid (serumkreatinienkonsentrasie, kreatinienopruiming en geskatte glomerulêre filtrasiekoers (gGFK)) in MIV/Vigs-pasiënte in Lesotho wat TDF-bevattende HAART-regimens neem, te ondersoek.

'n Waarnemende, beskrywende, retrospektiewe, longitudinale studie-ontwerp is geïmplementeer by Paballong HIV/AIDS Care Centre, geleë in die Berea-distrik, Lesotho. Twee honderd, vyf en vyftig deelnemers is ingeskryf vir die studie (56,0% vroulik, $n=143$); met 'n gemiddelde ouderdom van 39.76 ± 11.93 jaar en 59.00 ± 12.87 kg gemiddelde basislynliggaamsgewig. Die meeste deelnemers het begin met antiretrovirale behandeling by die WGO se kliniese stadium II (69,80%, $n=178$). Die meeste pasiënte het begin met die TDF/3TC/EFV-(84,70%, $n=216$) HAART-regimen. Die CD4-seltelling- en serumkreatinienbasislyn was onderskeidelik $328,16 \pm 167.25$ selle/ mm^3 en $83,39 \pm 37.50$ $\mu\text{mol/l}$. Drie en sewentig deelnemers (28,60%) was op antiretrovirale terapie vir > 42 maande.

Die uitslae vir kliniese uitkoms-ontledings het getoon dat, met die aanvang van behandeling op enige ouderdom, pasiënte'n geskatte liggaamsgewig toename van tot 0,10 kg getoon het vanaf die basislyn, gedurende die behandelingstydperk ($p < 0.05$). Vroulike pasiënte het meer gewig opgetel, geskat op 2,49 kg gedurende die behandelingstydperk as mans ($p < 0.05$). Die uitslae van CD4-seltelling het 'n toename in seltelling van 0,02 selle/ mm^3 in pasiënte wat op enige ouderdom deur die loop van die behandelingstydperk die behandeling begin gebruik het, getoon. In vergelyking met mans was daar by vroue 'n geskatte ekstra toename in CD4-seltelling van 69,13 cells/ mm^3 gedurende die behandelingstydperk ($p = 0.02$).

Die uitslae van nierveiligheidsontledings vanaf basislynvlakke gedurende die behandelingstydperk het 'n styging getoon in serumkreatinienkonsentrasie, geskat op 0,23 $\mu\text{mol/l}$ per dag vir enige ouderdom ($p = 0.004$). Vroue het ook meer stygings in serumkreatinienkonsentrasies getoon as mans, geskat op 12,14 $\mu\text{mol/l}$ daaglik gedurende die behandelingstydperk ($p < 0.05$). Kreatinienopruimingsresultate het 'n opvallende afname getoon in

glomerulêrefiltrasiekoers teen 1,10 ml/min gedurende die behandelingstydperk vir enige ouderdom ($p < 0.05$). Alhoewel dit nie statisties betekenisvol is nie, het vroue 'n addisionele afname in glomerulêrefiltrasiekoers gehad van 1,86 ml/min in vergelyking met mans ($p = 0.30$). Die liggaamsgewig het egter 'n statisties-betekenisvolle toename in glomerulêre filtrasiekoers voorspel, geskat op 1,49 ml/min gedurende die behandelingstydperk ($p < 0.05$). Die gGFK-resultate het aangedui dat geslag en ouderdom waarop antiretrovirale terapie begin is, tesame met die liggaamsgewig, risikofaktore is by die ontwikkeling van nierontoereikendheid gedurende die behandelingstydperk. Die gGFK het aansienlik afgeneem met 0,78 ml/min/1,73 m² in enige ouderdom ($p < 0.05$) terwyl vroue aansienlik meer in gevaar gestel is wat betref nierfunksie met 13,05 ml/min/1,73 m² in vergelyking met mans ($p < 0.05$). Alhoewel dit nie statisties betekenisvol is nie, is 'n daling in liggaamsgewig voorspel van 0,02 ml/min/1,73 m² ($p = 0.80$) gedurende die behandelingstydperk.

Ten slotte verbeter kliniese uitkomst wat, manifesteer as gewigstoename en styging in CD4-seltelling wanneer antiretrovirale terapie op enige ouderdom in aanvang neem. Vroue het 'n baie groter voorsprong bo mans om beter kliniese uitkomst te hê gedurende die behandelingstydperk. Die nierfunksie neem toenemend af namate daar op enige ouderdom begin is met antiretrovirale terapie. Vroue het 'n groter kans om nierskade op te te doen as mans.

Slutelwoorde: HAART-regimens wat tenofovir bevat, kliniese uitkomst, CD4-seltelling, liggaamsgewig, nierveiligheid, kreatinienopruiming, gGFK, Pabalong HIV/AIDS Care Centre, Lesotho.

LIST OF ACRONYMS

Acronyms relating to drug treatment

3TC	Lamivudine
ABC	Abacavir
ADRs	Adverse drug reactions
AZT	Zidovudine
d4T	Stavudine
EFV	Efavirenz
ETV	Entecavir
FIs	Fusion inhibitors
FTC	Emtricitabine
HAART	Highly active antiretroviral therapy
IDV	Indinavir
INTIs	Integrase inhibitors
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI/ NtRTI	Nucleoside/ nucleotide reverse transcriptase inhibitor
NSAIDs	Non-steroidal anti-inflammatory drugs
NVP	Nevirapine
PEP	Post-exposure prophylaxis
PIs	Protease inhibitors
TDF	Tenofovir
TasP	Treatment as prevention
PMTCT	Prevention of mother-to-child transmission
PrEP®	Pre-exposure prophylaxis

Acronyms relating to diseases, diagnosis and response to treatment

BMI	Body mass index
CCR5	Carbon-carbon chemokine receptor type 5
CD4/ CD ₈	Cluster of differentiation-4/ cluster of differentiation-8
CKD-EPI	Chronic kidney disease epidemiology collaboration
CXCR4	Carbon-X-carbon chemokine receptor type 4
eGFR	Estimated glomerular filtration rate
HIV/AIDS	Human immunodeficiency virus/ acquired immune deficiency syndrome
MDRD	Modified diet for renal disease
MRP4	Multidrug resistant protein-4
MSM	Men having sex with men

hOAT1	human organic acid transporter-1
PCP	<i>Pneumocystis carinii</i> pneumonia
HRQoL	Health related quality of life
RIFLE	Risk, injury, failure, loss and end-stage kidney
DNA/ RNA/ mRNA	Deoxyribonucleic acid/ ribonucleic acid/ messenger ribonucleic acid
SOPs	Standard operating procedures
TB	Tuberculosis
VCT	Voluntary counselling and testing

Acronyms relating to institutions

BCM	Baylor College of Medicine
CTRI	Clinical and Translational Research Institute
DHHS	Department of Health and Human Services
FDA	Food and Drug Administration
HREC	Health and Research Ethics Committee
IBM® SPSS®	International Business Machines Statistical Package for Social Sciences
K-DOQI	Kidney- Disease Outcomes Quality Initiatives
LMDPC	Lesotho Medical, Dental and Pharmacy Council
LNC	Lesotho Nursing Council
MOH/MOHSW	Ministry of Health/ Ministry of Health and Social Welfare
MSH	Management Sciences for Health
MUSA	Medicine Usage in South Africa
NIEHS	National Institute of Environmental Health Sciences
NWU	North-West University
U.S. PEPFAR	United States President's Emergency Plan for AIDS Relief
UNAIDS	United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
UNFPA	United Nations Fund for Population Activities
USA	United States of America
WHO	World Health Organization

Acronyms relating to statistics

CI	confidence interval
IQR	interquartile range
SD	standard deviation

LIST OF DEFINITIONS

Adult

According to Lesotho Penal Code Act, 2010 (6 of 2012) an adult is any person who has attained the age of 18 years

Baseline level

Findings and investigations relating to the beginning, before exposure or intervention (Merriam-Webster dictionary, 2016)

Clinical outcome

Broadly agreed measurable changes in health or quality of life resulting from therapeutic interventions (Hinds & Watson, 2008:16)

Creatinine clearance

Volume of blood cleared-off creatinine per unit time and a measure for approximating glomerular filtration rate (Harvard Medical School, 2016)

End-time

Time for the latest observation made for the patient (United Nations Children's Fund (UNICEF), 2014:2)

Estimated glomerular filtration rate

Estimated volume of blood filtered (by glomeruli) through the kidneys over a given period of time. A measure of level of kidney function and determinant of stage of kidney disease (Harvard Medical School, 2016)

Informed consent

Written agreement between the researcher and a patient (potential study participant) after being duly informed about the nature of the study, its significance, implications and risks associated with participation. The agreement is signed, dated and witnessed as an indicator that the patient volunteers to participate in the study as a study participant (Trochim, 2006)

Mediator

Someone who communicates about the study to potential study participants on behalf of the researcher (Merriam-Webster dictionary, 2016)

Nurse midwife

Licensed health practitioner educated in two disciplines of nursing and midwifery (WHO, 2012:4)

Patient

Someone who is under medical treatment (WHO, 2011)

Pharmacist

According to Medical, Dental and Pharmacy Order, 1970 (13 of 1970) a pharmacist is a person registered as such under the order.

Pharmacokinetic enhancer

A drug used to boost the effectiveness of another drug by interfering its breakdown and hence allowing the drug to remain in the body longer at higher concentration (DHHS, 2015:140)

Pharmacovigilance

The science and activity relating to detection, assessment, understanding and prevention of adverse drug reactions (WHO, 2006:21)

Primary health care facility

Facility that provides essential health care based on scientifically sound and socially acceptable methods and accessible to individuals and families in a community (Medical Subject Headings, 1995)

Prodrug

A chemical substance that is without pharmacological activity against a designated physiological target, but is metabolically converted into a drug with desired activity (de Montellano, 2013:213-228)

Regression analysis

Statistical analysis used to model the relationship between the dependent variable and one or more independent variables (Medical Subject Headings, 1980)

Renal safety

A judgment of the acceptability of the risk associated with a medical treatment to the kidneys (Harvard Medical School, 2016). For the purpose of this study the words “renal safety” and “renal toxicity” are used synonymously.

Risk

A measure of the probability and severity of harm to human health (WHO, 2011)

Standard Operating Procedures

Rules and procedures for performing an activity (National Institute of Environmental Health Sciences (NIEHS, 2015)

Tenofovir-containing regimens

A drug treatment containing three or more antiretroviral drugs that include tenofovir (DHHS, 2016)

Time-point

A specific point of time in a study whereby observation(s) is/are made (Trochim, 2006)

Treatment duration

Time course of treatment from the beginning of treatment to the latest treatment observation (English definition dictionary, 2016). For the purpose of this study the words “treatment duration” and “treatment period” are used synonymously.

MATHEMATICAL UNITS AND SIGNS

<	less than
=	equal to
>	greater than
$\leq$	less than or equal to
$\geq$	greater than or equal to
%	per cent
$\pm$	plus or minus
+	plus
*	multiply by
$\mu\text{mol/l}$	micromole per litre
cells/mm^3	cells per cubic millimetre
copies/ml	copies per millilitre
kg	kilogram
kg/m^2	kilograms per square metre
$\log_{10}$	common logarithm to base 10
ml/min	millilitres per minute
min/min/1.73m^2	millilitres per minute per 1.73 square metre
ml/kg/hr	millilitres per kilogram per hour

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CHAPTER 1: INTRODUCTION AND RESEARCH METHODOLOGY

1.1 Introduction

Tenofovir (TDF) inclusion in highly active antiretroviral therapy (HAART) regimens in the treatment of Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS) has clinically shown to have an excellent efficacy and safety outcomes when compared with HAART regimens containing other first-line antiretroviral drugs, such as abacavir (ABC), stavudine (d4T) and zidovudine (AZT) (Gallant *et al.*, 2004:200; Gallant *et al.*, 2006:259). Sax *et al.* (2009:5) have compared the outcomes of two antiretroviral regimens consisting of TDF-emtricitabine (FTC) and ABC-lamivudine (3TC) initiated as treatment for HIV-1 infection. The results of this partially blinded randomised study revealed that virologic failures were less common in the TDF-FTC group when compared to the ABC-3TC group; a comparison done according to criteria defined for both early and late virologic failure. The immunologic outcomes in terms of CD4 cell count were found similar in both regimen groups (Sax *et al.*, 2009:5).

Although TDF use has been associated with acceptable safety, several studies have reported a rare manifestation of renal disease in HAART regimens that include TDF (Chua *et al.*, 2012:1; Cooper *et al.*, 2010:358; Crum-Cianflone *et al.*, 2010:358; Johnson *et al.*, 2012:1; Kalyesubula & Perazella, 2011:3; Sadre *et al.*, 2012:18308). Crum-Cianflone *et al.* (2010:358) and Milinkovic *et al.* (2008:1) described the occurrence of the renal manifestation attributable to TDF being highly prevalent in patients with pre-existing renal disease, those whose HIV/AIDS is poorly controlled (low CD4 cell count), those who have been on TDF regimen for a long time, the elderly and females. Bygrave *et al.* (2011b:4) have consequently recommended continued and periodical investigation of the change in renal function in patients taking TDF-based antiretroviral regimens, although close monitoring and early detection of renal disease are difficult in resource-constrained settings such as Lesotho. According to Hagos and Wolf (2010:2056) TDF causes renal insufficiency manifesting by renal toxicity, proteinuria and ultimately renal failure.

The information above briefly describes the benefits as well as the risks of using TDF-containing HAART regimens in the treatment of HIV/AIDS. Therefore, the study evaluated the clinical outcomes and renal safety of HIV/AIDS patients being treated with TDF-containing HAART regimens.

In this chapter the background, problem statement, research aim and objectives, and research methodology will be discussed, followed by a brief discussion of the ethical consideration and a chapter division.

1.2 Background of the study

The outcomes of antiretroviral therapy are assessed by clinical assessment and laboratory monitoring (World Health Organization (WHO), 2016c:127). The Lesotho Ministry of Health (MOH) (2013:37) guidelines entailed that antiretroviral therapy clinical outcomes are assessed by changes in body weight, ability to perform daily tasks, response to signs and symptoms of HIV/AIDS and incidence of opportunistic diseases. According to the WHO (2000:1) laboratory monitoring is assessed by means of plasma viral suppression using plasma viral load test and immune system recovery by CD4 cell count. The plasma viral load is expressed as the number of viral ribonucleic acid (RNA) copies per millilitre of plasma. The main goal of antiretroviral therapy is to suppress plasma viral load below detectable limits and to maintain the undetectable levels (Pasternak *et al.*, 2013:1). According to Lesotho MOH (2016a:49) a plasma viral load of < 40 copies/ml is regarded as undetectable levels. Lima *et al.* (2009:195) have supported a change in CD4 cell count being useful in the assessment of antiretroviral therapy-mediated immunological response.

Laurent *et al.* (2011:832) conducted an open-label, non-inferiority and randomised clinical trial to assess the effectiveness and safety of clinical monitoring alone versus both laboratory and clinical monitoring. The trial revealed a small difference between these strategies. The authors of this trial suggested that clinical monitoring alone could be used temporarily to expand ART care in resource-limited settings. In settings of available resources, Sawe and McIntyre (2009:463) regarded viral testing as the gold standard to monitor ART. The clinical and immunological monitoring still provide benefits in resource-constrained settings nonetheless the WHO (2000:1) indicated that laboratory monitoring in ART focuses on markers of efficacy and toxicity of antiretroviral drugs.

Kalyesubula and Perazella (2011:7) found that similar to other medical therapies, antiretroviral therapy is associated with short- and long-term toxicities, including those affecting the kidneys. Many drugs including some antiretroviral agents are known to have a high nephrotoxicity profile, accounting for approximately 66% prevalence in the elderly population (people aged > 60 years) as compared to 39% prevalence in the younger population (Kohli *et al.*, 2000:215). Hagos and Wolf (2010:2056) mentioned that TDF causes renal insufficiency leading to renal failure. Gara *et al.* (2012:5) observed that renal tubular toxicity that occurs in patients taking TDF-containing HAART regimens is partially reversible when switching from TDF to entecavir (ETV) within two years of follow-up treatment. Reust (2011:1448) outlined that protease inhibitors such as indinavir and atazanavir have also been implicated in renal insufficiency manifesting by nephrolithiasis.

In a meta-analysis of eight studies, Neugarten *et al.* (2000:326) showed that the male sex is associated with rapid and progressively worsening renal outcomes, especially in patients with

pre-existing chronic renal failure. In a cross-sectional retrospective study that determined the prevalence of TDF-induced nephrotoxicity among HIV/AIDS patients, 717 patients with median age of 41 years (92% males) participated (Crum-Cianflone, 2010:358). Three percent of these patients had renal toxicity attributable to older age (odds ratio 2.0 per decade increase), low CD4 count (< 200 cells/mm³) and long-term treatment (odds ratio 1.5 on annual use). Again, 50% of those on TDF experienced a reduction in glomerular filtration rate within two years of treatment. Among those with glomerular filtration rate reductions, the female sex and low CD4 cell count were implicated.

The information above suggests that age, sex and antiretroviral agents, such as TDF and protease inhibitors, influence kidney function.

The HAART regimen that includes three or more antiretroviral drugs that are active against the HIV infection has revolutionised the management of HIV/AIDS. This therapy has been attributed to significant reductions in morbidities and mortalities associated with HIV/AIDS worldwide (Kalyesubula & Perazella, 2011:1). According to WHO (2010:20) the first-line antiretroviral regimen should consist of one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) and two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (one of NRTI should either be TDF, AZT, d4T or ABC). In Lesotho, TDF was introduced as a first-line drug in December 2010, after phasing out d4T due to poor toxicity profile (Johnson *et al.*, 2012:1; Ministry of Health and Social Welfare (MOHSW), 2010: ix; WHO, 2010:39).

Kim and Moon (2012:268) evaluated renal function through blood tests, including blood urea nitrogen, serum creatinine concentration, glomerular filtration rate and creatinine clearance. Glomerular filtration rate is measured as the plasma clearance of filtration marker and is regarded as the best overall determinant of kidney function (Stevens *et al.*, 2006:2473). Glomerular filtration rate measurements are based on the renal clearance of either endogenous or exogenous marker from plasma (Sirwal *et al.*, 2004:121). The ideal marker should be freely filtered through the glomerulus and not secreted or reabsorbed by the renal tubules, and it should be eliminated exclusively by kidneys (Sirwal *et al.*, 2004:121). Traditionally, a single plasma creatinine measurement was used to determine the glomerular function, diagnosis and staging of chronic kidney disease (Florkowski & Chew-Harris, 2011:75). Inadequacies of this single serum creatinine measurement led to the recommendation by the National Kidney Foundation Disease Outcomes Quality Initiative (K-DOQI) to use prediction equations that estimate glomerular filtration rate based on serum creatinine concentration (Florkowski & Chew-Harris, 2011:75).

Woodward *et al.* (2009:483) outlined that the renal proximal tubule is the major site for TDF toxicity since this is an area of a nephron where the excretion by secretion of the drug takes place. The secretion takes place here due to the expression of membrane transporters called human organic

acid transporter-1 (hOAT-1) and multidrug resistance protein-4 (MRP4) that excrete TDF by secretion (Hagos & Wolf, 2010:2065; Kohler *et al.*, 2009:5). Tenofovir is actively secreted at this site and any impairment or delayed elimination of this drug may lead to accumulation in the tubular cell. The drug accumulation disrupts mitochondrial biogenesis, leading to tubular toxicity. Several studies associated TDF renal toxicity with elevated serum creatinine concentration, hypophosphatemia and hypokalaemia (Chua *et al.*, 2012:1; Johnson *et al.*, 2012:1; Kalyesubula & Perazella, 2011:3; Pontrelli *et al.*, 2012:1). Kohler *et al.* (2009:5) found that TDF-treated mice exhibited sparse and irregularly shaped mitochondria.

The purpose of the present study was to evaluate the clinical outcomes relative to changes in body weight, CD4 cell count and plasma viral load; and the renal safety according to changes in serum creatinine concentration, creatinine clearance and estimated glomerular filtration rate (eGFR) in patients taking TDF-containing HAART regimens in relation to their treatment duration, baseline body weight, sex and age at antiretroviral initiation.

The study was conducted at the primary health centre called Paballong HIV/AIDS care centre found in Berea district, Lesotho. Primary health care facilities in Lesotho provide voluntary counselling and testing (VCT), antiretroviral therapy services, treatment for opportunistic diseases, counselling of patients and caregivers of HIV/AIDS patients. The aim of the study is to evaluate the clinical outcomes and renal safety in these patients so that relationships in clinical outcomes (beneficial) and renal toxicity (harmful) can be evaluated. Appropriate monitoring procedures can be made from these relationships in order to optimise beneficial outcomes and arrest or reverse progressive deteriorations in renal function and improve quality of life in HIV/AIDS patients treated with TDF-containing HAART regimens.

The study is justified because in Lesotho, TDF is one of the first-line drugs for the treatment of HIV/AIDS and most adult patients are initiated on a TDF-containing HAART regimen (MOHSW, 2010:51). There is further a need for routine clinical and laboratory monitoring of the effectiveness and safety of TDF-containing HAART regimens among these patients, which may attract more laboratory costs to the country. The results of the study will guide the use of TDF-containing HAART regimens in relation to the duration of treatment, baseline body weight, sex and age of patients at antiretroviral initiation.

1.3 Problem statement

Brennan *et al.* (2013:10) found that the use of TDF-containing HAART regimens is associated with improved clinical outcomes characterised by reduced single-drug substitutions, improved treatment durability and less toxicity. However, Crum-Cianflone *et al.* (2010:358) contended that a significant decline in renal function has been found with the use of TDF. This deteriorating renal function was associated with female sex, older age, low CD4 count and duration of TDF use.

In India, Cooper *et al.* (2010:502) conducted a systematic review and meta-analysis study of 17 studies in which nine of them were randomised clinical trials. The results of this study revealed a significantly greater reduction in renal function among patients who received TDF; accounting for a <2% prevalence and greater risk of developing acute renal failure when compared to control subjects (risk difference = 0.7%). Later in 2012 in India, Sadre *et al.* (2012:18308) found that the prevalence of TDF-induced acute kidney injury was 4.88%, which was higher (twice higher) than 0.5%-2.5% which was previously reported by Cooper *et al.* (2010:502). In Sadre *et al.* study, the prevalence was attributed to lower body weight, lower baseline creatinine clearance, advanced HIV/AIDS disease and the prevalence of co-morbidities.

The studies above have guided the identification of relevant parameters that contribute to the effectiveness and safety of TDF-containing HAART regimens. In the present study, the clinical outcomes in terms of changes in body weight, CD4 cell count and plasma viral load; renal safety relative to changes in serum creatinine concentration, creatinine clearance and eGFR were evaluated in patients taking TDF-containing HAART regimens with respect to their treatment duration, baseline body weight, sex and age at antiretroviral initiation.

1.4 Research questions

The following research questions were derived in order to address the research problem:

- Do TDF-containing HAART regimens improve clinical outcomes as determined by changes in body weight, CD4 cell count and plasma viral load in relation to patients' treatment duration, sex and age at antiretroviral initiation?
- Is there a relationship between the use of TDF-containing HAART regimens and renal decline manifesting by elevated serum creatinine concentration, decreased creatinine clearance and eGFR in relation to patients' treatment duration, baseline body weight, sex and age at antiretroviral initiation?

1.5 Research aim and specific objectives

The research aim and specific research objectives of the literature review and empirical study will be discussed below.

1.5.1 Research aim

The general aim of the study was to evaluate the clinical outcomes and renal safety in patients taking TDF-containing HAART regimens in Lesotho in relation to patients' baseline body weight, sex and age at antiretroviral initiation over treatment duration.

1.5.2 Specific objectives of the literature review

The specific research objectives of the literature review were the following:

- To describe therapeutic mechanism of action of TDF in the treatment of HIV/AIDS;
- To evaluate the therapeutic response to TDF-containing HAART regimens;
- To discuss conventional measures of the clinical outcomes in HIV/AIDS treatment;
- To describe renal toxicological mechanisms of action of TDF in the treatment of HIV/AIDS;
- To evaluate renal toxicological response to TDF-containing HAART regimens;
- To discuss conventional measures of renal safety in patients taking TDF-containing HAART regimens;
- To discuss patient factors influencing both the beneficial and harmful outcomes of TDF-containing HAART regimens.

1.5.3 Specific objectives of the empirical investigation

The specific research objectives of the empirical investigation were the following:

- To determine the change in body weight, CD4 cell count and plasma viral load in patients taking TDF-containing HAART regimens in relation to baseline body weight, sex and age at antiretroviral initiation over treatment duration;
- To determine renal safety in terms of change in serum creatinine concentration, creatinine clearance and eGFR in patients taking TDF-containing HAART regimens in relation to baseline body weight, sex and age at antiretroviral initiation over treatment duration.

1.6 Research methodology

The research methodology of the study consisted of two phases namely literature review and empirical investigation. These phases are discussed below.

1.6.1 Literature review

Taylor (2014) defined literature review as a compilation of published information about a topic by accredited scholars and researchers. Published information about a selected area is compiled to provide an evaluative report of studies in the literature that have been found to support the description, summary and clarification of the research area in question (Aldous *et al.*, 2011:18). Boote and Beile (2005:3) contended that a researcher cannot execute significant research if not having understood the literature in the field engaged, and therefore the literature review should be conducted to offer an understanding of what was done before, including the strengths and weaknesses of existing findings. The literature review of the present study is provided in Chapter 2 of this dissertation.

1.6.2 Empirical investigation

The WHO (2001:1) defined an empirical investigation as a research method where observations are made and data are collected on the health-related phenomena of interest in a defined population. In the present study, the following elements of empirical investigation were covered:

- Study design;
- Study site;
- Data source;
- Target population;
- Study population and sampling (including the inclusion and exclusion criteria);
- Recruitment of participants;
- Data collection.

1.6.2.1 Study design

The study followed a descriptive, observational, longitudinal retrospective design that evaluated the clinical outcomes and renal toxicity of TDF-containing HAART regimens. In a descriptive study, the information is gathered without changing the environment and involves either one-time interaction with a group of individuals (cross-sectional) or following individuals over time (longitudinal). According to Pearce (2012:396), a longitudinal study is a correlation study design that utilises repeated observations of the study participants over time. The study measures the observations from the same study participants at different time points. Generally, the study design allows for the assessment of both categorical and continuous data outcomes measured over the exposure period.

The design fitted the study well, as the investigation was based on routinely collected data that presented both past and present properties of the HAART combination in question (Waning & Montagne, 2001:47). Retrospective data were collected from individual patient's medical records kept at the study site.

1.6.2.2 Study site

The study was conducted at a primary health care facility called Paballong HIV/AIDS care centre located in Berea district in Lesotho. This facility is situated in the central area of Berea plateau called Sefikeng where people living in villages on the plateau. In 2014, 600 patients were served by the centre with antiretroviral therapy. Primary health care facilities in Lesotho presently provide voluntary counselling and testing (VCT), antiretroviral therapy services, treatment for opportunistic diseases and counselling of patients and the caregivers for HIV/AIDS patients. These facilities are the lowest public sector health care levels that bring health closest to individuals and family members living in a community. Since they are the first level of care where most patients would be encountered, the initiation of first-line HAART is usually done at these facilities. In Lesotho, patients on chronic treatment are served by primary health care facilities on different regularly intervals ranging from one- to three-month check-ups depending on control of their medical conditions. This means that patients that are well controlled would require less monitoring and hence be served at longer time intervals.

1.6.2.3 Data source

The data were obtained from patients' medical records kept at the facility. This data archive consisted of files of each patient who was served by the facility for every clinical encounter made. The data included information on the following:

- Patient demographic information such as patients' age, sex, village, contact details, caregiver's name and contact details;
- Counselling sessions done for pre- and post-HIV testing;
- Baseline clinical and laboratory data on physical examination, CD4 cell count, viral load, body weight, serum creatinine concentration, haemoglobin concentration, acid fast bacilli test for tuberculosis (TB);
- HAART regimen(s) initiated and switched with their dates;
- Prophylactic treatment for opportunistic infections such as pneumonia (caused by *Pneumocystis carinii*) and TB (caused by *Mycobacterium tuberculosis*);
- Treatment of opportunistic infections of HIV/AIDS;
- Pill count adherence measurements done;
- Medication adherence counselling sessions;
- Follow-up clinical and laboratory monitoring done at routine time intervals (mostly at 6-month interval).

The data had been generated by responsible personnel in the facility and all the information was gathered without the researcher's involvement and not for any specific research interest and purpose. Therefore, not all the information kept by the facility was used by the researcher; a predesigned data collection tool (refer to Appendix IV) was used to capture information that was relevant to the study in question.

The information captured using the data collection tool included the following:

- Patients' age, date of birth, sex and WHO HIV/AIDS clinical stage;
- Baseline information on CD4 cell count, serum creatinine, body weight, and corresponding dates taken;
- Antiretroviral therapy regimens initiated and their durations;
- Change of regimens, their dates and reason(s) for change;
- Follow-up information and dates taken CD4 cell count, serum creatinine and body weight.

1.6.2.4 Target population

The target population included all HIV/AIDS patients who were served by the centre since 2014 and backwards. The population of patients on antiretroviral therapy was estimated at 600 patients

in the year 2014. More than 50% of these patients were initiated on TDF-containing HAART regimens. According to Lesotho HIV/AIDS treatment guidelines, TDF is a first-line drug and many adult patients are initiated on a TDF-based regimen after phasing out d4T due to toxicity profile (MOHSW, 2010a:51). Also, TDF is given to people who are aged ≥ 12 years and weigh ≥ 35 kg (MOHSW, 2010a:53). However, the present study focused on patients aged ≥ 18 years.

1.6.2.5 Study population and sampling

The study population entailed both HIV-infected adult males and females aged ≥ 18 years who weighed ≥ 35 kg at baseline and attended the study site. Most of these patients resided in the villages that surround the facility and neither ethnicity nor language characteristics were used in selecting the participants. The study sample was obtained by taking a convenience sample from the HIV/AIDS patients served by the centre who complied with the inclusion and exclusion criteria outlined in section **1.6.2.5.1** and **1.6.2.5.2**, respectively. According to Dornyei and Csizer (2011:81), in a convenience sampling, the study population is selected only when they meet certain practical criteria such as geographical proximity, possession of key features that are related to the purpose of the study and voluntary participation.

The researcher accepted a 5% margin of error in this study at a 95% confidence level when calculating the sample size. The response distribution was assigned 50% to give the largest sample size. Therefore, using the Raosoft® sample size calculator, the recommended sample size was 235 (from 600 patients), which was rounded up to 250 potential participants who were needed to conduct the study (MaCorr Research®, 2014; Raosoft®, 2004).

However, upon data collection, the final sample size of 255 participants was obtained due to guidelines of power analysis in determining sample size.

1.6.2.5.1 Inclusion criteria

The study population included all targeted HIV/AIDS patients who were served by the facility and complied with the inclusion criteria was below:

- Patients who were on TDF/3TC/nevirapine (NVP) or TDF/3TC/efavirenz (EFV) regimens. These are the preferred TDF-containing HAART regimens used in Lesotho (MOH, 2016a:1);
- Patients who were initiated on non-TDF-containing HAART regimens but were later switched to TDF-containing regimens;
- Patients who were initiated on these TDF-containing HAART regimens since the year 2014 and backwards; meaning that different patients would have different treatment durations depending on the time they were initiated or switched on TDF-containing antiretroviral therapy.

- Patients who weighed $\geq 35\text{kg}$ prior to TDF-containing regimens. Lesotho HIV/AIDS treatment guidelines outline that TDF-containing HAART regimens are initiated to patients weighing $\geq 35\text{kg}$;
- Patients who were aged ≥ 18 years. According to Lesotho Penal Code Act, 2010 (6 of 2012) an adult is any person who has attained the age of 18 years. Therefore, the informed consent form was issued to these adult patients to provide permission to use their information.

Patients of the age and body weight ranges mentioned in the inclusion criteria do qualify for TDF-containing HAART regimens according to the Lesotho HIV/AIDS treatment guidelines (MOHSW, 2010:51).

1.6.2.5.2 Exclusion criteria

The study excluded patients with one or more of the factors below:

- Patients who had co-morbidities such as hypertension and diabetes mellitus were excluded because these diseases have been shown to affect renal function negatively; as does TDF and their inclusion would mislead the results of the study as the focus was only on TDF-containing HAART regimens (American Kidney Fund, 2010:3; Bhatti & Alkhayat, 2004:1073; National Kidney Foundation, 2007:6);
- Patient information during TB treatment was excluded because TB itself and its medications affect renal function (Eastwood *et al.*, 2001:1313);
- Patients on chronic use of tubular nephrotoxic drugs, including (1) protease inhibitors (ritonavir, lopinavir); (2) aminoglycoside antibiotics (streptomycin) and (3) aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac, ibuprofen); (Anzai & Endou, 2007:451; Naughton, 2008:745; Singh *et al.*, 2003:971).

1.6.2.6 Recruitment of participants

The following process was followed:

- A mediator was requested to assist with the identification of participants who complied with the inclusion criteria.
- The mediator signed the confidentiality agreement (refer to Appendix III) prior to the recruitment process.

- The mediator was a professional nurse working at the facility, holding a bachelor's degree in general nursing and midwifery registered currently with the Lesotho Nursing Council (LNC) as a nurse midwife.
- Since the mediator was a professional nurse working in the facility, all the trust to handle matters relating to his daily scope of work was entitled to him and therefore he could ensure adequate privacy, confidentiality and ethical conduct towards potential patients to be recruited as participants in the study.
- In the study the mediator was assisting in giving all the information about the study as well as communicating the informed consent with potential participants.
- The routine practice of the facility was that health talks were held daily at time intervals prior to consultations, meaning that the first batch of patients who arrived in the morning had their session, consulted and left the facility; the next batches could follow the routine until the facility is closed in the afternoon.
- The mediator briefly discussed general information with the patients about the study during these health talks. This was done to save time in the consulting room so that the mediator can focus on private and specific information with the patient.
- The mediator was communicating about the study in Sesotho (local language) and in English (second language). This was important to overcome possible problems that the participants may not understand the language of the mediator.
- The mediator alone identified all patient files that complied with the inclusion criteria from the filing office.
- For the selected files, a predesigned informed consent form (refer to Appendix I and Appendix II) written in both local (Sesotho) and second (English) language were orally communicated by the mediator to the patients in the consultation room as they came for their routine care visits. In the consultation room, the mediator was with each patient in order to assure privacy.
- The mediator emphasized the fact that only retrospective data was going to be used from their files and that no personal contact with the researcher was necessary.
- The mediator wrote the patient's file number on the informed consent form before the form was given to the patient.
- The forms were given in duplicate for patients' record purposes and for signing and returning to show participation.

- The selected patients had time up to their next visit to the clinic (almost a month later) to return the signed informed consent forms in two sealed boxes placed at the centre receptionist's office. This was done to give the selected potential participants enough time to think about the participation properly, to obtain a witness to sign and to discuss their participation with their family or caregivers. This also gave the patient the freedom to return or not return the informed consent form without any health team member's interference or awareness (including the mediator who issued the form) so that the patient may not be afraid that his/her refusal to participate in the study may change the way that his/her illness was taken care of, at the centre.
- The decisional capacity to enrol in this study was solely upon the patient even though the criteria demands might have been met and those willing to participate in the study showed by returning signed forms to the facility.
- At the end of two months, when almost all the patients had their next visit to the facility, the researcher opened the sealed boxes in privacy of his office at the facility.
- The recruitment process took place over a period of two months (60 working days) from October 2015 to December 2015 to ensure that all possible participants had an equal chance to be selected. It began on the date that the participants visited the centre for their routine monthly follow-up. Therefore, participants did not have any extra travelling costs to visit the facility.
- The recruitment took place after obtaining ethical approvals from the Lesotho Ministry of Health Research and Ethics Committee (refer to Appendix V), Health Research Ethics Committee (HREC), Faculty of Health Sciences, North-West University (refer to Appendix VI) and permission by the study site management (refer to Appendix VII).
- The researcher is ethically bound as a pharmacist (registered with Lesotho Medical, Dental and Pharmacy Council (LMDPC) as a pharmacist) to ensure privacy, anonymity, confidentiality and ethical conduct during the proceedings of the study including data collection.
- The researcher was the sole data collector and did not meet any of the participants during the data collection process.
- The researcher made a list of all the file numbers from signed consent forms in order to request the centre to provide with the files for the data gathering process.

- The data collection took place in an isolated entry restricted room at the study site where the researcher was alone to capture data from the files.
- The files were issued for data capturing and returned for filing on daily basis upon the request of the research in each working day. No files remained on the researcher's data collection room for use the next day.
- The data collection took place at the facility for up 60 days on daily working days from January 2016 to March 2016.
- The process continued until the sample size of 255 participants was obtained.

1.6.2.7 Data collection

1.6.2.7.1 Development of data collection tool (measuring instrument)

Kimberlin and Winterstein (2008:2276) define measurement as assigning numerical values to observations in order to quantify phenomena in terms of variables; developing and applying tools or tests to quantify these variables. The purpose of using measuring instruments or data collection tools is to enhance the quality of the research so that meaningful conclusions can be drawn from the test results of the study. The researcher designed the data collection tool that was used to capture the data (refer to Appendix IV).

1.6.2.7.2 Validation of data collection tool

The data collection tool was discussed by the researcher, study leaders and the statistician to ensure that the tool could capture data that would address the research questions. The validation was done by capturing data using the first 10 files at the study site before major data collection. In these files, it was evaluated roughly whether the tool captured majority of the variables that were needed. The purpose of validating the data collection tool first is to enhance the likelihood of success in the main study and minimise potential pitfalls in the study owing to deviations from the data collection tool (Charlesworth *et al.*, 2013:4). This was done to evaluate whether the tool will able to capture relevant information for the study. Indeed, the tool captured most of the required data. Therefore the large scale data gathering proceeded.

1.6.2.7.3 Process of data collection

The files of patients who had returned signed informed consent forms were the exact source of data. The data collection tool (refer to Appendix IV) was used to record data for the study only.

1.6.2.7.4 Data capturing

The data collection tool mainly entailed sections to capture patients' baseline demographical information and dates, CD4 cell count measurements, TDF-containing HAART regimen(s) initiated, treatment duration, change of HAART regimen (if any) and reasons for change, body weight measurements, serum creatinine concentration measurements. Some measurements were taken at initiation (baseline) of therapy and during therapy. The tools were printed and data were entered manually with a pen for each patient's information. The researcher then generated the data on a Microsoft® Excel® sheet for analysis later.

1.6.3 Study variables

Chernick and Friis (2003:46) and Knapp (2000:6) define a variable as a characteristic, quality or property that can vary from one subject to another and can be counted or quantitatively measured. There were both independent and dependent variables in this study. According to Knapp (2000:6), a variable is said to be independent if it can be applied by the investigator and results in an observable response referred to as a dependent variable. In other words, an independent variable serves as a predictor of the probable outcome; hence, it is also known as the stimulus or predictor variable while the dependent one is also called the response or outcome variable. **Table 1-1** below summarised the variables that were studied according to the aim of the study.

Table 1-1: Aims, specific objectives and variables of the empirical study

Aim	Specific objective	Variables	
		Independent	Dependent
Evaluation of clinical outcomes	To determine the change in body weight, CD4 cell count and plasma viral load in patients taking TDF-containing HAART regimens over treatment duration.	Age at antiretroviral initiation, sex and treatment duration	Body weight, CD4 cell count, plasma viral load
Evaluation of renal safety	To determine renal safety in terms of change in serum creatinine concentration, creatinine clearance and eGFR in patients taking TDF-containing HAART regimens over treatment duration.	Age at antiretroviral therapy initiation, sex, body weight and treatment duration	Serum creatinine concentration, creatinine clearance and eGFR

1.6.3.1 Independent variables

The independent variables of the study were (i) age at ARV initiation, (ii) body weight, (iii) sex, (iv) treatment duration and (v) TDF-containing HAART regimens.

1.6.3.1.1 Age

The patients' age was categorised into years in the ranges of ≥ 18 to ≤ 30 years, >30 to ≤ 40 years, >40 to ≤ 50 years, >50 to ≤ 60 years, >60 to ≤ 70 years and >70 years because kidney disease develops slowly over the years (Xu *et al.*, 2010:5). The findings that have led to the choice of age to be a contributing factor to renal deterioration showed that approximately 10% of nephrons are lost every decade of living and the majority of patients with renal disease would be elderly (Ahmed *et al.*, 2010:839; AlAhmadi & AbuButain, 2013).

1.6.3.1.2 Body weight

The data was also categorised into body weights in kilograms in the ranges of ≥ 35 to ≤ 50 kg, >50 to ≤ 65 kg, >65 to ≤ 80 kg, >80 to ≤ 95 kg and >95 kg. According to Yacovino and Aleksunes (2012:10), the expression and function of renal transporters involved in secretion and re-absorption of drugs, nutrients and toxins are influenced by several factors, which include weight gain. As a result, the regulation of renal transport may be altered by body weight and may result in drug toxicity. This becomes the rationale for the consideration of body weight as one of the predictor factors of renal function.

1.6.3.1.3 WHO HIV/AIDS clinical stage

Patients were categorized into their clinical stage at baseline and the results are presented on the demographic characteristics of the study population. However, in China, Huang *et al.* (2015:6) found that advanced WHO clinical stage at baseline experienced immunological and virological failure.

1.6.3.1.4 Sex

Sex was categorised into males and females. Neto *et al.* (2016:15) and Soldin *et al.* (2010:2) viewed sex-related variations in frequencies of adverse events being due to pharmacokinetic and pharmacodynamic factors; women being the most susceptible group to drug toxicity at therapeutic doses used in general.

1.6.3.1.5 Treatment duration

The treatment duration was categorised in months in six-month time intervals, that is ≥ 0 to ≤ 6 months, ≥ 6 to ≤ 12 months, >12 to ≤ 18 months, ≥ 18 to ≤ 24 months, >24 to ≤ 36 months, >36 to ≤ 42 months and >42 months (MOHSW, 2010:61; Xu *et al.*, 2010:5). Crum-Cianflone (2010:358) has shown that the prevalence of TDF-induced nephrotoxicity was attributable to older age and TDF treatment duration.

1.6.3.1.6 TDF-containing HAART drug regimens

TDF-boosted protease inhibitor combination ART regimens are associated with a greater serum creatinine concentration and a declined renal function when compared to the control (Cao *et al.*, 2013:7).

1.6.3.2 Dependent variables

The dependent variables of the study included (i) body weight, (ii) CD4 cell count, (iii) plasma viral load, (iv) serum creatinine concentration, (v) creatinine clearance and (vi) eGFR. The study determined the clinical outcomes and renal toxicity in patients taking TDF-containing HAART regimens. The changes in body weight and CD4 cell count were used to assess clinical outcomes, while changes in serum creatinine concentration, creatinine clearance and eGFR calculations were used to determine renal safety in terms of age, sex, body weight and treatment duration.

The viral load measurement was not found in the data source.

1.6.3.2.1 Serum creatinine concentration

Drug-induced nephrotoxicity has been associated with limitation or failure of the kidneys to get rid of substances including serum creatinine that have to be excreted by the body through the urine. This often results in elevated levels of serum creatinine, which become reliable markers of kidney disease. According to Mychaleckyj *et al.* (2012:1012), the use of fenofibrate has been associated with elevated plasma creatinine levels after three months of therapy. The plasma creatinine measured is also used to determine the glomerular function, diagnosis and staging of chronic kidney disease (Florkowski & Chew-Harris, 2011:75). These have led serum creatinine concentration to become the dependent variable of the study and the level has been found to be influenced by several factors including age, sex, body weight and treatment duration.

Internationally accepted equations were applied in mathematical analyses of the data and they were Cockcroft-Gault equation and the Modification of Diet in Renal Disease study (MDRD) equation to calculate renal function (Levey *et al.*, 2007:771; MOHSW, 2010:64). These equations were used to calculate creatinine clearance and eGFR respectively.

$\text{Creatinine clearance (in ml/min)} = \frac{(140 - \text{age}) \times \text{weight (in kg)} \times 1.23 \text{ (and multiply by 0.85 if female)}}{\text{divided by serum creatining (in } \frac{\mu\text{mol}}{\text{L}})}$
--

Equation 1-1: Cockcroft-Gault equation

and

$\text{eGFR (mL/min/1.73 m}^2\text{)} = 175 \times (\text{S}_{\text{cr}/88.4})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black})$
--

Equation 1-2: Modification of diet in renal disease study equation

These creatinine-based equations have been studied extensively and have been widely used simultaneously to calculate renal function in terms of glomerular filtration rate (Stevens *et al.*, 2006:2476). The Cockcroft-Gault equation has been shown to overestimate the glomerular filtration rate by approximately 10 to 20% as it used to calculate glomerular filtration rate indirectly. Hence, the MDRD equation has been used to adjust for the overestimation by determining the eGFR directly (Sirwal *et al.*, 2004:122).

1.6.3.2.2 CD4 cell count

The CD4 cell count is expected to rise approximately by 90-150 cells/mm³ following successful initiation of ART (WHO, 2000:2).

1.6.3.2.3 Plasma viral load

The ultimate goal of combination antiretroviral therapy is to reduce the plasma viral load to below detectable limits and to maintain the undetectable levels (Pasternak *et al.*, 2013:1; WHO, 2000:1).

1.6.3.2.4 Body weight

According to Ongoina *et al.* (2010:4), uninterrupted HAART has been found to be effective in preventing or reversing the rising AIDS-defining illness such as the wasting syndrome in developing countries.

The database was prepared on a 2007 Microsoft® Excel® sheet and exported to the International Business Machines Statistical Package for the Social Sciences (IBM® SPSS®) Version 22® Statistical software (IBM United States Software Announcement, 2013).

1.6.4 Reliability and validity of the study

The information documented in the patients' medical records was information entered by the nurse in charge and the laboratory findings from tests done. The nurse entered important information such as the date of birth, WHO clinical HIV/AIDS stage, body weight, sex, dates and regimens initiated. Laboratory results were obtained from the district laboratory where they were generated according to the national laboratory standard operating procedures (SOPs), irrespective of the researcher's attention (Mwase *et al.*, 2010:85). Demographic information and other measurements were generated by the relevant personnel working in the centre. The researcher himself was the one capturing the data from the files to the collection tool as accurately

as they were made available. The mathematical analyses of the data applied the internationally accepted equations for renal function.

1.7 Statistical analysis

Utts and Heckard (2007:1) define statistics as a collection of principles and procedures of gathering data and analysing information to guide decision-making when faced with uncertainty. According to Knapp (2000:11), the methodology in statistics is divided mainly into two branches namely descriptive and inferential statistics. Descriptive statistics are concerned with the organisation, presentation and summarisation of data, while the inferential statistics are a set of procedures applied to draw conclusions about the population that the data were sampled from (Knapp, 2000:11). The two branches were applied in the study as outlined below.

1.7.1 Descriptive statistics

The data collected was summarised using descriptive statistics. Discrete data including sex, WHO clinical HIV/AIDS stage and antiretroviral regimens initiated was prepared in categories and percentages from the categories. Continuous data such as age at antiretroviral initiation in years, body weight in kilograms and treatment duration in months was firstly categorised into intervals and percentages were derived from the intervals. Both the age at antiretroviral initiation in years, body weight in kilograms, CD4 cell count in cells/mm³ and serum creatinine concentration in $\mu\text{mol/l}$ were expressed in terms of the mean and median. The standard deviation for the mean and the interquartile range for the median were used as measures of dispersion at 95% confidence level.

The specific objectives of empirical investigation determined the change in variables over treatment duration, which means that there were observations of the variables recorded on patients' files at baseline, during treatment and at end-time. The minimum time points and they included the baseline and at least one follow-up point; while the maximum time point included the baseline and follow-up points during treatment relatively at six-month interval and the end-time point which was the last observation of the follow-up.

1.7.2 Inferential statistics

Using the Shapiro-Wilk test, a normality test was done to evaluate whether the continuous data emanated from a normally distributed population or skewed distributed population. According to the test, p -values ≥ 0.05 showed normal distribution while p -values < 0.05 showed that the data is either skewed to the right (positive) or skewed to the left (negative) (Razali & Wah, 2011:25).

In order to adjust for missing data and fitting the data based on assumptions, linear mixed statistical modelling was done and linear regression analysis was performed on the data. The analysis evaluated the effect of one independent variable on the change in the dependent variable at a time. The combined effect of the identified independent variables was also evaluated per dependent variable. For all the dependent variables, their change relative to the independent variables was determined from baseline observations to end-line observations over the treatment duration. The vector quantity was used to show declines and increments, that is, negative value changes showed declines in the dependent variable while positive values changes showed increments in the dependent variable.

1.7.3 Statistical significance of the results

The p -value of 0.05 was used as a measure of statistical significance for the change in the dependent variable relative to identified independent variable(s) over treatment duration. The p -value of ≤ 0.05 indicated the statistically significant change while there was no statistically significant change at p -values > 0.05 .

1.8 Ethical considerations

1.8.1 Ethical approval and permission to conduct the study

Ethical approval was first obtained from the Ministry of Health Research and Ethics Committee, Lesotho (refer to Appendix V) and the initial title while working on the protocol with the study leaders read as follows: “The renal safety profile of tenofovir as used in antiretroviral therapy in Lesotho (ID43-2014)”. The protocol was later submitted to the Health Research Ethics Committee of the Faculty of Health Sciences at the NWU after making more adjustments and the title was expanded to: “Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing regimens in Lesotho (NWU-00084-15-S1)” (refer to Appendix VI), with the same design as the previous title. Ultimately, permission to issue informed consent forms to patients and collect data was granted by the management of the Paballong HIV/AIDS care centre upon submission of ethical approvals from Ministry of Health Research and Ethics Committee and NWU Health Research Ethics Committee by the researcher (refer to Appendix V & Appendix VI). Patients showed permission to access their files by returning signed informed consent forms (refer to Appendix I & Appendix II). The project began as soon as when these ethical requirements were met.

1.8.2 Assurance of anonymity

All participants’ information that was gathered from the files was assigned anonymous codes to conceal real identity and to ensure anonymity on the data collection tool. Prior to the issuing of

informed consent forms by the mediator, a tamperproof sealed box was placed in the receptionist's office where signed and unsigned forms were returned to minimise identification of study participants among the attendants' population. Since the mediator was the one issuing the informed consent forms, the researcher did not have any contact with the participants. The researcher only had access to the files of participants who returned signed the informed consent forms.

1.8.3 Assurance of confidentiality

The researcher ensured the highest level of confidentiality of patient information found in the medical records that were used. The information gathered from the files was captured and stored in a password protected computer system with an up-to-date antivirus software programme and only the researcher had access to such electronic information. The computer was kept in a locked office that denied access by unauthorised personnel. The researcher, study leaders and the statistician gained access to the information for statistical analysis and interpretation purposes; thus the researcher transferred the information electronically to the archive of the research entity Medicine Usage of South Africa (MUSA), North-West University, Potchefstroom.

1.8.4 Archive of data

After data collection, hard copies were kept in a locked safe to deny access to unauthorised personnel. These hard copies will be kept safe for seven years and will be shredded and burned afterwards. Electronic data captured on the Microsoft® Excel® sheet from the hard copies by the researcher was kept on a password protected computer system. The electronic version of the data was then submitted by the researcher for safe keeping at the entry restricted unit for archives of Medicine Usage in South Africa for a minimum period of seven years and shall be discarded afterwards. The locked cupboard was used to store hard copies. The data manager of Medicine Usage in South Africa is responsible for the handling of the data and no one was granted access to data except the study leaders and the statistician.

1.8.5 Risk-benefit analysis

According to the United States of America (USA) Department of Health and Human Services (DHHS) (2009:4), a risk is defined as the magnitude and probability of harm or discomfort anticipated in a research study while a benefit is said to be the desired outcome of the research study. The risks to participants are reasonable in relation to anticipated benefits that may be expected to result from the study. The risk can be minimised by employing procedures that are consistent with proven research design that limits unnecessary exposure to harm (DHHS, 2013:1).

1.8.6 Expected output of the study

The study intended to evaluate both the clinical outcomes and renal safety among HIV/AIDS patients taking TDF-containing HAART regimens. The study variables that have an effect on these two parameters have been outlined and the expectation was to quantify their effect on clinical outcomes and renal safety.

The study was expected to reveal the following output:

- A rise in CD4 cell count and body weight relative to treatment duration.
- A decline in plasma viral load relative to treatment duration.
- An increasing renal toxicity relative to aging and treatment duration.
- A higher risk of developing renal compromise in females than in males.

1.8.7 Benefits of the study

The direct and indirect benefits of the study will be discussed below.

1.8.7.1 Direct benefits

The study was a retrospective study that had no physical contact with the participants, and therefore there were no direct benefits to the participants from the study.

1.8.7.2 Indirect benefits

The results of the study are intended to be discussed with the facility health team. The discussions will be done so that significant changes can be made on patients' care based on available evidence for the benefit of the patients. The results will positively influence the treatment approach towards using TDF-containing HAART regimens with the aim of minimising inherent adverse effects of the drug combination and optimising the outcome of the drug regimens.

- At facility level, the decision on eligibility of TDF-containing HAART regimens will be closely attended to, and close monitoring related to patient variation discussed will be done accordingly.
- The results of the study will also influence decision-making at national level. In this way, the results of this study will be extrapolated to other facilities and adjustments will be made in accordance to optimise drug therapy while minimising adverse events.

- Again, the results of the study will offer new knowledge that will be useful to teachers and learners in institutions of higher learning.

The use of TDF-containing HAART regimens is directly influenced by the Lesotho HIV/AIDS guidelines since the drug is categorised as first-line and many people are initiated on HAART regimens that contain the drug; therefore changes can be made on the guidelines where needed (MOHSW, 2010a:48).

The study was considered highly beneficial not only to the patients using the facility but also to the MOH Lesotho, health professionals involved in HIV/AIDS care in general; as well as HIV/AIDS treated patients including those who were eligible to be enrolled in the study, but did not participate.

1.8.8 Anticipated risk and precautions

There was no direct harm imposed on the participants of the study as there was no physical participation by the patients in the study and the researcher did not meet them anywhere. However, since the mediator was the nurse working at the facility, patients could hesitate to participate in the study for a variety of reasons, including fear of stigmatization and their understanding of the precautions of participation and believing that the study might have negative impact on services offered to them. According to Rick and Briner (2000:312) the individuals' mood may have a considerable impact on the way they respond the moment they are being engaged into an activity. The informed consent clearly articulated voluntary participation in the study, which the mediator emphasised and assured transparency. Therefore, discussion of the study using the informed consent eliminated the anticipated psychosocial and health risks that participants could associate with their participation.

Again, the issuing of informed consent forms would become extra work not only for the mediator, but also an extra burden to the patients, as more time may be spent in the consulting room. Since there was brief general discussion given to the group of patients prior to consulting, lesser time was spent in the consulting room.

Therefore, study was considered a low risk since there was no physical harm imposed on the patients. Therefore, the benefits of the study were anticipated to outweigh the potential risks.

1.9 Division of chapters

The division of chapters will be as follows:

Chapter 2: Clinical outcomes and renal safety

Chapter 3: Results and discussion

Chapter 4: Conclusion, limitations and recommendations

1.10 Chapter summary

In this chapter the background, problem statement, research aim and objectives, and research methodology were discussed, followed by a brief discussion of the ethical consideration and a chapter division. Aspects of the literature review will be discussed in Chapter 2.

CHAPTER 2: CLINICAL OUTCOMES AND RENAL SAFETY

2.1 Introduction

This chapter is the phase of the study that provides background information about the present study, using relevant published literature sources from other studies. The literature focuses on therapeutic and renal toxicological mechanism(s) of action of TDF in the treatment of HIV/AIDS.

The response to TDF-containing HAART regimens is also evaluated in terms of desired outcomes and renal safety. The desired outcomes namely clinical, immunological and virological are covered in this chapter. The renal safety measures covered in this chapter include direct and indirect measures of renal function, using relevant conventional mathematical equations. Lastly, the chapter covers patient factors influencing both the therapeutic (beneficial) and toxicological (harmful) outcomes of TDF-containing HAART regimens.

2.2 Background of HIV/AIDS epidemic

This section on the background of HIV/AIDS entails the global and regional coverage of HIV infection. The findings are strengthened by statistics that gives the objective analysis of the situation of HIV/AIDS epidemic.

2.2.1 Global epidemic of HIV/AIDS

The Acquired Immune Deficiency Syndrome (AIDS) was first recognised amongst homosexual men in the United States of America in 1981 (Gallo & Montagnier, 2003:2283). Later in 1983, Human Immunodeficiency Virus (HIV) was identified as the causal agent of the syndrome (United Nations Programme on HIV/AIDS (UNAIDS) & WHO, 2003:3). This virus is a retrovirus that belongs to the taxonomical family of *Retroviridae*, subfamily *Orthoretrovirinae* and genus name *Lentivirus* (Gallo & Montagnier, 2003:2283). There are two types of HIVs namely HIV-1 and HIV-2 which both fit the taxonomical hierarchy mentioned (International Agency for Research on Cancer, 2012:215). In the mid-80s, the HIV infection was found to have spread largely with separate epidemics in different parts of the world. Each of these epidemics had own distinct origin, geographical distribution and specific key populations affected. The epidemics also involved the type of HIV and frequencies of risk behaviours and practices (UNAIDS & WHO, 2003:3). Acquired Immune Deficiency Syndrome (AIDS) is now the deadliest epidemic of the 21st century and the leading cause of infectious diseases mortality, surpassing tuberculosis (TB) and malaria infections (DHHS, 2004:17). Since 2004, Kaplan (2013:7) found that HIV infection has already affected more than 60 million people worldwide.

Human Immunodeficiency Virus (HIV) infection continues to be a major global public health challenge that has claimed approximately 36.9 million people living with the infection at the end of 2014 (UNAIDS, 2015:3; WHO, 2016a). Of the 36.9 million people, 2 million people were newly infected while 2.6 million were children aged < 15 years (WHO, 2016a). Again, 17.1 million of the 36.9 million did not know that they have HIV infection and needed to be reached with testing services; while around 22 million of those who knew that they have HIV infection did not have access to antiretroviral treatment (including 1.8 million children) (UNAIDS, 2015:3). According to WHO (2016a), the vast majority of people living with HIV are in low- to middle-income countries. By far from the year 2000, it is estimated that 34 million people died from AIDS-related causes and these included 1.2 million deaths which occurred in 2014 only (WHO, 2016a).

2.2.2 The coverage of HIV/AIDS in the sub-Saharan African region

The sub-Saharan region of Africa is the mostly affected region of the world, with almost 1 in 20 adults (4.9%) living with HIV and accounting for 69% of the people living with HIV infection globally (UNAIDS, 2012:8). In 2013, it was estimated that 24.7 million people lived with HIV infection in the region, accounting for 71% of the worldwide data (UNAIDS, 2014b:27). Again in 2013, there were estimated 1.5 million new HIV infections and 1.1 million AIDS-related deaths (UNAIDS, 2014b:27). The HIV infection prevalence for the region was found to be 4.7%, with variations between the divisions of the region and by countries including Lesotho. Southern Africa is so called the 'epicentre' of global HIV epidemic because it is the worst affected division of sub-Saharan Africa (UNAIDS, 2014b:27).

The Lesotho Ministry of Health (MOH) (2015:2) asserted that the country is facing an overall epidemic that affects a variety of sub-populations at various modes of transmission. The latest estimates show that for adults aged 15-49 years, the HIV infection prevalence is 25.0% (MOH, 2016b:235). There were estimated 360, 000 people living with HIV infection and 16, 000 AIDS-related deaths were reported in 2012 (MOH, 2012:26). According to UNAIDS (2014b:277) HIV incidence in Lesotho has reduced significantly since 2005 from 30,000 new infections to 26,000 new infections in 2013. There are a number of affected key populations that have been attributed to high HIV prevalence in Lesotho. Not limited to the list, MOHSW (2012) highlighted that women and young girls, orphans and children, prison inmates, prison staff, men who have sex with men (MSM) are amongst the key populations affected by HIV infection in Lesotho.

In Lesotho, MOH (2016b:245) found that the urban areas are more burdened with HIV infection than their rural counterparts, with prevalence of 30% and 21% respectively. While the major proportion of new HIV infections in sub-Saharan Africa are found in the adult population aged 25 years and above, HIV infection disproportionately affects women (UNAIDS, 2014b:31). Women have higher HIV infection prevalence of 29.7% while men prevalence is 18.6 % according to

Lesotho national demographic health survey done in 2014 (MOH, 2016b:243). This sex imbalance in HIV infection prevalence was found to begin early in life, where the infection is almost twice as prevalent in young women (13.0 %) than young men (6.0 %) in the ages 15-24 years (MOH, 2016:239). Human Immunodeficiency Virus infection epidemic covered greater proportions that have altered family life for many young people in the country. According to UNAIDS (2015) the epidemic has been associated with prevailing circumstances of many orphaned children in Lesotho (estimated 150, 000 orphans in 2015).

2.2.3 Key populations affected by HIV/AIDS in Lesotho

Prisoners are a key population affected by HIV infection in Lesotho. According to UNAIDS (2014b:149), 31.4% of the male inmates are living with HIV infection within the prison environment. The prison inmates and prison staff have an increased perceived vulnerability to HIV infection acquisition. The vulnerability has been associated with reasons ranging from unmet health needs, overcrowding, sexual violence and unsafe sexual practices (UNAIDS, 2014b:149). For prison inmates, males (76.7%) and females (61.6%) stated an increased risk of contracting HIV infection within the prison environment (UNAIDS, 2014b:149). The findings for prison staff reported that 80.8% of males and 71.5% of females being at risk of contracting HIV infection. There is limited research being done on men having sex with other men in Lesotho. Baral *et al.* (2011) however reported 11.6% prevalence of HIV infection among men having sex with other men in Lesotho.

The sex workers are not left out in this regard. The prevalence of HIV infection among sex workers was estimated to be 72% (almost 3 times higher than for the adult population) (MOH, 2015:3). This is largely due to widespread violence, criminalisation, stigmatisation and discrimination, lack of targeted programmes and funding. The factory workers come subsequent to sex workers with the prevalence of 42.7% (MOH, 2015:3). In a study conducted by UNAIDS (2010:27) on modes of HIV infection transmission, using the predesigned HIV infection modes of transmission model in the methodology, most new infections were from low-risk heterosexual sex (31.87%) and from gays, transsexuals and men who have sex with men (33.26%).

The statistics above brought an understanding of the HIV/AIDS epidemic among the Lesotho population.

2.3 Treatment and access to ART for HIV/AIDS

This section of the literature covers treatment programmes relative to access to antiretroviral therapy for treatment and prevention of HIV infection. The implementation of antiretroviral therapy programmes is also covered.

2.3.1 Access to antiretroviral therapy

The antiretroviral therapy programmes have been scaled up significantly in the sub-Saharan Africa over the past decade, although there are still some challenges. United Nations Programme on HIV/AIDS (UNAIDS) (2013:5) witnessed this with findings by revealing 68% of people living with HIV infection in sub-Saharan Africa having access to antiretroviral treatment under the WHO 2010 guidelines (those with a baseline CD4 cell count of ≤ 350 cells/mm³). Moreover, the WHO 2013 guidelines consequently improved the eligibility for treatment by expanding the initiation of antiretroviral therapy to those people with a CD4 cell count of ≤ 500 cells/mm³. This improvement reduced antiretroviral therapy coverage to 39% in 2013 (UNAIDS, 2014b:29). In 2016, World Health Organization (WHO) (2016c:74) strongly recommends that all adults living with HIV infection should be initiated on antiretroviral therapy regardless of CD4 cell count. As of March 2015, 15 million people living with HIV infection worldwide were receiving antiretroviral treatment (including 823 000 children) (WHO, 2016b). This is representing 41% of those people in need of antiretroviral therapy. Of these 15 million people, 13.5 million patients were from low- and middle-income countries (WHO, 2016b).

There is greater success for access to antiretroviral therapy in low- and middle- income countries. The scaling-up of access to treatment is in the hearts of new global treatment targets for the year 2020 since the aim is to end the AIDS epidemic as a public health threat by the year 2030 (WHO, 2016c:iv).

2.3.2 Antiretroviral therapy as prevention

There is a significant progress made in the use of antiretroviral treatment for prevention of HIV infection. The initiatives in place include prevention of mother-to-child transmission (PMTCT), post-exposure prophylaxis (PEP), pre-exposure prophylaxis (PreP®) and antiretroviral therapy as prevention (TasP) (Cohen *et al.*, 2011:503; Thomas *et al.*, 2015:7; UNAIDS, 2015:3; UNAIDS, 2010:65). In 2009, United Nations Programme on HIV/AIDS (UNAIDS) (2010:65) estimated that 370, 000 children were newly infected with HIV following mother-to-child transmission. Transmission can occur during pregnancy, labour, delivery, and breastfeeding (Marino, 2015:8). In Ethiopia, a five-year cohort study (2006-2010) revealed a remarkable improvement in potential coverage of PMTCT services due to rapid increase in PMTCT sites (Nigatu & Woldegebriel, 2011:8). Studies conducted in Botswana and Malawi demonstrated greater effectiveness of various antiretroviral regimens for PMTCT. These studies revealed effectiveness of antiretroviral therapy in PMTCT at 26-34 weeks of pregnancy, up to six months post-delivery and in extended infant prophylaxis for 28 weeks (Chasela *et al.*, 2010:2272; Shapiro *et al.*, 2010:2292).

In Botswana, Shapiro *et al.* (2010:2292) conducted cohort study from July 2006 to May 2008 (two years) on pregnant women infected with HIV-1. The study revealed similar rates of HIV-1 RNA suppression to <400 copies/ml in both at delivery and throughout breastfeeding. In total, only 1.1% of the infants were infected with HIV-1 at six months of age during breastfeeding. These findings suggested an effective strategy for prevention of mother-to-child transmission to be initiating antiretroviral therapy as early as possible in pregnancy throughout six months of breastfeeding while allowing beneficial outcomes of breastfeeding to the infants. In Malawi, Chasela *et al.* (2010:2272) showed that both maternal antiretroviral regimen and infant prophylaxis during 28 weeks of breastfeeding were effective in reducing post-natal HIV-1 transmission. Binagwaho *et al.* (2013:10) established that all PMTCT regimen options were cost saving when compared to non-PMTCT interventions. Failure to prevent transmission through mother-to-child is therefore found totally unacceptable from an ethical, financial and public health perspective. In 2014, 73% of pregnant women living with HIV infection had access to antiretroviral treatment to prevent transmission to their babies worldwide (WHO, 2016b).

Thomas *et al.* (2015:7) claimed that PEP is a successful method to prevent HIV infection after sexual exposure. The Smith *et al.* (2005:18) study on animal and human models demonstrated that antiretroviral therapy initiated within 48-72 hours of sexual intercourse, injection-drug use, and other substantial non-occupational HIV infection exposures, and continued for 28 days reduces the likelihood of HIV transmission. In a review done by Anglemyer *et al.* (2013:17) on antiretroviral therapy for prevention of HIV transmission in HIV sero-discordant couples, they found that antiretroviral therapy was associated with diminished risk of transmission of HIV infection within such couples.

Oral PreP® in HIV infection is a daily use of antiretroviral treatment by HIV-uninfected individuals to block the acquisition of HIV infection. PreP® has also been shown to prevent HIV infection in diverse population groups including gays, men who have sex with other men, heterosexual men and women, transgender people and people who inject drugs (UNAIDS, 2015:3). Pre-exposure Prophylaxis reduces HIV infection transmission by up to 90% when compared with no intervention (UNAIDS, 2015:3). It is however important to take it correctly and ensure that the patient is actually adherent to the treatment.

There has also been growing evidence of the benefits of HIV treatment as a prevention (TasP) method for a number of years. In 2011, a landmark study conducted in nine countries including African countries showed that early initiation of antiretroviral treatment to HIV-sero-positive partner in a sero-discordant couple reduced HIV infection transmission to the HIV-sero-negative partner by 96% (Cohen *et al.*, 2011:503). Follow-up studies conducted in three African countries namely Kenya, Uganda and Botswana reported significant reductions in HIV infection

transmission in sero-discordant couples and numbers of new infections were reduced (Baeten *et al.*, 2012:409; Thigpen *et al.*, 2012:433).

2.3.3 Implementation of highly active antiretroviral therapy

As of September 2015, the WHO (2015) announced the “treat all” recommendation that removes the limitations on eligibility for antiretroviral therapy initiation relative to CD4 cell count criteria. The recommendation entails that all populations and age groups are now eligible for antiretroviral therapy and treatment should begin as soon as possible after diagnosis. According to the United Nations Fund for Population Activities (UNFPA) (2016), Lesotho became the first African country to launch the “test and treat” strategy in June 2016. The goal of this scaled-up treatment initiative is to attain the 90-90-90 global targets. The targets entail that by the year 2020, 90% of the total population should be tested for and diagnosed if found living with HIV infection. Of those diagnosed, 90% of them should be on lifelong antiretroviral therapy and 90% of those on antiretroviral therapy should be virally suppressed (MOH, 2016a:ix). These new policies are expected to help avert more than 21 million AIDS-related deaths and 28 million new HIV infections globally by 2030.

The global implementation of highly active antiretroviral therapy has been attributed to reductions in morbidities and mortalities associated with HIV infection (Crabtree-Ramirez *et al.*, 2010:378; Kalyesubula & Perazella, 2011:1; Lohse *et al.*, 2008:57; Resino *et al.* 2004:1611). This therapeutic approach includes at least three-drug combination of antiretroviral drugs as the first line treatment. The drug combination should consist either of:

- two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI); or
- one Nucleotide Reverse Transcriptase Inhibitor (NtRTI), one NRTI and one NNRTI; or
- two NRTIs and one Protease Inhibitor (PI) (WHO, 2010:39).

In Lesotho, the above-mentioned drug classes are available and are used in HAART first line. The drugs available include zidovudine (AZT), lamivudine (3TC) and abacavir (ABC) as NRTIs, lopinavir (LPV) and ritonavir (RTV) as PIs. TDF is the only NtRTI available (MOH, 2013:29). According to Baylor College of Medicine (BCM) (2010:45) the goals of HAART are to attain maximal and durable suppression of HIV replication, restore and preserve immune function, reduce HIV-related morbidity and mortality and ultimately improve the quality of life.

The highly active antiretroviral therapy (HAART) initiative allows for multiple mechanism approach by which the antiretroviral drugs given arrest the replication of the HIV at different points of its life

cycle (Arts & Hazuda, 2012:4). The duo NRTIs and NtRTIs compete with viral nucleotides to inhibit the viral enzyme called reverse transcriptase that is responsible for the conversion of the viral ribonucleic acid (RNA) to viral deoxyribonucleic acid (DNA), while NNRTIs instead bind on the enzyme's catalytic site and denature it, so that the enzyme cannot convert RNA to DNA too (Arts & Hazuda, 2012:8). Protease inhibitors block the proteolytic cleavage of the precursor polypeptides into mature structural and functional proteins required for the survival of the HIV in the host cell (De Clercq, 2009:315).

2.4 The impact of antiretroviral therapy

This section covers the efficacy and safety outcomes of antiretroviral therapy. The efficacy of this therapy is entailed as direct and indirect benefits to patients taking treatment and those not taking treatment but are at risk of being HIV infected. The adverse reactions to antiretroviral drugs as safety concerns about antiretroviral therapy are also entailed in this section.

2.4.1 Life-saving benefits of antiretroviral therapy

The life-saving benefits of antiretroviral therapy have been markedly observed. Prior to initiation of antiretroviral therapy in Thailand, about 80% of people presenting at clinics with AIDS-related illnesses died within two years (Chasombat *et al.*, 2009:511). Upon initiation of antiretroviral therapy in this country, even the most severely ill people living with HIV infection today have at least 80% chance of survival after two years of treatment (Chasombat *et al.*, 2009:511). In Brazil, mortality rates in AIDS-related causes declined dramatically in the years 1986-1991 from 9.2 deaths per 100 people-years to 1.4 deaths per 100 people-years in the years 2007-2009 (Grinsztejn *et al.*, 2013:7); whereas there was no change in mortality rates amongst non-AIDS-related causes. Meanwhile in China, mortality rates declined from 45.7 per 100 person-years in 2002 to 9.2 per 100 person-years in 2011. The decline in mortality rates was attributed to increment in coverage of antiretroviral treatment from almost 0 to 63% (Zhang *et al.*, 2011:523).

The global reduction in prevalence of HIV infection over the past 15 years led to significant declines in the number of people dying from AIDS-related illnesses (UNAIDS, 2012:12). Worldwide annual AIDS-related deaths declined from 2.3 million (2.1-2.6 million) in 2005 to less than 1.7 million (1.5-1.9 million) in 2011 (UNAIDS, 2012:12). By the end of 2012, the scaling up of antiretroviral therapy coverage has reduced an estimated 4.2 million deaths experienced in the previous decade in the low- and middle-income countries (UNAIDS, 2012:12). Since the introduction and scaling-up of HAART, the number of people dying from AIDS-related causes in sub-Saharan Africa declined by 32% from 2005 to 2011, although the region still accounted for 70% of all people dying from AIDS in 2011 worldwide (UNAIDS, 2012:12).

The antiretroviral therapy increases the quality-of-life since people become healthier once they are stable on antiretroviral therapy. In South Africa, Bradshaw *et al.* (2012:10) found that life expectancy from birth rose from 56.5 to 60 years from the years 2009 and 2011. The gain is largely attributed to scaled-up antiretroviral therapy coverage and the prevention of mother-to-child transmission programmes (Bradshaw *et al.*, 2012:10). In rural KwaZulu-Natal (still in South Africa), Bor *et al.* (2013:15) found that the overall adult life expectancy increased by more than 11 years between the years 2003 and 2011.

Studies conducted more recently in low-and middle-income countries confirmed that elevations in life expectancy of HIV infected people receiving antiretroviral therapy were as impressive as those that were earlier encountered in the high-income countries (Johnson *et al.*, 2013:8; Mills *et al.*, 2011:213). In South Africa, Johnson *et al.* (2013:8) revealed that data from six HIV infection treatment programmes in three provinces namely Western Cape, Gauteng and KwaZulu-Natal showed that adults obtained about 80% of normal life expectancy if antiretroviral treatment was initiated before their CD4 cell count dropped to <200 cells/mm³. Moreover, in Uganda, a 20-year old person living with HIV infection could expect to live an additional time of 27 years if receiving antiretroviral therapy (Mills *et al.*, 2011:213). The antiretroviral therapy does not only save lives but elongates life expectancy of people living with HIV infection.

2.4.2 Impact of antiretroviral therapy on prevalence of opportunistic infections

The antiretroviral therapy is also associated with significant decrements in prevalence of many opportunistic infections. Low *et al.* (2016:7) conducted a systematic review on the effects of antiretroviral therapy on major HIV infection-related opportunistic infections amongst adults in low- and middle-income countries. The review has shown that the rates of most opportunistic infections fell to lower levels when compared with those observed in many high-income countries. The decline was the greatest for opportunistic infections such as pulmonary and extra-pulmonary TB, *Pneumocystis jiroveci* pneumonia, oral candidiasis, toxoplasmosis, shingles and Kaposi's sarcoma. The reduction in the risk of developing these diseases ranged from 98% to 61% during the first year of antiretroviral therapy. The reduction partially resulted from concurrent scaled up chemoprophylactic treatment for protozoan and fungal infections (Low *et al.*, 2016:8).

Since HIV infection increases the risk of activation of latent TB to active TB, active TB control is very challenging in countries with high prevalence of HIV infection (Middelkoop *et al.*, 2011:268; Zachariah *et al.*, 2011:937). However, studies conducted in resource-limited settings have confirmed that antiretroviral therapy is strongly associated with a reduction in the incidence of TB (Lawn *et al.*, 2005:2115; Lawn & Wood, 2005:1785; Pettit *et al.*, 2011:312). In a meta-analysis conducted by Suthar *et al.* (2012a:9) on observational studies done on low-and middle-income countries, antiretroviral therapy was found to reduce the risk of TB by up to 65%. Earlier initiation

of antiretroviral therapy may be a key strategy for reducing HIV infection-associated TB. Suthar *et al.* (2012a:9) found preventive benefit to active TB occurred even among people with high CD4 cell counts.

Studies conducted at national levels in Malawi (Zachariah *et al.*, 2011:937) and South Africa (Middelkoop *et al.*, 2011:268) revealed that when antiretroviral therapy coverage in a population reaches a high level, TB notification rates declined. Zachariah *et al.* (2011:937) observed that in areas that achieved high antiretroviral therapy coverage, there is very encouraging evidence of significant decline in TB case notification rates for new and recurrent TB cases. Furthermore, Middelkoop *et al.* (2011:268) asserted that high coverage of antiretroviral programme can reduce the TB notification rates and TB case fatality within a community that is heavily affected by both HIV infection and TB epidemics. The reduction in TB infection has been attributed predominantly to the decrease in TB rates in HIV-infected patients who received antiretroviral therapy and may be the result of both active TB screening and improved immune function following antiretroviral therapy initiation (Middelkoop *et al.*, 2011:268).

2.4.3 Impact of antiretroviral therapy on HIV transmission

The antiretroviral therapy has also been proven to prevent HIV transmission and thus reducing the incidence of HIV infection (UNAIDS, 2012). In 2001, 3.2 million people acquired HIV infection, this number fell to 2.5 million in 2011 (700, 000 less) (UNAIDS, 2012). The rate of people acquiring HIV infection fell by $\geq 50\%$ in 25 low- and middle-income countries during that same period (2001-2011) (UNAIDS, 2012). In 2011, a model study done by Schwartzlander *et al.* (2011:2039) estimated that combined effort of HIV/AIDS prevention interventions coupled with antiretroviral treatment coverage of up to 80% could reduce the number of people getting infected with HIV globally from three million per year to 1.2 million by the year 2025.

Since 2009, the number of children acquiring HIV infection has been significantly declining globally (UNAIDS, 2014a:9). This is due to rapidly expanding PMTCT programmes and efficacies of antiretroviral drug regimens. The number of children acquiring HIV infection declined by 43% between 2009 and the end of 2013 in the priority countries (including Malawi, South Africa, Botswana, Zimbabwe, Namibia, Mozambique, Ethiopia and Ghana), from 350, 000 in 2009 to 199, 000 in 2013 (UNAIDS, 2014a:9). In 21 African priority countries which accounted for about 90% of all pregnant women living with HIV infection and newly HIV infected children globally, mother-to-child transmission rates declined in overall from an estimated 33% (30-38%) in 2005 to 26% (24-30%) in 2009 and lastly down to 17 % (15-20%) in 2012 (UNAIDS, 2013:35).

The efficacy of PreP[®] has been assessed in randomised clinical trials among men who have sex with other men, sero-discordant couples, sexually active young adults and injecting-drug users

(Baeten *et al.*, 2012:409; Choopanya *et al.*, 2013:2088; Grant *et al.*, 2010:2588; Thigpen *et al.*, 2012:433). In each of these trials, the efficacy of antiretroviral therapy was closely linked to adherence such that high adherence led to the reduction in HIV infection incidence by >90% (Anderson *et al.*, 2012:113). Pre-exposure prophylaxis involves the challenge of identifying the populations that most need additional prevention support and that have sufficient familiarity with pre-exposure prophylaxis; and are willing and able to use it.

2.4.4 The safety concerns about antiretroviral therapy

There is considerable experience in the developed world with the use of antiretroviral drugs. Antiretroviral drugs are associated with significant safety concerns including serious adverse drug reactions (ADRs), with both short- and long-term effects. As of 2010, over five million people worldwide have access to antiretroviral drugs (WHO, 2010). The scaling up of antiretroviral therapy has called for a greater need for monitoring and promoting safety and effectiveness of these essential medicines. The resource-limited countries run a risk with the scaling up interventions to safety monitoring due to lack of structures, systems or resources necessary to support medicine safety activities (Management Sciences for Health (MSH), 2009:1). Loss of confidence in the safety of antiretroviral drugs could lead to poor or non-adherence, development of drug resistance or inappropriate switching to more toxic or expensive medicines. A sustainable pharmacovigilance system can help achieve comprehensive, safe and effective healthcare.

Adverse drug reactions are widely accepted as one of the most significant factors influencing the treatment outcomes (Guo *et al.*, 2010:207; Mills *et al.*, 2006:2061). These are more evident in the treatment of chronic illnesses as studies have reported a substantial impact on chronic illnesses, resulting in decrement of health-related quality of life (HRQoL) (Guo *et al.*, 2010:207). Adverse drug reactions are often attributed to greater challenges brought by antiretroviral drugs. In a systematic review, Mills *et al.* (2006:2061) identified barriers towards adherence in developing countries which included ADR experiences that remained challenging and resulting in noteworthy morbidity and treatment failures.

The review conducted by Subbaraman *et al.* (2007:1097) has illuminated a few common overlapping toxicities between antiretroviral therapy and opportunistic infections and their treatment especially in patients receiving the TB treatment. The initiation of antiretroviral therapy at advanced AIDS clinical stages has implications beyond the risk of morbidity and mortality due to opportunistic infections (Subbaraman *et al.*, 2007:1097). Low CD4 cell count at initiation of antiretroviral therapy is a risk factor for multiple adverse events from the antiretroviral drugs such as peripheral neuropathy, lactic acidosis and myelosuppression (McComsey & Lohergan, 2004:34).

2.5 Mechanisms of action of antiretroviral drugs

There are six classes of antiretroviral agents that are currently available in clinical practice to date. The classes are NRTIs/NtRTIs, NNRTIs, PIs, integrase inhibitors (INSTIs), fusion inhibitors (FIs) and chemokine receptor antagonists (Rathbun, 2016:1). The six classes are distinct in terms of their molecular mechanisms and resistance profiles (Arts & Hazuda, 2012:4). All these drugs attempt to arrest the replication of the HIV at different stages of the virus life cycle. Rathbun (2016:1) asserted that the choice and use of these agents in clinical practice is largely based on several considerations including ease or complexity of use, side effect profile, efficacy based on clinical evidence, clinician preference as well as practice guidelines.

2.5.1 Nucleoside reverse transcriptase inhibitors (NRTIs)/ Nucleotide reverse transcriptase inhibitors (NtRTIs)

The first class of antiretroviral drugs to be approved by FDA in 1987 were the NRTIs/NtRTIs (Young, 1988:243). The prototypical drug that was approved at the time was AZT. Other drugs in this class are stavudine (d4T), 3TC, ABC and TDF. Generally, these drugs are prodrugs that enter the host cell and become active drugs in the body upon metabolism by phosphorylation (Miller, 2002:17). Miller (2002:17) further indicated that the difference between NRTIs and NtRTIs is that NtRTIs require fewer steps of activation (two-step phosphorylation) as they already possess one phosphate moiety in their original prodrug structure; as opposed to NRTIs which lack the initial phosphate moiety and hence will require more activation steps (three-step phosphorylation). Tenofovir is the only NtRTI in the above-mentioned drug examples.

These drugs are structural analogues to host cell nucleotides in their active phosphorylated form. According to Weller and Williams (2001:1410) since the HIV is an RNA virus, its RNA has to be copied into a proviral DNA which gets incorporated into the host cell DNA. The copying process is regulated by the specific HIV DNA polymerase enzyme called reverse transcriptase that converts viral RNA to proviral DNA. The NtRTIs and NRTIs inhibit the enzyme because the enzyme cannot distinguish the drugs from the natural nucleotides (building blocks) of viral DNA as the copying process is being attempted (Elion & Witt, 2003:2). They are thus incorporated into the DNA chain, terminating its elongation, then resulting in incomplete and non-functional DNA (Miller, 2002:17). These drugs are not only the competitive inhibitors of the enzymes but also the terminators of the proviral DNA strand (Weller & Williams, 2001:1410). When the copying process fails, the incomplete and non-functional proviral DNA becomes susceptible to destruction by the host cellular enzymes (Elion & Witt, 2003:2).

The use of NRTIs/NtRTIs is associated generally with mitochondrial toxicities namely lactic acidosis, hepatic steatosis, lipodystrophy, pancreatitis and peripheral neuropathy (DHHS,

2016:13). According to Cote *et al.* (2002:816) this is due to high binding power of the drugs to the mitochondrial DNA polymerase- γ enzyme, with the descending order of affinities: d4T, 3TC, AZT, ABC and TDF. The individual drug's adverse effects include cardiovascular effects and hypersensitivity reactions from ABC and bone marrow suppression from AZT (DHHS, 2016:13; Sabin *et al.*, 2008:1425). Despite the fact that antiretroviral therapy decreases the susceptibility to developing chronic kidney disease coupled with CD4 cell recovery and viral suppression, some initial regimens including TDF increase the risk of developing renal toxicity (Kalayjian *et al.*, 2012:1914).

2.5.2 Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

There are currently six FDA-approved NNRTIs since the approval of the prototypical NNRTI called nevirapine (NVP) which was approved in 1996. Other drugs are efavirenz (EFV), delavirdine, etravirine, doravirine and rilpivirine (Arts & Hazuda, 2012:4). In contrast to NtRTIs/NRTIs, NNRTIs do not require phosphorylation to become active hence they are not prodrugs (Miller, 2002:17). The HIV reverse transcriptase enzyme is a heterodimer composed of two subunits namely the p66 and p51. The p66 subunit has the binding site for the NNRTIs. The non-competitive binding to this site induces a conformational change that denatures the enzyme (Miller, 2002:17; Sluis-Cremer & Tachedjian, 2008:7). The denatured enzyme is unable to incorporate nucleotides and hence DNA strand is also terminated. In addition, NNRTIs are regarded as more highly specific for HIV-1 reverse transcriptase than the NtRTIs/NRTIs (which also affect host mitochondrial DNA polymerase- γ enzyme), implying lesser adverse effects (Xia *et al.*, 2007:1733).

Rash is the common side effect for all NNRTIs and it usually develops in the beginning weeks of treatment with NNRTI-containing regimens. The rash that occurs in NVP-based regimens is associated with severe hepatotoxicity while somnolence, insomnia, dizziness, impaired concentration, abnormal dreams, depression, psychosis and suicidal ideation may accompany the rash in EFV treated patients (DHHS, 2016:10).

2.5.3 Protease inhibitors (PIs)

The first PI to be approved by FDA is saquinavir in 1995 prior to NNRTI approval (Arts & Hazuda, 2012:4). There are currently 10 agents under this class that are available for use in clinical practice; namely ritonavir, lopinavir, nelfinavir, fosamprenavir, inidinavir, amprenavir, saquinavir, atazanavir, tipranavir and darunavir (Palmisano & Vella, 2011:46). According to Hill and Balkin (2009:35) ritonavir is used in sub-optimal doses (optimal dose in 600mg) as a pharmacokinetic enhancer for lopinavir, darunavir, atazanavir and tipranavir. Protease-mediated maturation of HIV particles is essential for the virus infective consequences. According to Adamson (2012:2) protease inhibitors target and inhibit the HIV protease enzyme. The inhibition is said to be

sufficient to prevent cleavage events of viral proteins that are necessary for viral maturation; hence leading to production of immature non-infectious viral particles. The PIs elicit activity against both HIV-1 and HIV-2 clinical isolates. The development of PIs provided a second mechanistic class of drugs which made antiretroviral therapy combination possible and these drugs have remained a key component of HIV infected patient treatment regimen right up to the current day.

The metabolic adverse effects including dyslipidaemia, insulin resistance and lipodystrophy; and gastrointestinal adverse effects such as diarrhoea, nausea and vomiting are common in patients taking PIs (Hill & Balkin, 2009:37; Hruz *et al.*, 2001:549). These adverse effects are more common in the boosted-PI since the booster drug is also a PI and the fact that the boosted-PI attains higher and sustained plasma concentrations due to pharmacokinetic enhancement (Hill & Balkin, 2009:37).

2.5.4 Newer classes

World Health Organization (2010:39) declared that the three above-mentioned classes of antiretroviral drugs are integral members of the HAART combination. These classes are probably the primitive therapeutic classes that gained huge clinical experience in the 20th century and they are still useful even in the 21st century; although newer classes have been approved. According to Espeseth *et al.* (2000:11249) the integrase enzyme was the most recent HIV enzyme that became a successful target for drug development. The HIV integrase enzyme serves the purpose of translocation and insertion of the proviral DNA into the host DNA to allow for transcription of proviral messenger RNAs that are translated into viral proteins. Raltegravir is the first integrase inhibitor approved in 2007 and this drug progressed through the clinical development (Shimura *et al.*, 2008:722). Integrase inhibitors are thought to bind to the active site of the integrase and inhibiting the proviral DNA strand integration into the host DNA (Espeseth *et al.*, 2000:11248). The integration is a twostep process that entails processing of host cell cytoplasm to allow for transfer of proviral DNA into the nucleus and then covalent linkage of the proviral DNA with the host DNA. These two steps are catalysed by the HIV integrase enzyme, hence they are blocked by the inhibitors of the enzyme (Hazuda, 2010:514). The most common adverse effects to raltegravir are insomnia, headache, dizziness, nausea and fatigue (DHHS, 2016). Raltegravir can cause serious life-threatening skin reactions, allergic reactions and liver problems (DHHS, 2016).

Viral entry currently represents one of the most attractive targets in the identification of novel drugs for HIV infection treatment. According to Briz *et al.* (2006:619) the main steps in the viral entry entail the attachment of the viral gp120 to the host CD4 T cell. The attachment induces conformational change to the HIV such that its gp41 is bound to the CD4 T cell via Carbon-Carbon chemokine receptor type five (CCR5) and Carbon-X-Carbon Chemokine receptor type four

(CXCR4) co-receptors and lastly fusion of the viral membrane with the host cell (CD4 T cell) membrane to allow immersion of viral contents into host CD4 T cell interior (Briz *et al.*, 2006:625). Entry inhibitors are a new generation of antiretroviral drugs represented only by one drug called enfuvirtide that is currently approved by FDA for the treatment of the HIV infection as a fusion inhibitor. In addition, there are also entry inhibitors belonging to the chemokine receptor antagonists' class. Patients treated with enfuvirtide complained of the inflammation at the injection site (Lalezari *et al.*, 2003:2183).

Maraviroc is the only chemokine receptor antagonist approved by FDA in 2007 as a CCR5 inhibitor (Artz & Hazuda, 2012:4). Carbon-Carbon chemokine receptor type five inhibitors are allosteric non-competitive inhibitors that bind to the CCR5 co-receptor and induce the conformational change to the receptor such that the V3 loop of the viral gp120 is unable to recognise the CD4 T cell (Dragic *et al.*, 2000:5643; Latinovic *et al.*, 2009:2). The most frequently reported adverse effects with the use of maraviroc included upper respiratory infections, musculoskeletal and connective tissue signs and symptoms, sleep disturbances, herpes infection, abdominal pain and postural dizziness (Lieberman-Blum *et al.*, 2008:1242).

In summary of drug classes, there are currently over 25 antiretroviral drugs available to treat the HIV infection worldwide. According to Arhel and Kirchhoff (2009:313) majority of these agents target the HIV reverse transcriptase and protease enzymes. More recently, viral integrase and entry inhibitors have been approved. The use of these drugs is associated with different risks of causing adverse events, thus their use especially in combinations should consider overlapping toxicities that may pose risks in using these drugs.

2.6 Treatment with tenofovir-containing HAART regimens

The antiretroviral regimens for initiation adopted by most national treatment programmes in most resource-limited settings included two NRTIs/NtRTI and one NNRTI. The four TDF-containing HAART regimen combination derived from antiretroviral drugs have saved hundreds of thousands of lives and provided hope to millions of others. The regimens are TDF/3TC/NVP, TDF/FTC/NVP, TDF/3TC/EFV and TDF/FTC/EFV. According to Louie *et al.* (2003:1154) TDF is more potent and less toxic than AZT and d4T, with the median decline in plasma viral load in subjects receiving TDF, AZT or d4T mono-therapy at 1.4 log₁₀, 0.5 log₁₀, and 0.5 log₁₀, respectively.

In a randomised, open-label non-inferiority trial, Pozniak *et al.* (2006:538) enrolled 517 antiretroviral-naïve, HIV-infected patients to receive either TDF/FTC/EFV or AZT/3TC/EFV. Through the 96th week of treatment, more patients who received TDF/FTC significantly achieved and maintained a plasma viral level of < 400 copies/ml (75% versus 62%). The TDF/FTC group also demonstrated a significantly greater increase in CD4 cell counts (270 versus 237 cells/mm³;

$p = 0.036$). Moreover, Arribas *et al.* (2008:77) conducted a long-term follow-up of the study for over 144 weeks in which TDF/FTC-based regimen confirmed the superior ability over the AZT/FTC-based regimen to suppress viral load without causing the lipodystrophy (seen with AZT/3TC-containing regimens) and with less effect on lipids.

Gallant *et al.* (2004:198) also conducted a randomised, placebo controlled study, comparing TDF or d4T in combination with 3TC and EFV in 602 treatment-naïve patients. The study results showed equivalences in the percentage of subjects with HIV RNA < 50 copies/ml at week 48 and through 144 with less lipodystrophy prevalence and more favourable lipid profiles in the TDF treated group. In the report on outcomes and toxicities among patients on TDF, AZT and d4T-based first-line antiretroviral therapy in a routine treatment cohort study in Lesotho, Bygrave *et al.* (2011a:77) have provided further evidence that a TDF-based first-line regimen is supportive of simplified care by reducing the rate of regimen substitutions compared with d4T-based and AZT-based regimens. In Zambia, Chi *et al.* (2010:70) observed that TDF is associated with similar clinical and programmatic outcomes as AZT and d4T, but appears to be better tolerated.

Although TDF regimens have been proven to be efficacious, potential long-term adverse effects on kidney function which limit its use for patients at high risk for renal complications were reported. According to Horberg *et al.* (2010:68) TDF had a statistically significant negative impact on renal function of patients. It is also significantly associated with a greater risk of developing proximal tubular toxicity manifesting as Fanconi syndrome.

The clinical trials and short-term studies failed to show evidence of renal toxicity in patients taking TDF-based regimens. In a 48 week randomised, double-blind study, Schooley *et al.* (2002:1262) observed that the safety profile of TDF was similar to placebo through 24 weeks of treatment. Gallant and Moore (2009:1975) were engaged in a 103-week study evaluating renal function with use of a TDF-containing initial antiretroviral regimen; the outcome was consistent with clinical trials in that the results supported the use of TDF as a component of the initial antiretroviral regimen, and suggests that the eGFR should be monitored more closely when TDF is used with a boosted PI.

Gallant *et al.* (2008:2162) further conducted a 144-week, multicentre, randomised, double-blind, active-controlled trial to evaluate the efficacy and safety of TDF compared with d4T, in combination with 3TC and EFV in antiretroviral-naïve patients. The results demonstrated small decreases in glomerular filtration rate over time in antiretroviral-naïve patients with adequate renal function at baseline who are treated with TDF. This is consistent with data reported from cohort studies conducted by Winston *et al.* (2006:109) and Nelson *et al.* (2007:1278). Nelson *et al.* (2007:1278) investigated the safety of TDF for the treatment of HIV infection in adults. They found that the incidence of renal serious adverse events of any kind in the TDF-expanded access

programme was 0.5% in the first four years. This was similar to the 0.3% rate reported for TDF-treated patients in the smaller cohort study of Winston *et al.* (2006:109).

2.7 Therapeutic outcomes of tenofovir-containing HAART regimens

The effectiveness of antiretroviral therapy is monitored by the assessment of patients' clinical status, immunologic and virologic functions (MOH, 2013:37). However, the virologic monitoring is the gold standard of monitoring antiretroviral therapy efficacy (Sawe & McIntyre, 2009:463). These outcomes of effectiveness to antiretroviral therapy are covered in detail in this section.

2.7.1 Clinical outcomes of tenofovir-containing HAART regimens

The clinical indices useful for assessing the response to antiretroviral therapy include betterment feeling and improved energy to perform daily tasks, gain in body weight, growth in children, response of current signs and symptoms and reduced incidence of opportunistic diseases (Beard *et al.*, 2008:7; MOH, 2013:37; Mukherjee *et al.*, 2014:6). In a systematic analysis done by Beard *et al.* (2008:6), HIV/AIDS patients on antiretroviral therapy had improved emotional, mental and physical wellbeing in comparison to those who were not receiving the treatment. Work attendance, productivity and performance also appeared to improve amongst treated patients following initiation of antiretroviral therapy. Mukherjee *et al.* (2014:6) had furthermore shown that antiretroviral therapy is effective and safe in HIV-infected children and has been associated with improvements in growth indicators over long-term treatment.

In Lesotho, the useful indices in monitoring clinical response to antiretroviral therapy include:

- Whether the patient feels better and has more energy to perform daily tasks?
- Does the patient gain weight?
- Is the patient improving from the original signs and symptoms of presenting illness?
- Whether the patient is free from moderate or severe opportunistic infections? (MOH, 2013:37).

2.7.2 Immunological outcomes of tenofovir-containing HAART regimens

Cooney (2002:233) recognised a number of surrogate markers of immunology function but has indicated that the CD4 cell count is the only currently recommended marker of immunologic response to antiretroviral therapy. Lima *et al.* (2009:195) have supported the assessment of antiretroviral therapy-mediated immunologic response using a change in CD4 cell count. The rise in CD4 cell count has been correlated with durable treatment with antiretroviral therapy and the

evidence that the CD4 cell count could return to values observed in HIV negative individuals has been obtained by Smith *et al.* (2003:969).

2.7.3 Virological outcomes of tenofovir-containing HAART regimens

The plasma viral load is traditionally used as the biomarker of HIV replication. The viral load is expressed as the number of viral RNA copies per millilitre of plasma. The level of HIV RNA in patient plasma remains an important marker for determining the success of antiretroviral therapy in treated patients (Palmer, 2013). According to Meintjes *et al.* (2014:127) the virology measure of antiretroviral therapy treatment success is defined by a decline in plasma HIV RNA concentration < 50 copies/ml within six months of 95-105% adherence to antiretroviral therapy and sustained thereafter. Therefore, the main goal of antiretroviral therapy is to suppress plasma viral concentration to below detectable limits and to maintain the undetectable levels (Pasternak *et al.*, 2013:1).

In a randomised, open-label, non-inferiority trial done by Laurent *et al.* (2011:832) to assess the effectiveness and safety of clinical monitoring alone against both laboratory and clinical monitoring, they found that the small difference noted between these two approaches suggested that clinical monitoring alone temporarily be useful to expand antiretroviral therapy in resource-limited settings. In settings of available resources, viral load testing as the gold standard to monitor antiretroviral therapy and it should be done (Sawe & McIntyre, 2009:463). However, clinical and immunological monitoring still provides benefits in resource-limited settings. According to WHO (2000:1) laboratory monitoring in antiretroviral therapy focuses on markers on efficacy and toxicities of the drugs.

2.8 Renal toxicological outcomes of tenofovir-containing HAART regimens

This section entails the adverse effects of HAART regimens. More details are provided on renal adverse effects attributable to TDF. The evaluation of renal function and toxicity is also covered.

2.8.1 Adverse effects of tenofovir-containing HAART regimens

The implementation of HAART has provided an extraordinary clinical benefit in HIV-infected patients by lowering morbidity and mortality associated with the infection (Crabtree-Ramirez *et al.*, 2010:378; Kalyesubula & Perazella, 2011:1; Lohse *et al.*, 2008:57; Resino *et al.* 2004:1611). However, Fortuny *et al.* (2015:40) have realised that complications of long-term use of antiretroviral drug use remain a major problem especially in paediatric HIV-infected patients. These complications have been correlated with a major impact on the quality of life and adherence to treatment amongst these patients. Moreover, there is a foreseeable progression into early cardiovascular and cerebrovascular accidents, renal failure, diabetes mellitus or pathologic

fractures when the children and adolescence population enters their third 3rd and 4th decades of their life.

The Food and Drug Administration (FDA) approves drugs for use by patients when the benefits of using these drugs are thought to outweigh the risk associated with these drugs, not because there are no inherent risks to using the drugs (U.S. Food and Drug Administration, 2016:2). According to Calmy *et al.* (2009:165), there are several life-threatening adverse effects related to antiretroviral drugs and some of these effects have been noticed following drug approval. The adverse effects of antiretroviral drugs are lactic acidosis, hepatotoxicity, hypersensitivity, genotoxicity, cardiovascular diseases, cerebrovascular accidents, dyslipidaemia, lipodystrophy, renal diseases, osteoporosis and osteonecrosis (Calmy *et al.*, 2009:165; DHHS, 2016:13; Hill & Balkin, 2009:37; Hruz *et al.*, 2001:549; Kalayjian *et al.*, 2012:1914; Sabin *et al.*, 2008:1425).

Maagaard *et al.* (2006:57) have shown that depletion in peripheral plasma mitochondrial DNA concentration is associated with NRTI/NtRTI exposure in HIV-infected patients. The depletion could result in lactic acidosis (hyperlactatemia) and a wide spectrum of clinical abnormalities such as generalised neuropathy (autonomic and peripheral), myopathy (cardiac and skeletal), pancreatitis, steatohepatitis and lipoatrophy (Calmy *et al.*, 2009:165). Despite the fact that most cases of NNRTI-induced hepatotoxicity are asymptomatic, the rates of symptomatic liver toxicity events in patients treated with nevirapine are greater than in patients treated with EFV (Rivero *et al.*, 2007:345). Renal toxicity has been mainly associated with two antiretroviral drugs, namely indinavir (IDV) and TDF. Boyd (2007:1162) evaluated that IDV renal toxicity generally occurs with renal colic or as gradual onset tubule-interstitial nephritis or rarely as acute renal failure.

2.8.2 Renal toxicity of TDF

The renal proximal tubule is the major site for TDF toxicity since this is the site at the nephron where the drug is excreted by secretion (Woodward *et al.*, 2009:483). Hagos and Wolf (2010:2065) and Kohler *et al.* (2009:5) associated the drug with this site due to the presence of transporters belonging to the solute carrier family 22 (SLC22) called human organic ion transporter-1 (hOAT-1) and adenosine triphosphate-binding cassette transporter family member called multidrug resistance protein-4 (MRP-4). These are transporters of TDF from the blood circulation into the proximal tubule intracellular space and secreting the drug into the urinary space respectively as shown in figure 1 below.

The renal toxicity of TDF occurs as a result of both or one of (i) excessive proximal tubular uptake of TDF from blood by hOAT-1 which accumulates the drug intracellularly (proximal tubule cell) and (ii) following disrupted secretion of TDF into the urine by MRP-4 that also poses a risk to accumulation of the drug inside proximal tubule cell. According to Kohler *et al.* (2011:7) the

expression of kidney transporters may vary between individuals and also due to certain genetic regulation and down-regulation of genes that encode for transporters' synthesis by TDF. Lerma and Nissenson (2012:81) and Fernandez-Fernandez *et al.* (2011:8) contend that the most common renal toxic effects of TDF are acute kidney injury and Fanconi syndrome; rarely nephrogenic diabetes insipidus.

In a case report of a 37-year old later presenting HIV-positive male patient with no medical history of risk factors related to renal toxicity, TDF-containing HAART regimen was initiated. Two days following initiation, renal function laboratory tests revealed steadily increasing creatinine and urea and attaining maximal level at 6th day despite intravenous volume substitution. Schleenvoigt *et al.* (2011:564) concluded that the patient developed acute kidney injury according to the Risk, Injury, Failure, Loss and End-stage kidney (RIFLE) criteria. The RIFLE criteria indicate that kidney injury is confirmed at twice upper limit of normal of serum creatinine concentration and at < 0.5 ml/kg/hr urine output in 12 hours (Hoste *et al.*, 2006:3).

Leem *et al.* (2014:246) have characterised Fanconi syndrome by urinary loss of phosphate, bicarbonate, potassium, glucose, amino acids and low-molecular weight proteins. Therefore, Min *et al.* (2013:1) regarded Fanconi syndrome as a generalised proximal tubule transport defect that may be associated with drug-induced acute tubule-interstitial nephritis. Several drugs including TDF cause renal failure associated with proximal tubule tubule-interstitial nephritis that may result in partial or complete Fanconi syndrome (Fernandez-Fernandez *et al.*, 2011:8).

2.8.3 Evaluation of renal function and toxicity

Kim and Moon (2012:268) contend that evaluation of renal function through blood tests involves measurements of blood urea nitrogen and serum creatinine concentration. The renal clearance concept was put in place to express the relationship between excretion per unit time and the plasma concentration which becomes the index of kidneys' ability to clear blood of any substance. Although not identified yet, an ideal marker should be freely filtered through the glomerulus, neither secreted nor reabsorbed by the renal tubule and it should be eliminated by the kidneys only (Sirwal *et al.*, 2004:121).

Traditionally, a single plasma creatinine measurement was used to determine the glomerular function, diagnosis and staging of chronic kidney disease (Florkowski & Chew-Harris, 2011:75). However, due to several factors including muscle mass, diet, sex, age and ethnicity that affect plasma creatinine, exogenous markers were used. According to Stevens *et al.* (2006:2473) the use of exogenous markers such as inulin is complex, expensive and difficult to perform in routine clinical practice.

Kim and Moon (2012:268) further outline that calculations on glomerular filtration rate and creatinine clearance are also useful in evaluation of renal function. Glomerular filtration rate is measured as the urinary or plasma clearance of an ideal filtration marker and is accepted as the best overall measure of kidney function (Stevens *et al.*, 2006:2473). Glomerular filtration rate measurements are based on renal clearance of a marker in plasma, usually expressed as the volume of marker-free blood per unit time (Sirwal *et al.*, 2004:121).

Inadequacies of single serum creatinine measurement led to the recommendation from the National Kidney Foundation Disease Outcomes Quality Initiative (K-DOQI) to use prediction equations that estimate glomerular filtration rate based on serum creatinine (Florkowski & Chew-Harris, 2011:75). Cockcroft and Gault equation was then developed to predict the creatinine clearance based on age, sex, body weight, plasma creatinine and height, together with correction factors (Florkowski & Chew-Harris, 2011:76; Stevens *et al.*, 2006:2476). The equation had many inherent limitations although helpful; this led to the development of the MDRD study equation to adjust for body surface area since the Cockcroft and Gault equation was claimed to overestimate glomerular filtration rate.

Recently, the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) has been developed and been considered as a more accurate measure of eGFR even though it has its own limitations (Willems *et al.*, 2013:1). Age has been found to influence the results from these equations. The other equation(s) overestimate eGFR in the elderly patients while others underestimate the eGFR in these patients. According to Willems *et al.* (2013:4) Cockcroft and Gault equation gives lower estimates of renal function in comparison with MDRD study and CKD-EPI. Again, the prevalence of renal toxicity at the age of 85 years and above was highest with Cockcroft and Gault and lowest with MDRD study and CKD-EPI in the middle. MDRD study was also found to be a good predictor of mortality.

2.9 Determinants of antiretroviral therapy outcomes

This section provides details of various factors influencing the outcomes of antiretroviral therapy. There are factors with positive influence and negative influence on the antiretroviral therapy outcomes. The indicators of response to therapy are also covered. These factors are classified into those evaluated by the present study and those that worth consideration but were not used by the present study.

2.9.1 Determinants of the research interest

This is the subsection of a group of factors influencing outcomes to antiretroviral therapy and indicators of response to antiretroviral therapy. The factors under this subsection presents the factors that the study has used them as study variables.

2.9.1.1 Age

The United Nations Programme on HIV/AIDS (2006:83) revealed that an estimate of 2.8 million adults aged ≥ 50 years worldwide are living with HIV/AIDS in the years 2000 to 2005. According to Greig *et al.* (2012:34) 1 in 10 HIV-infected adults in the sub-Saharan Africa are ≥ 50 years. Furthermore, reports in sub-Saharan Africa suggested that older patients are more likely to develop antiretroviral-related adverse effects when compared to younger counterparts. These outcomes were suggested to result from late antiretroviral therapy initiation and/or delayed CD4 T cell recovery in the elderly (Greig *et al.*, 2012:34). In a prospective observational study conducted by Bakanda *et al.* (2011:704) on association of age and survival in Uganda, the study unveiled that elderly patients receiving antiretroviral therapy have comparatively poorer survival outcome than the non-elderly cohort with the disease. In Zambia, Vinikoor *et al.* (2014:954) added that initiating antiretroviral therapy in older age is associated with decreased immune recovery and increased mortality when compared to initiating it at younger age. The clinical improvement of the body weight was also associated with age differences at initiation of antiretroviral therapy. The antiretroviral therapy initiation at early ages had the greatest weight recovery than in the higher ages (McGrath *et al.*, 2011:351).

Violari *et al.* (2008:8) strongly recommended that for HIV infected infants, immediate antiretroviral therapy initiation should be due to better survival benefits. In a trial conducted by Laughton *et al.* (2012:6) the findings revealed that early antiretroviral therapy in infants improves neurodevelopmental outcomes. The initiation of antiretroviral therapy demonstrated that excellent growth outcomes can be achieved in virally suppressed children on treatment (Shiau *et al.*, 2013:8). The outcome is strong evidence that early antiretroviral therapy initiation plays an essential role in the rate of growth recovery in young children living with HIV infection.

However, Resino *et al.* (2008:7) argued in their retrospective cohort study conducted in Spain from 1996 to 2006 (10 years) that having low CD4 cell count at baseline hinders the immune recovery in majority of children, even for over eight years of follow up. On the other hand, those with rapid immune reconstitution experienced high rates of metabolic disorders. Although children who have lower CD4 threshold have reduced likelihood of effective disease progression, it may not be optimal for them to achieve and maintain desirable peak CD4 level (Lewis *et al.*, 2012:555). While it is well-known that antiretroviral therapy initiation leads to viral suppression coupled with recovery of immunological functions in most HIV-infected children, determinants of immune recovery needs to be understood. Ruel *et al.* (2009:4) explored the dynamics of T-cell activation in HIV-infected African paediatric patients following antiretroviral therapy initiation. This prospective study conducted in Uganda revealed that children who were on antiretroviral therapy for at least 24 weeks experienced significant reduction in levels of immune activation when compared to antiretroviral therapy naïve HIV-infected patients. Generally, early initiation of

antiretroviral therapy in children infected with HIV-1 significantly improves the immunologic outcomes within four years of treatment (Yin *et al.*, 2016:1110).

AlAhmadi and AbuButain (2013) indicated that each of the two fully functional human kidneys contains approximately one million nephrons. Weinstein and Anderson (2010:1) added on to outline that glomerular filtration rate approaches adult levels by the end of two years of life and maintained until the fourth decade of life. Beyond the fourth decade, AlAhmadi and AbuButain (2013) contend that approximately 10% of the nephrons are lost in each kidney at every 10 years gained. Renal aging is a complex multi-factorial process that predisposes to acute kidney injury in the elderly population. This is due to a decreased tissue repair in the aging kidney (Wang *et al.*, 2014:15367).

The evidence that HIV-infected patients develop age-related diseases at a faster pace has threatened the goal of providing infected people with normal lifetime treatment. Although there are many factors that can increase risk of renal disease, antiretroviral agents, including PIs appear to pose a greater risk of developing and exacerbating renal impairment than other drugs (Weinstein *et al.*, 2016).

2.9.1.2 Sex

Although mechanisms underlying sex differences in childhood infections are poorly understood, sex difference susceptibility and mortality from infectious diseases in children play role in greater disease burden and death (Muenchhoff & Goulder, 2014:124). In an observational cohort study conducted in South Africa, Mori *et al.* (2015:11) focused on sex differences in paediatric HIV infection and specifically on the initiation of antiretroviral therapy and post-treatment outcome. The cohort study showed that females have higher CD4 cell counts from birth amongst both HIV-infected and the uninfected group. The observation was coupled with improved immune recovery on antiretroviral therapy in females compared to males upon treatment initiation. Therefore, the recommendation from the study was to initiate antiretroviral therapy to males based on CD4 criterion while females could be initiated based on clinical disease progression since there was increased mortality in females as a result of later than optimal initiation of ART.

In the review of sex differences on retention and survival among HIV-1 infected adults on antiretroviral therapy in Malawi, Taylor-Smith *et al.* (2010:52) found that HIV-infected men (health workers, teachers, police and army personnel) have significantly higher mortality than women counterparts. The difference was observed to be due to men starting treatment at later clinical stage than women. In the previous report on the same country (Malawi), Chen *et al.* (2008:517) exposed that males generally sought medical care at a later clinical stage due to the culture of

masculinity. Across the various occupations, females had better rates of survival and losses on follow up compared to worst outcomes observed in males.

However, setting the PMTCT initiative as the counterexample, women have been prioritised by funding agencies, international organisations and local governments (U.S. President's Emergency Plan for AIDS Relief (PEPFAR), 2011). According to Braitstein *et al.* (2008:49) these efforts have proportionally resulted in greater enrolment of women in HIV testing and treatment. Therefore, males seek medical attention in advanced disease, which is strongly associated with poorer outcomes. This is why several studies have found that males have higher mortality following antiretroviral therapy initiation than females (Cornell *et al.*, 2012:6; DeSilva *et al.*, 2009:4; Mills *et al.*, 2011:6). The reasons that were proposed from several investigations for this difference included inequalities in health care systems in low-to-middle-income countries, poorer treatment adherence, irregular clinic attendance due to responsibilities and work-related matters, higher baseline mortality rate for males in general population and potentially biological differences (Cornell *et al.*, 2012:6; Druyts *et al.*, 2013:424).

The correlations between sex differences and individual's ability to tolerate antiretroviral drugs have increasingly being evaluated. A range of observations suggested that women are more likely to experience adverse effects from antiretroviral drugs than men (Ofotokun & Pomeroy, 2003:58). Nevirapine-associated rash and antiretroviral therapy-associated metabolic disorders have been reported to be more frequent in women (Bersoff-Matcha *et al.*, 2001:127; Pernerstorfer-Schoen *et al.*, 2001:732). According to Ofotokun (2005:81) women experience greater toxicity with all antiretroviral agents. Although the sex differences in the effects of these drugs may partly be due to physiologic and hormonal dimorphism between men and women, variations in these drugs' metabolising enzymes and transporters may also be implicated.

Sex differences in renal nitric oxide levels are related to relationship between nitric oxide and prostaglandin E₂, which is a stimulant of renal endothelial nitric oxide synthase (Weinstein & Anderson, 2010:4). Nitric oxide has overall protective effect in kidneys due to decreased matrix production and mesangial cell growth (Weinstein & Anderson, 2010:4). According to Baylis (2009:393) premenopausal women are substantially protected from age-dependent decline in renal function that occurs in men. This is because the female sex resists this decline via oestrogen-stimulated nitric oxide production.

2.9.1.3 Treatment duration

The acquisition of HIV infection leads to immunological damage that is not full reversible by antiretroviral therapy especially in late stages (Kelley *et al.*, 2009:792). Hecht *et al.* (2006:732) found that a short course of antiretroviral therapy at HIV primary infection may preserve the

immune function. Two clinical trials conducted in 2012 by Hogan *et al.* (2012:94) and Grijzen *et al.* (2012:10) evaluated the effectiveness of short course antiretroviral therapy versus deferred treatment on plasma HIV RNA levels. These trials unveiled a clear clinical benefit of temporary antiretroviral therapy treatment. Again, the findings revealed those patients on short course to have the modest need for subsequent long term antiretroviral therapy initiation than the untreated group. Fidler *et al.* (2013:216) also conducted a 48-week open-label, randomised controlled trial on adults primarily infected with HIV. The results of the trial showed that 48-week antiretroviral therapy course delayed the CD4 cell count decline to <350 cells/mm³.

Consistently, a cohort study conducted at Themba Lethu clinic, Johannesburg in South Africa (four-year follow-up) by Sanne *et al.* (2009:9) has unveiled that following a good long-retention on antiretroviral therapy that was observed, there were excellent clinical, immunologic and virological outcomes. Although long term antiretroviral therapy treatment for more than 10 years does not completely eradicate the HIV virus, Buzon *et al.* (2014:10062) emphasised that maintenance of low residual viral reservoirs together with HIV-1 specific CD₈ T cells, can make patients with early HIV infected treatment respond in a clinically significant manner. The early initiation of treatment at the time of seroconversion may improve the viral control by limiting the extension of viral reservoirs. Since the viral reservoirs occur rapidly upon acquisition of the infection without antiretroviral therapy, early treatment initiation has been shown to lower viral reservoir sizes and transcription level in blood (Malatinkova *et al.*, 2015:8).

2.9.1.4 Body weight

In Nepal between 2006 and 2011, higher mortality in HIV-infected patients was associated with poor baseline clinical features including low body weight (>45 kg), advanced WHO clinical stage and bedridden performance status (Bhatta *et al.*, 2013). However, in another study conducted by Setegn *et al.* (2015:6) in south western Ethiopia, the odds of mortality were higher in patients with Body Mass Index (BMI) ≥ 25 kg/m² than those with normal BMI (18.5 - 24.9 kg/m²). These obesity-related higher odds of dying were suggested to be associated with other chronic co-morbidities related to overweight and obesity namely diabetes mellitus, hypertension and chronic kidney disease.

Obesity is an independent risk factor of chronic kidney disease. While obesity is a risk factor for two major causes of chronic kidney disease namely diabetes mellitus and hypertension, obesity itself may increase susceptibility to chronic kidney disease via several potential mechanisms such as central adiposity that links obesity and overweight to the development of metabolic syndrome (Kramer, 2006:13). According to Ejerblad *et al.* (2006:1697), men and women who reported a BMI of ≥ 25 kg/m² at age of 20 years had a significant three-fold increased risk for chronic renal failure compared with patients with BMI <25 kg/m². While antiretroviral therapy is associated with

clinical improvements including renal function over time, overall rates of decline in eGFR were however still significant in patients successfully treated with antiretroviral therapy (Choi *et al.*, 2009:2148).

2.9.1.5 CD4 cell count

There exists strong evidence that the CD4 cell count prior to antiretroviral therapy initiation is a determinant of survival and death (Sterne, 2009:1359). Again, trends in CD4 cell count upon antiretroviral therapy commencement vary relative to baseline values. The parameter has a variety of attributes to offer in HIV/AIDS clinical management. The CD4 cell count is an important indicator of immunological status of the patient at baseline, immunological response to antiretroviral therapy relative to treatment durability, an eligibility criterion for initiating antiretroviral therapy and treatment for opportunistic infections and a determinant for detection of treatment failure (MOH, 2016a:29). According to Lesotho MOH (2016a:30) CD4 cell count can be used to categorise the immunological status of HIV-infected patients, whereby CD4 counts of >500, 350-499, 200-349 and < 200 cells/mm³ indicated no significant immunodeficiency, evidence of mild immunodeficiency, moderate immunodeficiency and severe immunodeficiency respectively.

The degree of CD4 cell count depletion prior to initiating antiretroviral therapy is the most consistent measure of immune recovery upon antiretroviral therapy commencement. According to Lawn *et al.* (2005:2147) most early deaths occur in patients with advanced immunodeficiency (median CD4 of 94 cells/mm³), who have not been initiated on antiretroviral therapy. In the meta-analysis conducted on 18 HIV infection cohort studies on timing of antiretroviral therapy initiation, Sterne (2009:1359) found that in resource-constrained settings, deferring antiretroviral therapy initiation until CD4 cell count declines to less than 350 cells/mm³ actually possess more risk to developing endpoint AIDS or death when compared to patients initiated on treatment at CD4 cell count between 350 and 450 cells/mm³. As such, the excess of AIDS or deaths occur in patients who started antiretroviral therapy at lower CD4 cell count threshold. On the other hand, initiation of antiretroviral therapy with higher CD4 cell counts at baseline is associated with clinical advantages and a better immune recovery.

Despite baseline characteristics including CD4 cell count, infants on antiretroviral therapy in Kampala, Uganda showed a significant elevation of CD4 from 23% to 30% in the first six months of antiretroviral therapy ($p<0.001$) (Tukei *et al.*, 2013). The results were replicates of findings documented by Janssen *et al.* (2010:417) when evaluating paediatric survival from HIV/AIDS treatment in rural primary care in KwaZulu Natal, South Africa, whereby a substantial increase in CD4 percentage from 17% to 22% ($p<0.001$) was observed following 6-12 months of treatment. Adults aged >30 years were found to have long-term immunologic reconstitution despite having almost identical baseline CD4 cell count with adolescents and young adults (Nachega *et al.*,

2009:5). The reason for the difference was attributed to poor adherence amongst the adolescents and young adults.

Patients who were initiated on antiretroviral therapy at lower baseline mean CD4 cell count had a lower incremental rate and experienced first-line antiretroviral therapy immunologic failure at the 7th year of treatment (Musa *et al.*, 2015:307). Upon switching to second-line regimen, the experienced elevations in CD4 to values comparable to those experienced while in active stage of the first-line regimen.

2.9.1.6 Plasma viral load

The HIV viral load refers to the number of HIV RNA copies per millilitre of plasma. While the HIV mainly resides intracellular, plasma measurement is an accurate reflection of the burden of HIV infection and the extent of viral multiplication. The measurement provides guide to initiation of antiretroviral therapy, useful to assess the risk of disease progression and is critical for monitoring virological response to treatment. Higher baseline plasma viral load is a known predictor of antiretroviral treatment failure (Lundgren *et al.*, 2002:179). Therefore, Sawe and McIntyre (2009:463) regarded viral testing as the gold standard to monitor antiretroviral therapy. Plasma viral load has been regarded as the best predictor of clinical outcome, followed by CD4 cell count. According to Mylonakis *et al.* (2001:485) plasma viral load and CD4 cell count provided more prognostic information than either predictor alone.

There exists an inverse relationship between the CD4 cell count and survival as higher HIV RNA levels are associated with more rapid decline in CD4 cell count and thus greater disease progression (Thiebaut *et al.*, 2005:16). According to Goujard *et al.* (2006:713) both CD4 cell count and plasma viral load are independent predictors of disease progression following primary HIV infection in untreated patients. In permissible resources, the inclusion of plasma viral load testing prior to antiretroviral therapy initiation could be a useful way to increase prevention benefits as it allows identification of infected persons with highest risk of HIV transmission (Murnane *et al.*, 2012:4).

In a prospective interventional study conducted in rural communities of South Africa and Uganda on evaluating viral suppression following antiretroviral therapy initiation, Barnabas *et al.* (2014:9) found that with 12 months of antiretroviral therapy to eligible patients, 80% of them were virally suppressed. According to Patel *et al.* (2007:1652) 73% of pregnant women who initiated antiretroviral therapy during antenatal care had undetectable plasma viral loads at the time of delivery while the remaining had generally low detectable viral loads upon delivery. Robbins *et al.* (2007:32) showed that when using the virologic failure criteria alone in their study, 27.2% of the patients experienced at least one episode of treatment failure as evidenced by HIV RNA levels

>400 copies/ml during the two-year study period. In South Africa, 8-17% of patients who failed first line treatment in five years of treatment were attributable to virologic treatment failure (Boulle *et al.*, 2010:568).

2.9.1.7 Serum creatinine concentration

Creatinine is a by-product of muscle metabolism that is chiefly removed through filtration from blood by the kidneys. The serum creatinine measurement is the most widely used and commonly accepted measure of kidney function in clinical settings. However, serum creatinine may be misleading when determining kidney function (Swedko *et al.*, 2003:356). For example, patients with low muscle mass may have approximate near-normal serum creatinine levels while having a severely compromised renal function and vice versa.

The National Kidney Foundation (NKF) (2002:81) contended that eGFR should be calculated from serum creatinine concentration and other influential parameters such as age, sex, race and body size. This can assist physicians with the early detection of renal disease. Several serum creatinine-based equations for estimating renal function in adults have been previously discussed (refer to **section 2.11.3**). The most commonly used one are the Cockcroft-Gault equation for estimating urinary creatinine clearance and the Modification of Diet in Renal Disease Study prediction equation for glomerular filtration rate.

Cohen and Kimmel (2007:2117) found that untreated patients with HIV infection develop a form of renal disease referred to as HIV-associated nephropathy characterised by rapid progression to renal toxicity. According to Kamga *et al.* (2011:36) HIV/AIDS patients have reduced creatinine clearance and thus glomerular filtration rate compared to uninfected counterparts. The reduction in kidney function was not attributed to antiretroviral therapy; rather, the initiation of antiretroviral therapy may instead improve kidney function since it reduces AIDS-related kidney disease by depleting renal HIV reservoirs. However, antiretroviral agents such as TDF and protease inhibitors are known to cause renal toxicity of their own (Pujari *et al.*, 2014:6; Reust, 2011:1450).

2.9.2 Other determinants not investigated by the study

This section consists of factors influencing outcomes to antiretroviral therapy that were not used by the present study as variables, but are factors and indicators to consider in evaluation of outcomes to antiretroviral therapy.

2.9.2.1 WHO clinical staging

In a prospective cohort study conducted by Huang *et al.* (2015:6) in China (2013-2014) on the risk factors to antiretroviral therapy treatment outcomes, patients who were on advanced WHO

clinical stage (stage IV) at baseline experienced immunological and virological failure. These findings are comparable to those found in a retrospective cohort study conducted by Ayele *et al.* (2015:6) (2007-2011) in four selected health facilities of southern Ethiopia. In this study, it was found that the WHO clinical stage was a strong predictor of mortality. According to the results of the study, patients with baseline advanced clinical stages (III & IV) had almost twice the risk of death within the cohort study period (five years) when compared to those who were in earlier stages (I & II). Other African countries including Ethiopia, South Africa and Cameroon had previously shown that WHO clinical stage gives a very strong prediction of mortality even following attainment and maintenance of CD4 cell count at goal (Fatti *et al.*, 2010:8; Sieleunou *et al.*, 2009:42).

The low-income countries in the continents of Africa, Asia and South America had the highest rates of mortality during the first months of antiretroviral therapy compared to the high-income countries (Europe and North America). The rates were explained to be related to lower baseline CD4 cell counts coupled with advanced HIV infection clinical stages in the low-income countries (Egger & Braitstein, 2006:822). In an antiretroviral therapy effectiveness assessment done in Malawi following scaling up of the treatment, Ferradini *et al.* (2006:1338) identified four independent predictors of mortality at baseline namely BMI of $<18.5 \text{ kg/m}^2$, CD4 cell count $< 50 \text{ cells/mm}^3$, male sex and WHO clinical stage IV without CD4 test.

Ayele *et al.* (2015:6) suggested that the possible reason attributed to the escalating mortality due to advanced clinical stage being increased susceptibility for contracting opportunistic infections. All participants enrolled in a prospective, cross sectional study done by Kong *et al.* (2007:66) in Cambodia were in advanced HIV/AIDS clinical stages and had opportunistic infections ($n = 100$). Most of these patients had absolute CD4 cell count $< 50 \text{ cells/mm}^3$ (88%). According to Ngowi *et al.* (2008:5) in evaluation of pulmonary TB among people living with HIV/AIDS in northern Tanzania, positive pulmonary TB test results were obtained only from patients at HIV/AIDS clinical stage III and IV in that cross sectional study.

2.9.2.2 Opportunistic infections

Generally, HIV-infected patients are at risk of developing opportunistic infections including pulmonary TB, cryptococcal meningitis, *Pneumocystis jirovecii* Pneumonia (Corbett *et al.*, 2002:1257; Holmes *et al.*, 2003:658). According to Lawn *et al.* (2008:6), in many resource limited settings of Africa, TB is the most common opportunistic AIDS-defining cause of mortality in patients initiating antiretroviral therapy. Jarvin *et al.* (2010:3) observed that multiple occurrences of opportunistic infections may be suggestive of possible interactions between HIV-associated opportunistic infections. In this study when assessing whether HIV-associated TB is a risk factor for developing cryptococcal disease, Jarvin *et al.* (2010:3) found that up to a two-year history of

pulmonary TB may be an independent predictor of the consequential development of cryptococcal meningitis.

The multivariate Cox model analysis conducted by Ayele *et al.* (2015:4) revealed that the risk of dying in patients with two or more opportunistic infections following antiretroviral therapy initiation was almost three-fold compared to patients who had no opportunistic infections initially. Ferrener *et al.* (2013:197) further suggested that there are possible interactions within opportunistic infections that need attention as some do influence even the immune system recovery by antiretroviral therapy. There are common interactions between bacteria and fungi that are medically important (Spear *et al.*, 2008:3). According to Peleg *et al.* (2010:247) these interactions are highly complex and their occurrence depends on variety of factors ranging from environmental, pathogen and host factors. For example, in an investigation to evaluate the association between the levels of vaginal interleukin-8 cytokines with *Candida albicans* and lactobacilli in HIV-infected women, Spear *et al.* (2008:3) found that there is a positive association in the lower genital tract interleukin-8 cytokines with *Candida albicans*. In contrast, there was a negative association between the levels of vaginal interleukin-8 cytokines and lactobacilli.

Although the initiation of antiretroviral therapy reduces the incidences of opportunistic infections and improves quality of life (Volberding & Sullivan, 2002:104), the development of opportunistic infections after initiation of antiretroviral therapy is an indication of treatment failure and poor immunological response to antiretroviral therapy (Ayele *et al.*, 2015:8). Negative changes in haemoglobin concentration and body weight occurred in 15.4% and 16% of patients respectively in a retrospective cohort study done by Rajasekaran *et al.* (2007) on HIV-infected patients enrolled in a government-sponsored antiretroviral programme. Treatment failure was then confirmed in those patients following drug adherence greater than 95% (92.7%) with a decrease in CD4 cell count to or below the baseline and before treatment. There was 50% decrease either from the on-treatment peak CD4 cell count value or CD4 cell count persistently maintained at < 100 cells/mm³.

In an eight-year (1994-2003) cohort study conducted on 581 HIV-infected patients on antiretroviral therapy, 126 (21.7%) patients were diagnosed with chronic hepatitis B viral infection (Sheng *et al.*, 2004:1474). These hepatitis B co-infected patients had higher rates of virologic failure than those without the infection. In another cross-sectional study conducted in Tanzania, Mgelea *et al.* (2014:2) unveiled that 39.0% of the patients who were confirmed to have virologic, immunologic and clinical failure had also a history of opportunistic infections while on antiretroviral therapy. Pulmonary TB was the mostly reported opportunistic infection in the study.

While the retrospective cohort conducted in Canada (a developed setting) in the HAART era indicated that AIDS-defining events are no longer main causes of death as opposed to during

pre-HAART era (Krentz *et al.*, 2005:105), mortality patterns differ widely in resource-limited settings. In resource-limited settings, patients seek medical care at advanced immunocompromised stages when opportunistic infections are out of control (Egger & Braitstein, 2006:822). The initiation of antiretroviral therapy in resource-limited settings was associated with higher mortality rates in the first months of treatment when compared to high-income countries (Egger & Braitstein, 2006:822).

2.9.2.3 Co-morbidities

Krentz *et al.* (2005:105) had indicated that HAART has improved the overall health status of majority of HIV-infected patients by increasing, stabilising or delaying the decline CD4 cell count in the general population. These improvements have been witnessed by significant reductions in mortality due to AIDS-related causes as well as increased survival as shown by extended mean age. However, the study also revealed that the risk of dying from causes not related to AIDS has increased owing to a variety of reasons. This finding was replicated in the prospective multicentre observational cohort study (eight years) conducted by Palella *et al.* (2006:33) in Chicago. The study has shown that the proportion of deaths due to non-AIDS-related diseases has increased owing to non-AIDS-related malignancies, cardiovascular and hepatic causes.

In the era of HAART, Bedimo *et al.* (2009:7) disclosed that following the adjustments of age, sex and race, the incidence of non-AIDS defining malignancies is significantly higher in the HIV-infected population than the uninfected group. Furthermore, Bonnet *et al.* (2009:637) proceeded to contend that mortality rates due to AIDS-defining cancers in HIV-infected patients stabilised between the years 2000 and 2005 while the non-AIDS-defining lung, nose and throat, digestive and pancreatic, anal, skin and breast cancers increased proportion of deaths in HIV-infected people.

According to Marin *et al.* (2009:1750) HIV infection may contribute to cancer development due to chronic cytokine-mediated inflammation that occurs in immune-compromised patients, leading to the development of non-AIDS defining cancers and reduced ability of the body to control cancer-causing viruses. The cardiovascular and hepatic diseases were the next most common causes of death in the findings of Cowell *et al.* (2015:612). Similarly, Morlat *et al.* (2014:1188) disclosed that the proportion of deaths owing to cardiovascular causes increased from 8% to 10% between 2000 and 2005. In 2010, 13% and 30% of the deaths occurred due to hepatitis B and C infections respectively. The high prevalence on non-AIDS-related cardiovascular and hepatic diseases has in part also been attributed to side effects of antiretroviral therapy including hyperlipidaemia, metabolic syndrome and insulin resistance (Morlat *et al.*, 2014:1188). HIV-infected adults on nucleoside-analogue and protease inhibitor therapy experienced metabolic and body-fat

abnormalities (Grinspoon & Carr, 2005:59). With the newer drugs in these classes, specific metabolic toxicities have decreased (Brown & Glebsy, 2012:18).

2.9.2.4 HAART Regimen at baseline

The recommended ART regimens for initiation have variations in treatment outcomes as shown by several studies across high-income and resource-constrained settings. In a randomised non-inferiority trial conducted by Pozniak *et al.* (2006:539) on investigating therapeutic outcomes with AZT/3TC/EFV and TDF/FTC/EFV regimens on treatment naïve patients, more durable antiviral response, excellent safety and favourable resistance profile were obtained from the TDF/FTC/EFV group. Four regimens namely TDF/3TC/EFV, TDF/3TC/NVP, AZT/3TC/EFV and AZT/3TC/EFV were also evaluated in a retrospective review of patients' medical chart information. The review exposed an outstanding efficacy of TDF/3TC/EFV versus the other regimens on 14 - month treatment course (Pillay *et al.*, 2013:11).

The initiation of antiretroviral therapy requires minimisation of both short- and long-term adverse effects upon initiation of treatment. While the AZT/3TC nucleoside backbone has been used over several years, Pozniak *et al.* (2006:539) stated that the regimen increases the risk for short- and long-term adverse effects when compared to the use of TDF/3TC backbone as the alternative. According to Gallant *et al.* (2004:199) long-term follow-up treatment on antiretroviral naïve patients who entered the three-year clinical trial and randomised to TDF showed similar renal safety profile compared with controls. There is also a significant clinical experience gained with the use of boosted-PI in some first line considerations. Both boosted-atazanavir and -darunavir have shown an outstanding virologic suppression at 96th week of treatment versus boosted-lopinavir (Llibre, 2009:221).

2.9.2.5 Chemoprophylaxis

Damtie *et al.* (2013) correlated CD4 cell count with common opportunistic infections among HIV-infected patients on antiretroviral therapy. The study brought up the highest prevalence of opportunistic infections being mostly in patients with CD4 cell counts < 200 cells/mm³, followed by those with CD4 cell counts in the range 200-350 cells/mm³ and lastly, in those with more than 350cells/mm³. The most commonly encountered opportunistic infections were hierarchically in the order of TB (9.72%), oral candidiasis (5%), diarrhoeal diseases (3.3%), skin fungal infections (1.67%) and pneumonia (1.38%). These findings were comparable to those obtained by Ghate *et al.* (2009:5) in their prospective study whereby both pulmonary TB and extra-pulmonary TB had the highest incidence followed by oral candidiasis. The herpes zoster and cryptococcal infections were also found. In addition, HIV-associated hepatitis B infection was also observed in both high-income and resource-limited countries (Askari *et al.*, 2014:709). The inclusion of

chemoprophylactic therapy against these infection-causing opportunistic organisms is vital for the improvement of controlled HIV treatment and reduction of morbidities and mortalities associated with HIV-infection.

The isoniazid prophylactic therapy is recommended for the prevention of active TB in HIV-infected patients (Martin & Black, 2012:1). Assebe *et al.* (2015) conferred a significant reduction of active TB incidence following isoniazid prophylactic therapy among HIV-infected patients. In a meta-analysis of randomised clinical trials conducted by Ayele *et al.* (2015:16), there was more protection against the active TB with tuberculin skin test on HIV-infected patients than the tuberculin skin test on uninfected population. In the evaluation of isoniazid prophylactic therapy amongst TB exposed children aged ≤ 15 years regardless of their HIV status; Ayieko *et al.* (2014:9) found a 59% reduction in the risk of active TB due to the use of isoniazid prophylactic therapy.

There is existing quality and consistent evidence that co-trimoxazole prophylactic therapy is protective reduces morbidity and mortality amongst HIV-infected adults in sub-Saharan Africa. According to Hassani *et al.* (2015:267) the strongest mortality reduction evidence has been observed in adults with the lowest CD4 cell counts while the morbidity reduction evidence extended to those with high CD4 cell counts even in malaria endemic areas. Co-trimoxazole is a combination of two antibiotics namely trimethoprim and sulphamethoxazole that provide coverage over the opportunistic gram-positive and gram-negative bacteria, protozoa (including malaria-causing organism) and fungi such as the common pneumonia-causing *Pneumocystic jirovecii* (PCP) (Suthar *et al.*, 2012b:128). In South Africa, Badri *et al.* (2001:1147) found low incidence of PCP in HIV-infected adults on co-trimoxazole. There was also significant reduction in mortality as well as the prevalence of opportunistic infections.

Despite the availability of antiretroviral therapy, cryptococcal meningitis is still associated with high mortality (Govender & Dlamini, 2014), accounting for 12.7% cumulative incidence of symptomatic relapse in 79% HIV-infected patients who inadequately received secondary fluconazole prophylaxis (Narvis *et al.*, 2010:6). According to McCarthy and Meintjes (2007:27) the South African guidelines on the prevention, diagnosis and management of cryptococcal meningitis and disseminated cryptococcosis in HIV-infected patients require that all patients with previous episode of cryptococcosis should receive secondary prophylaxis of fluconazole 200 mg once daily for life or until CD4 cell count rises to >200 cells/mm³ for more than six months of antiretroviral therapy. Since *Candida albicans* is the common fungal organism responsible for many localised fungal infections in humans, patients with impaired immunity such as those living with HIV/AIDS may require antifungal prophylaxis to reduce the risk of infections due to the pathogen. Fluconazole remains the first line agent for use against *Candida albicans* infections because of excellent efficacy, safety, suitability for use by children and adults with compromised

immune system (Garcia-Cuesta *et al.*, 2014:582), although resistance has been reported in the 20th century.

While the TDF/3TC backbone HAART is the recommended first line regimen as previously discussed (refer to **section 2.9.2.4**), the two drugs are also active against hepatitis B virus (Isaakidis *et al.*, 2012:240). This means that as part of prophylaxis for HIV-associated Hepatitis B infection the combination relatively reduces the burden on HIV-Hepatitis B co-infection.

2.9.2.6 Treatment adherence

Adherence to antiretroviral therapy is crucial for long-term immunological and virological successes and adherence remains as one of the greatest public health concerns. In a 24-month follow-up assessment of adherence to antiretroviral therapy in Cambodia, Spire *et al.* (2008:700) found that high adherence rate is associated with favourable outcomes at the end of the assessment period. The outcomes were similar to those reported by Ferradini *et al.* (2006:1341) in Malawi on a 9.5-month follow-up assessment. Mills *et al.* (2006:685) suggested that favourable adherence in sub-Saharan African patients can be achieved. Schaecher (2013:235) has implicated positive treatment adherence in patient with HIV infection in terms of better management of the disease and identifying and directly addressing barriers to adherence through multidisciplinary care, educational and medication management initiatives.

Poor and/or non-adherence to treatment have detrimental consequences on care of HIV/AIDS patients. There are a variety of reasons behind poor adherence ranging from patient and family/caregiver-, medication-, socio-environment- and health care delivery-related factors (Reda & Biadgilign, 2012:4). In a cross-sectional multi-centre study conducted by Wakibi *et al.* (2011:5) in Kenya, 82% of the patients enrolled were non-adherent to antiretroviral therapy, claiming their non-adherence was attributable to proximity to the clinic for refill, difficulty in fitting therapy into own schedule and a range of personal reasons for skipping doses.

2.9.2.7 Plasma haemoglobin

The CD4 cell count and plasma viral load are the most important biomarkers of HIV/AIDS disease stage and progression in infected patients. Other factors including haematological status can also predict the prognosis (De Santis *et al.*, 2011). Obirikorang and Yeboah (2009:6) have witnessed a strong relationship between haemoglobin levels and AIDS-defining illness and death, such that in settings where sophisticated laboratory markers of plasma viral load or CD4 cell count are unavailable, haemoglobin measurements may be done to identify patients at highest risk of disease progression.

In a cross-sectional analysis of longitudinal cohort study on HIV-infected and uninfected women in Rwanda, Munyazesa *et al.* (2012:7) revealed a high prevalence of anaemia in both groups. The anaemia was more common with the HIV-infected than the uninfected group, especially those with advanced disease progression as shown by lower CD4 cell counts. In China, Shen *et al.* (2013:5) found that anaemia was highly prevalent in adults newly diagnosed with HIV/AIDS in the older age and lower CD4 cell count patients.

The anaemia associated with HIV infection responds quite well to initiation of antiretroviral therapy. In a retrospective study conducted by Tesfaye and Enawgaw (2014:4) in Ethiopia on prevalence of anaemia prior and following antiretroviral therapy initiation on HIV-infected patients, the prevalence of anaemia declined together with elevations in mean CD4 cell count upon antiretroviral therapy initiation. Antiretroviral therapy reverses HIV associated anaemia. In Tanzania, majority of people who were enrolled into HIV treatment were anaemic. The haemoglobin levels increased significantly within 12 months of antiretroviral therapy initiation (Johannessen *et al.*, 2011:7). Several antiretroviral drugs including AZT are however known to cause anaemia. AZT-containing antiretroviral therapy regimen was associated with persistent anaemia 12 months following regimen intake in patients who had anaemia prior to antiretroviral therapy initiation (Akilimali *et al.*, 2015:9).

2.10 Chapter summary

TDF-containing HAART regimens were found to have better clinical, immunologic and virological outcomes when compared to AZT- and d4T-backbone regimens. While these HAART-regimens have found clinically to improve quality of life, these drugs are however harmful to patients' body parts including kidneys. Therefore, antiretroviral therapy is a double-edged sword since the benefits of the therapy are accompanied by risks of giving. These beneficial and harmful outcomes of antiretroviral therapy are positively (therapeutically) and negatively (toxicologically) influenced by several factors. These factors including age, sex, body weight, clinical, biochemical, immunological and virological parameters are implicated prior to treatment initiation and during treatment. They are therefore the determinants of the therapeutic and toxicological outcomes of treatment.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Chapter introduction

This chapter consists of results of the empirical investigation described in chapter 1. The evaluation of consistency (and deviations) of these results with findings from other studies of comparable nature is also covered in this chapter.

3.2 Characteristics of the study population

The demographic data of the participants are presented in the **Tables 3-1** and **Table 3-2**. There were 255 participants enrolled in the study, having more females than males engaged; with 143 (56.10%) females and 112 (43.90%) males. The majority of participants fell in the age group ranging between 30 to 40 years (40.40%) with the mean age of 39.76 ± 11.93 years. Also, upon initiation of therapy most participants' body weights ranged from 50 to 65 kg with 59.00 ± 12.87 kg as the mean body weight for all participants. Most of the patients were initiated on antiretroviral therapy at WHO clinical stage II, accounting for 178 (69.80%) participants. Those at stage III were 67 (26.30%). Most participants were initiated on the antiretroviral regimen TDF/3TC/EFV ($n=216$, 84.70%), followed by 22 (8.6%) participants who were initiated on TDF/3TC/NVP. Although more participants have been on treatment for the past 6 to 12 months (15.30%) there were comparable numbers of participants in the other categories of treatment duration at the encounter of the study.

The median CD4 cell count for the participants prior to initiation of antiretroviral therapy was 308.00 (201.50-433.50) cells/mm³ while the median serum creatinine at baseline was 78.00 (66.00-87.00) $\mu\text{mol/l}$. The test of normality for the continuous data revealed normal distribution for the CD4 cell count data ($p=0.88$) and body weight data ($p=0.06$) variables; when using the Shapiro-Wilk test at a p value of 0.05. The inference behind the normal distribution of CD4 cell count and body weight is that the data composed of a balanced distribution. This means that the data sets between the lower values and the higher values gave the mean and median at the peak of the distribution curve; showing a symmetrical data distribution. The implications of a normally distributed data are that the distribution allows for analysis of best choice as many statistical tests are mostly applicable in normally distributed data sets.

However, the Shapiro-Wilk test also revealed that both age at antiretroviral therapy initiation and serum creatinine concentration were skewed to the right (positive) with $p=0.04$ and $p<0.05$ respectively. The conclusion that can be drawn from this output may be that there were more lower bound values than the upper bound values in the data, such that the mean of the data was on the right of the peak of the distribution curve showing asymmetric distribution of the data skewed to the right. With age at antiretroviral therapy initiation, most patients were initiated on

antiretroviral therapy at younger age compared to those fewer patients who were initiated on antiretroviral therapy at older age. Upon therapy initiation, the serum creatinine concentrations of most patients were lesser than those few people whose serum creatinine concentrations were higher, implying that participants had normal renal function.

In general, the study engaged mostly young people with well-functioning kidneys at baseline. This may skew the data and certain statistical tests would not be appropriate to use (especially parametric tests) if data distribution is too skewed. Therefore, the analysis was performed by non-parametric tests (linear regression) to cover for the skewed data and to enhance the likelihood of reliable results.

Table 3-1: Categorical data of the study participants

Variable (n)	Category	N (valid %)
Sex (N=255)	Female	143 (56.10)
	Male	112 (43.90)
Age at initiation (years) (N=255)	≥18 to ≤30	38 (14.90)
	>30 to ≤40	103 (40.40)
	>40 to ≤50	53 (20.80)
	>50 to ≤60	45 (17.60)
	>60 to ≤70	15 (5.90)
	>70	1 (0.40)
Treatment duration (months) (N=255)	≥0 to ≤6	24 (9.40)
	>6 to ≤12	39 (15.30)
	>12 to ≤18	27 (10.60)
	>18 to ≤24	28 (11.00)
	>24 to ≤30	25 (9.80)
	>30 to ≤36	23 (9.00)
	>36 to ≤42	16 (6.30)
	> 42	73 (28.60)
Body weight (kg) (N=92)*	≥35 to ≤50	24 (26.10)
	>50 to ≤65	53 (57.60)
	>65 to ≤80	10 (10.90)
	>80 to ≤95	4 (4.30)
	>95	1 (1.10)
WHO HIV/AIDS Clinical stage (N=255)	Stage I	9 (3.50)
	Stage II	178 (69.80)
	Stage III	67 (26.30)
	Stage IV	1 (0.40)
ART regimen initiated (N=255)	TDF/3TC/NVP	22 (8.60)
	TDF/3TC/EFV	216 (84.70)
	d4T/3TC/NVP°	5 (2.00)
	d4T/3TC/EFV°	7 (2.70)
	AZT/3TC/NVP°	2 (0.80)
	AZT/3TC/EFV°	2 (0.80)
	ABC/3TC/EFV°	1 (0.40)

* Only 92 participants had baseline body weight, the rest had missing baseline body weights.

°These are participants who were firstly initiated on the non-TDF containing regimens such as d4T- and AZT-containing regimens. However, due to drugs being phased out (d4T) and developing toxicity to drugs (AZT and ABC), they were later switched to TDF-containing regimens; their information was extracted from initiation of TDF-containing regimens and considered baseline to the present study.

Table 3-2: Continuous data of the study participants

Variable (n)	Mean ($\pm$ SD)	Median (IQR)	95 % CI
Age at initiation (years) (N=255)	39.76 ($\pm$ 11.93)	38.00 (30.00-48.00)	35.78-43.74
Treatment duration (months) (N=255)	31.25 ($\pm$ 20.99)	27.00 (13.00-50.50)	28.67-33.83
CD4 cell count (cells/mm ³) (N=255)	328.16 ($\pm$ 167.25)	308.00 (201.50-433.50)	272.40-383.93
Body weight (Kg) (N=92)	59.00($\pm$ 12.87)	56.000 (50.5-63.50)	54.71 -63.29
Serum creatinine concentration (μ mol/l) (N=255)	83.39 ($\pm$ 37.50)	78.000 (66.00-87.00)	70.88-95.88

The statistical summary of the study participants has several comparable characteristics with study participants obtained from other studies of similar nature and settings, though some deviations exist. In a multicentre cohort study conducted on 933 participants in rural Lesotho on renal safety of TDF-containing first line regimens, there were more females (61.95%) enrolled than males (Bygrave *et al.*, 2011b:3). Of the 485 participants enrolled in a retrospective study done by Mugomeri *et al.* (2014:35) in Roma health service area, Lesotho, 60.21% (n=292) of the participants were females. The study evaluated the effect of tenofovir on renal function in HIV-positive patients in Lesotho. These two studies are in agreement in that females are more encountered in clinical HIV/AIDS treatment settings than their male counterparts.

The above studies again showed consistency on age at ART initiation of participants. Bygrave *et al.* (2011a:77) found a median age at baseline of 39.0 (30.8-49.2) years in the two-year follow-up study. Mugomeri *et al.* (2014:35) also had median ages of 42 (17) and 38 (16) years for the non-

TDF and TDF subgroups respectively. In South Africa, the median age at ART initiation was found to be 36.6 (31.3-43.2) years in patients initiated on ART from 2004 to 2011 in a cohort study done on 35 000 HIV-infected patients (61.9% females) at Themba-Lethu Clinic, Johannesburg (Brennan *et al.*, 2013:5). The concordance derived in these findings versus the results of the current study entails that although patients can be enrolled for HIV/AIDS care at any age, most patients in this clinic get initiated for ART at ages around 40 years.

The World Health Organization (WHO) clinical staging prior to ART initiation has also been investigated. In a cross-sectional retrospective and prospective study done in Malawi on 1 308 HIV-infected patients about assessment effectiveness of scaled-up and simplified HAART, Ferradini *et al.* (2006:1338) found that 58% (n=230) of the participants enrolled were on stage III (69% females and 34.0 (28.9-40.3) years median age). In a systematic review done by Egger (2006:819) most participants were initiated on antiretroviral therapy at WHO clinical stage III and IV in both low-income 36% (n=1733) and high-income 23% (n=5 075) settings respectively. Most of these patients were initiated on antiretroviral therapy at ages between 30-39 years (45% stage III and 47% stage IV) and were mostly females with 51% (n=2 461) in low-income settings while males dominated in the high-income settings (75%, n=16 731). The impression derived at this far is that patients seek for HIV/AIDS medical care at advanced WHO clinical stages.

The body weight at initiation of antiretroviral therapy also deserves attention. In their study, Mugomeri *et al.* (2014:35) had statistically significant ($p>0.001$) difference in body weights between non-TDF and TDF subgroups. The TDF group had lower median body weight of 54.0 (12.0) kg at baseline. Another comparison was done to TDF-based regimen group versus ABC-based regimen group in a single centre retrospective cohort (2004-2009) study done in Japan (Nishijima *et al.*, 2012:4). The study enrolled 503 HIV/AIDS patients who were initiated either on TDF- or ABC-based regimens (Nishijima *et al.*, 2012:4). At baseline, the participants in both groups (TDF- and ABC-based regimens) had median body weight of 64.0 (58.0-70.9) kg. The study engaged mostly males (98.5% TDF-based regimes and 97.4% ABC-based regimens) at median ages of 36 (31 - 44) and 37 (31 - 43) years respectively.

The most commonly used TDF-containing HAART regimen at initiation in the current study was TDF/3TC/EFV. In the Bygrave *et al.* (2011a:76) study the three-drug HAART combination had TDF, AZT or d4T. All regimens initiated contained 3TC (100%) and either NVP (25.8%) or EFV (74.2%). Those patients who were on TDF-containing HAART regimens were almost exclusively on EFV (99.8%, n=568). Brennan *et al.* (2013:5) study also had most patients initiated mostly on EFV (90.2%, n=17 775). The Lesotho National guidelines on the use of antiretroviral therapy for HIV prevention and treatment stated that the preferred first line antiretroviral therapy regimen for adults and adolescents is TDF/3TC/EFV (MOH, 2016a:1).

Since the immunological status in terms of CD4 cell count was the eligibility criteria for antiretroviral therapy initiation before introduction of the “test and treat initiative” which began in June 2016, the baseline CD4 cell count had to be known. The “test and treat initiative” is a new development which contends that irrespective of the CD4 cell count, patients who tested HIV-positive should be initiated on antiretroviral therapy (MOH, 2016:1). The median baseline CD4 cell counts for the current study is however higher than findings obtained from Bygrave *et al.* (2011a:76), Brennan *et al.* (2013:5) and Mugomeri *et al.* (2014:35) which were 211 (119-284) cells/mm³, 105 (40-179) cells/mm³ and 167 (180.5) cells/mm³ respectively.

The antiretroviral therapy guideline recommends that baseline renal function in terms of creatinine clearance should be evaluated prior to initiating TDF-containing HAART regimen (MOH, 2016a:47). The creatinine clearance was calculated by the Cockcroft-Gault equation which requires age (years), sex (male or female), and body weight (kg) and serum creatinine concentration (μmol/l) of the patient as its parameters. The median serum creatinine found in the current study at baseline was slightly higher than the one found by Nishijima *et al.* (2012:4) as they found 65.42 (59.23 - 74.26) μmol/l.

3.3 Results of the linear mixed model regression analysis

The study had two major aims namely evaluation of both clinical and renal safety outcomes in which the specific objectives emanated from and thus the relevant variables (independent and dependent) were identified. Using the IBM®SPSS® statistical software, linear mixed model regression analysis was performed to estimate fixed effects of the independent variables on the change in dependent variables over treatment duration. The independent variables namely treatment duration, age at antiretroviral therapy initiation, sex and body weight were used in the analysis to predict changes in the dependent variables. According to the aims, the dependent variables were measures of clinical outcomes (body weight, CD4 cell count and plasma viral load) and measures of the renal safety (serum creatinine concentration, creatinine clearance and estimated glomerular filtration rate). All the data from 255 participants was used for the analysis from baseline findings, within the treatment duration up to end-time findings (latest measurements from patients' medical records).

3.3.1 Interpretation of the results presented in the estimates of fixed effects

The interpretation of the results of estimates of fixed effects entails that over treatment duration expressed in annual intervals, the effect of change is given on the independent variables column. The declines in the change are indicated by negative values while increments in the change are shown by positive (without signs) values. These changes are computed by the intercept of the estimate plus the estimate itself. The intercept reflects the baseline estimate of the variable;

meaning that from baseline, the dependent variable will either decline (negative) or increase (positive) of treatment duration in annual basis. The estimate of the change in the dependent variable is the product of the change and the treatment duration over one year of treatment with antiretroviral therapy. In comparison of more than one independent variable, the overall change in the dependent variable equals to the sum of the intercept and products of each independent variable change and its treatment duration. Variables of reference (male) are assigned zero value while those which change is evaluated against (female, age at ART initiation and body weight) are assigned value of one.

3.3.2 The results for evaluation of clinical outcomes

The analysis determined the effects of treatment duration, sex and age at antiretroviral therapy initiation (independent variables) on the change in the dependent variables namely body weight (clinical indicator), CD4 cell count (immunological indicator) and plasma viral load (virological indicator) as measures of the clinical outcomes. The results of the analysis are presented in tables from **Table 3-3** to **Table 3-18**.

3.3.2.1 Change in body weight

Table 3-3 to **Table 3-10** present the estimated effects of the individual and combined effects of the independent variables on the change in body weight. **Table 3-3** shows that body weight is independent of treatment duration. As such, there was no statistically significant difference in the change in body weight relative to treatment duration ($p=0.23$) at 95% confidence level.

Table 3-3: Body weight according to treatment duration: Type III tests of effects

Type III tests of effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	5293.09	98543.01	0.00
Treatment duration	1	186.35	1.42	0.23

Table 3-4 reveals the estimates of the fixed effects and shows a decline in body weight of 0.003 kg over treatment duration that is not statistically significant ($p=0.23$). This means that from 62.02 kg, body weight decreased insignificantly by 0.003 kg over one year treatment with antiretroviral therapy; that is $62.02 + (-0.003) * 1 \text{ year} = \mathbf{62.017 \text{ kg}}$.

Table 3-4: Body weight according to treatment duration: Estimates of fixed effects

Estimates of fixed effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	62.02	0.20	5293.08	313.91	0.00	61.63	62.40
Treatment duration	-0.003	0.002	186.35	-1.19	0.23	-0.007	0.002

The results in **Table 3-5** indicate the effect of sex on the change in body weight over treatment duration. These results show a statistically significant difference in the change in body weight between males and females over the treatment duration ($p < 0.05$).

Table 3-5: Body weight according to treatment duration and sex: Type III tests of effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	5295.96	98593.81	0.00
Treatment duration	1	186.27	1.58	0.21
Sex	1	5414.04	41.37	0.00

Table 3-6: Body weight according to treatment duration and sex: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	60.69	0.28	5398.80	213.07	0.00	60.13	61.25
Treatment duration	-0.003	0.002	186.27	-1.25	0.21	-0.007	0.002
[Sex=female]	2.49	0.39	5414.04	6.43	0.000	1.73	3.25
[Sex=male]	0	0					

The results from **Table 3-6** outline a statistically significant increase in body weight over treatment duration relative to sex of the patients ($p < 0.05$). In these results, females are found to have 2.49 kg more increase in body weight than males at 95% confidence interval. The results are interpreted that males are 2.49 kg lower in body weight increase than females over one year of treatment. This means that for males, the change in body weight is given by $60.69 \text{ kg} + 2.49 * 0 + (-0.003) * 1 \text{ year} = \mathbf{60.69 \text{ kg}}$; while for females, body weight increased to $60.69 \text{ kg} + 2.49 * 1 + (-0.003) * 1 \text{ year} = \mathbf{63.18 \text{ kg}}$ over one year of treatment.

Table 3-7 reveals the effect of age at antiretroviral therapy initiation on change in body weight over treatment duration. This table shows that a change in body weight is dependent of the age at antiretroviral therapy initiation at 95% confidence level ($p < 0.05$).

Table 3-7: Body weight according to treatment duration and age at antiretroviral therapy initiation: Type III tests of effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	5415.10	5893.31	0.00
Treatment duration	1	188.75	1.41	0.23
Age at antiretroviral therapy initiation	1	5415.41	25.17	0.00

Table 3-8: Body weight according to treatment duration and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	58.33	0.76	5415.10	76.768	0.00	56.84	59.82
Treatment duration	-0.003	0.002	188.75	-1.19	0.24	-0.01	0.002
Age at ART initiation	0.09	0.02	5415.41	5.02	0.00	0.05	0.12

Table 3-8 depicts that 0.09 kg increase in body weight relative to the age at antiretroviral therapy initiation over treatment duration is statistically significant at 95% confidence level ($p < 0.05$). The

results reveal that due to age at ART initiation, patients gained weight significantly from baseline. That is, $58.33 \text{ kg} + 0.09 * 1 + (-0.003) * 1 \text{ year} = \mathbf{58.42 \text{ kg}}$.

Table 3-9: Body weight according treatment duration, sex and age at antiretroviral therapy initiation: Type III tests of effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	5414.10	5810.25	0.00
Treatment duration	1	188.66	1.58	0.21
Sex	1	5413.30	46.65	0.00
Age at ART initiation	1	5414.39	30.43	0.00

Table 3-9 represents the results of the combined effect of sex and age at antiretroviral therapy initiation on change in body weight over the treatment duration. The results indicated that body weight is dependent of sex and age at antiretroviral therapy initiation, with a statistically significant change in the body weight relative to sex and age at antiretroviral therapy initiation over treatment duration at 95% confidence interval ($p < 0.05$).

The results presented in **Table 3-10** unveil that females have 2.64 kg more increment in body weight over treatment duration than males ($p < 0.05$). In both males and females, there is also 0.10 kg increase in body weight over treatment duration relative to the age at antiretroviral therapy initiation at 95% confidence interval ($p < 0.05$). This table infers that when body weight is evaluated from the combined effect of sex and age at ART initiation, females gained weight from 56.57 kg to **59.30 kg** ($56.57 \text{ kg} + 2.64 * 1 + 0.10 * 1 + (-.003) * 1 \text{ year}$) at any age over one year of treatment. Males also gained weight but to a lesser extend from 56.57 kg to **56.67 kg** ($56.57 \text{ kg} + 2.64 * 0 + 0.10 * 1 + (-0.003) * 1 \text{ year}$) at any age over one year of treatment.

Table 3-10: Body weight according treatment duration, sex and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	Df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	56.57	0.80	5414.89	70.75	0.00	55.10	58.13
Treatment duration	-0.003	0.002	188.66	-1.26	0.21	-0.01	0.002
[Sex=female]	2.64	0.39	5413.30	6.83	0.00	1.89	3.40
[Sex=male]	0	0					
Age at ART initiation	0.10	0.017	5414.39	5.52	0.00	0.06	0.13

These results of the study presented in above tables (**Table 3-3, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9 and 3-10**) relating to the change in body weight relative to the identified independent variables are comparable to findings of other studies of similar nature. In Nigeria, Olawumi *et al.* (2008:313) conducted a 14-month follow-up study on 185 patients determining the effect of HAART on body weight over treatment duration. The mean pre-antiretroviral therapy body weight for these patients was 52.1±10.6 kg, which increased significantly over 1 to 14-month treatment duration following antiretroviral therapy initiation to 59.0±10.5 kg ($p=0.01$) (Olawumi *et al.*, 2008:313). Again, another observational cohort study was done in Nigeria between March and December 2007 (10 months) by Denué *et al.* (2013:90) on 120 newly HIV/AIDS diagnosed patients. The results of the study showed that the percentage of both overweight and obese patients increased from 14.4% to 22% and 2.5% to 6.4% respectively from baseline levels following HAART (Denué *et al.*, 2013:91). In general, the HAART initiation in HIV infected patients confers clinical improvement manifesting as weight gain from baseline levels proportional to treatment duration (Denué *et al.*, 2013:91; Olawumi *et al.*, 2008:314).

In a review done by Squires *et al.* (2003:43) on the impact of sex on antiretroviral therapy outcomes, they found that there is comparable response to antiretroviral therapy amongst men and women. However, there were sex-based changes owing to differences in pharmacokinetic parameters to the antiretroviral agents. The HIV-infected patients on antiretroviral therapy are increasingly gaining weight in the United States as shown by Crum-Cianflone *et al.* (2010:8), in a male predominating multicentre observational cohort study that enrolled 4 586 HIV-infected participants. In North Carolina USA, Lakey *et al.* (2013:438) studied weight changes in 92 HIV infected adults on antiretroviral therapy in a 12-month cohort study. The results of the study

revealed a greater increase in body weight among the HIV-infected females compared to their male counterparts from baseline levels. Additionally, these high rates of getting overweight (from 64.1% to 80%) and/or obese (from 35.5% to 53%) were higher in HIV-infected females than the uninfected females. Women were found to be more likely to have higher body mass indices following long term HAART; though HAART was not a risk of obesity in HIV-infected women (Sharma *et al.*, 2014:7). The study was a longitudinal multicentre observational study done on 1,177 HIV-infected women. In overall, in both sexes, patients acknowledge weight gain following antiretroviral therapy initiation and women are found to experience more weight gain (Crum-Cianflone *et al.*, 2010:8; Lakey *et al.*, 2013:438; Sharma *et al.*, 2014:7; Squires *et al.*, 2003:43).

The age at antiretroviral therapy initiation is one of the predictors of weight gain. Yuh *et al.* (2015:1854) conducted a cohort study on 20,602 HIV-infected patients. The multivariate analyses of weight change relative to age and other factors showed that weight gain was evidenced by positive and increased magnitude of regression coefficients in patients aged ≥ 50 years. The change in body weight relative to body weight is also associated with age differences at initiation of antiretroviral therapy. The antiretroviral therapy initiation at early ages had the greatest weight recovery than in the higher ages (McGrathet *et al.*, 2011:351). There was a slowed linear gain in weight relative to advanced age in HIV-1 infected children on HAART study done by McGrathet *et al.* (2011:351) in a HIV treatment clinic in Kenya ($n=173$). In a retrospective cohort study (1998-2006) done on 196 children aged between 0 and 12 years who were initiated on antiretroviral therapy in Brazil, Diniz *et al.* (2011:133) found that the increase in weight following antiretroviral therapy initiation was more in younger patients than older children ($p=0.02$). In summary, initiation of antiretroviral therapy at younger age is associated with better body weight recovery than initiation at older age although ART initiation at any age generally leads to weight gain.

3.3.2.2 Change in CD4 cell count

The results of the individual and combined effects of the independent variables on the change in CD4 cell count are tabulated from **Table 3-11** to **Table 3-18** below.

Table 3-11 portrays that CD4 cell count is dependent of treatment duration. A statistically significant difference in change in CD4 cell count over treatment duration was observed ($p<0.05$) at 95% confidence interval.

Table 3-11: CD4 cell count according to treatment duration: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	1302.72	2382.27	0.00
Treatment duration	1	106.61	57.10	0.00

The statistically significant daily estimated increase of 0.22 cells/mm³ in CD4 cell count was obtained at 95% confidence interval as shown by **Table 3-12** ($p < 0.05$). The results of this table infer that CD4 cell count rose significantly from 270.96 cells/mm³ to 271.18 cells/mm³ ($270.96 + 0.22 * 1 \text{ year}$) per year of treatment.

Table 3-12: CD4 cell count according to treatment duration: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	<i>p</i> value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	270.96	5.55	1302.72	48.81	0.00	260.07	281.86
Treatment duration	0.22	0.03	106.61	7.56	0.00	0.16	0.27

Table 3-13 represents the effect of sex on the change in CD4 cell count over treatment duration. There is a statistically significant difference in the change in CD4 cell count between females and males ($p < 0.05$) over the treatment duration at 95% confidence interval.

Table 3-13: CD4 cell count according to treatment duration and sex: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	1308.64	2363.06	0.00
Treatment duration	1	104.22	60.52	0.00
Sex	1	1591.73	41.89	0.00

Table 3-14: CD4 cell count according to treatment duration and sex: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	T	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	235.73	7.79	1467.26	30.26	0.00	220.45	251.01
Treatment duration	0.21	0.03	104.22	7.78	0.00	0.15	0.26
[Sex=female]	66.88	10.33	1591.73	6.47	0.00	46.61	87.15
[Sex=male]	0	0					

The results in **Table 3-14** illustrate that there is a statistically significant increase in CD4 cell count over treatment duration in both sexes ($p < 0.05$) at 95% confidence interval. Females were found to have increment in CD4 cell count estimated at 66.88 cells/mm³ more than males over treatment duration. The CD4 cell count for females increased from 235.73 cells/mm³ to **302.82 cells/mm³** ($235.73 \text{ cells/mm}^3 + 66.88 * 1 + 0.21 * 1 \text{ year}$) while males experienced annual increase in CD4 cell count from 235.73 cells/mm³ to **235.94 cells/mm³** ($235.73 \text{ cells/mm}^3 + 66.88 * 0 + 0.21 * 1 \text{ year}$).

Table 3-15 determined the effect of age at antiretroviral therapy initiation on the change in CD4 cell count over treatment duration. The change in CD4 cell count is dependent of age at antiretroviral therapy initiation, with a statistically significant difference at 95% confidence interval ($p = 0.02$).

Table 3-15: CD4 cell count according to treatment duration and age at antiretroviral therapy initiation: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1583.60	120.04	0.00
Treatment duration	1	105.66	55.09	0.00
Age at antiretroviral therapy initiation	1	1588.52	5.16	0.02

In **Table 3-16**, the results demonstrate a statistically significant increase in CD4 cell count of 1.09 cells/mm³ over the treatment duration relative to the age of the patient at antiretroviral therapy

initiation at 95% confidence interval ($p=0.02$). The results reveal that due to age at ART initiation, patients' CD4 cell count increased significantly from baseline to **227.14 cells/mm³**. That is, $225.84 \text{ cells/mm}^3 + 1.09 * 1 + 0.21 * 1 \text{ year} = \mathbf{227.14 \text{ cells/mm}^3}$.

Table 3-16: CD4 cell count according to treatment duration and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	225.84	20.61	1583.60	10.96	0.00	185.41	266.27
Treatment duration	0.21	0.03	105.66	7.42	0.00	0.16	0.27
Age at antiretroviral therapy initiation	1.09	0.48	1588.51	2.27	0.02	0.15	2.03

Table 3-17: CD4 cell count according to treatment duration, sex and age at antiretroviral therapy initiation: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1590.26	109.67	0.00
Treatment duration	1	103.01	58.14	0.00
Sex	1	1588.70	44.63	0.00
Age at antiretroviral therapy initiation	1	1591.99	7.77	0.01

Table 3-17 reflects the results of the combined effects of sex and age at antiretroviral therapy initiation on change in CD4 cell count over treatment duration. The CD4 cell count is dependent of both sex and age at antiretroviral therapy initiation. This is shown by a statistically significant change in CD4 cell count in relation to sex and age at antiretroviral therapy initiation over treatment duration($p=0.01$) at 95% confidence interval.

The estimates of the fixed effects are shown on **Table 3-18**. There is a statistically significant increase in CD4 cell count in both sexes ($p<0.05$) 95% confidence interval. In addition, females

have more increment in CD4 cell count of 69.13cells/mm³ than males over treatment duration. There is also a statistically significant increase in CD4 cell count by 1.32 cells/mm³ due to age at antiretroviral therapy initiation over treatment duration at 95% confidence interval ($p<0.05$). These results portray that when the change in CD4 cell count is evaluated from the combined effect of sex and age at ART initiation, females experienced increase in CD4 cell count from 179.67 cells/mm³ to **250.32 cells/mm³** ($179.67 \text{ cells/mm}^3 + 69.13 * 1 + 1.32 * 1 + 0.20 * 1 \text{ year}$) at any age annually over treatment. Males have however experienced lesser elevations in CD4 cell count over one year of treatment from 179.67 cells/mm³ to **181.19 cells/mm³** ($179.67 \text{ cells/mm}^3 + 69.13 * 0 + 1.32 * 1 + 0.20 * 1 \text{ year}$) at any age.

Table 3-18: CD4 cell count according to treatment duration, sex and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	T	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	179.67	21.58	1585.19	8.33	0.00	137.33	222.10
Treatment duration	0.20	0.03	103.01	7.62	0.00	0.15	0.27
[Sex=female]	69.13	10.35	1588.70	6.68	0.00	48.83	89.43
[Sex=male]	0	0					
Age at antiretroviral therapy initiation	1.32	0.47	1591.99	2.78	0.00	0.39	2.25

The immunological outcomes as exemplified by the change in the CD4 cell count over treatment duration relative to the identified predictor variables are replicates of findings from other studies. Maskew *et al.* (2013:116) did a cohort study in Themba Lethu Clinic South Africa, enrolling 32 000 HIV infected patients from 2004 to 2009. The evaluation of the immunologic recovery to antiretroviral therapy in this study revealed the median CD4 cell count recovery from baseline levels of 100 (41-163) and 81 (21-151) cells/mm³ to 443 and 353 cells/mm³ in both females and males respectively at 36-month treatment duration. The results of this study infer that irrespective of sex, patients recover immunologically over antiretroviral therapy treatment duration. Furthermore, the same study demonstrated better median CD4 cell count recoveries were found with females than males. At 12th month of antiretroviral therapy females had 166 cells/mm³ while

males had 139 cells/mm³ and the values increased up to 343 (females) and 272 cells/mm³ (males) in at 36th month treatment duration.

In a systematic review conducted on 65 published observational cohort studies from North American and Europe (39 studies) and Latin America, Asia and Africa (26 studies), the consensus was made that in both high-income and resource-constrained settings, females have far more improved survival than males following antiretroviral therapy initiation (Castilho *et al.*, 2014:453). Chow *et al.* (2015:7) conducted a longitudinal cohort on 103 HIV-1 infected patients on antiretroviral therapy evaluating the CD4 cell recovery in combination antiretroviral therapy-experienced patients. The study found that HIV-1 infected women have 2.7 times significantly greater opportunity for obtaining CD4 cell counts ≥ 350 cells/mm³ than their men counterparts, implying better immunological outcomes in women. Although both sexes experience elevated CD4 cell count over treatment duration, females obtain greater values than males.

The immunological outcomes of antiretroviral therapy are influenced both negatively and positively by age at antiretroviral therapy initiation (Centre for AIDS Information and Advocacy, 2011:26; Weller *et al.*, 2008:1470). In Europe, Weller *et al.* (2008:1470) conducted a collaborative observational study using 33 cohort studies that included 49 921 patients who were initiated on antiretroviral therapy. The study evaluated antiretroviral therapy outcomes in children, adolescents and older HIV-infected patients in terms of CD4 cell recovery. The results were that although reasonable responses were observed across the younger age groups (those aged < 2 years [72%], 2-5 years [82%] and 6-12 years [78%]), the CD4 cell recovery was poorer in the older age group (those aged ≥ 60 years had 57%). These immunological outcomes were most improved in the children subgroups. According to Centre for AIDS Information and Advocacy (2011:26) initiating antiretroviral therapy in older age is associated with lowered chances of immunological recovery in the first two years of treatment. Patients aged ≥ 60 years had 0.74 chances to achieve 100 cells/mm³ gain in CD4 cell count while those aged from 30 to 39 years had 0.92 chances to gain CD4 cell count by 100 cells/mm³ and females in general had 1.13 opportunity to gain CD4 cell count by 100 cells/mm³. The initiation of antiretroviral therapy at younger age is associated with better CD4 cell recovery (Weller *et al.*, 2008:1470) while poor recoveries are observed with antiretroviral therapy initiation at older age (Centre for AIDS Information and Advocacy, 2011:26).

3.3.2.3 Plasma viral load

The study had also anticipated to evaluate the clinical outcomes using the plasma viral load. However, the plasma viral load measure was not included as the treatment monitoring parameter at the time of study. Therefore, there was no analysis about plasma viral load as there was no data about the parameter on the data source.

3.3.3 The results for evaluation of renal safety

The study had also determined the effect of treatment duration, sex, age at antiretroviral therapy initiation and body weight on the change in renal function as measured by serum creatinine concentration and calculated by creatinine clearance (using Cockcroft-Gault equation) and estimated glomerular filtration rate (using Modification of Diet in Renal Disease Study equation). The results of the analysis are presented in tables below (from **Table 3-19** to **Table 3-49**).

3.3.3.1 Change in serum creatinine concentration

The analyses of the effects of the independent variables on the change in serum creatinine concentration are presented in **Tables 3-19, 3-20, 3-21, 3-22, 3-23, 3-24, 3-25, 3-26, 3-27 and 3-28**. **Table 3-19** indicates that the change in serum creatinine is independent of treatment duration. There was no statistical significant difference in the change of serum creatinine over treatment duration at 95% confidence interval ($p=0.13$).

Table 3-19: Serum creatinine concentration according to treatment duration: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	996.18	5998.74	0.00
Treatment duration	1	90.88	2.38	0.13

Although not statistically significant at 95% confidence interval, the estimates of fixed effects in **Table 3-20** unveil an increase in serum creatinine concentration of 0.004 μ mol/l over treatment duration($p=0.13$). This increase in serum creatinine concentration indicates that from baseline, serum creatinine concentration increased insignificantly to 81.13 μ mol/l + 0.004 * 1 year = **81.134 μ mol/l** over one year treatment duration.

**Table 3-20: Serum creatinine concentration according to treatment duration:
Estimates of fixed effects**

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	81.13	1.05	996.18	77.45	0.00	79.06	83.17
Treatment duration	0.004	0.003	90.88	1.54	0.13	-0.001	0.01

**Table 3-21: Serum creatinine concentration according to treatment duration and sex:
Type III tests of fixed effects**

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	995.64	6323.32	0.00
Treatment duration	1	89.13	2.95	0.09
Sex	1	965.67	57.34	0.00

Table 3-21 depicts the effect of sex on the change in serum creatinine over treatment duration. The change in serum creatinine is dependent on sex, as shown by a statistically significant difference in the change in serum creatinine in males and females ($p < 0.05$) at 95% confidence interval.

The results presented in **Table 3-22** indicate a statistically significant decline in serum creatinine in both sexes over treatment duration at 95% confidence interval ($p < 0.05$). Females are found to have more worsened decline of 13.22 $\mu\text{mol/l}$ than males. According to the table, from baseline, females experienced significantly worsened decline to **75.04 $\mu\text{mol/l}$** ($88.26 \mu\text{mol/l} + (-13.22) * 1 + 0.004 * 1 \text{ year}$) in serum creatinine concentration while males' serum creatinine concentration remained at **88.26 $\mu\text{mol/l}$** over one year of treatment.

Table 3-22: Serum creatinine concentration according to treatment duration and sex: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	88.26	1.39	1121.20	63.44	0.00	85.53	90.99
Treatment duration	0.004	0.003	89.13	1.72	0.09	-0.0007	0.01
[Sex=female]	-13.22	1.75	965.67	-7.57	0.00	-16.65	-9.80
[Sex=male]	0	0					

The change in serum creatinine concentration over treatment duration depends on age at antiretroviral therapy initiation. **Table 3-23** results indicate a statistically significant difference in change in serum creatinine relative to age at antiretroviral therapy initiation over treatment duration at 95% confidence interval ($p < 0.05$).

Table 3-23: Serum creatinine concentration according to treatment duration and age at antiretroviral therapy initiation: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1006.72	389.95	0.00
Treatment duration	1	96.30	1.46	0.23
Age at antiretroviral therapy initiation	1	968.68	14.84	0.00

The estimates of fixed effects in **Table 3-24** reveal a statistically significant increase in serum creatinine concentration by 0.31 $\mu\text{mol/l}$ due to age at antiretroviral therapy initiation over treatment duration at 95% confidence interval ($p < 0.05$). The results infer that due to age at ART initiation, in overall, patients had increasing serum creatinine concentrations from baseline. Serum creatinine concentration increased significantly from baseline to **68.71 $\mu\text{mol/l}$** ($68.40 \mu\text{mol/l} + 0.31 \times 1 + 0.003 \times 1 \text{ year}$) over one year of treatment.

Table 3-24: Serum creatinine concentration according to treatment duration and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	68.40	3.46	1006.72	19.74	0.00	61.60	75.19
Treatment duration	0.003	0.003	96.30	1.21	0.23	-0.002	0.009
Age at antiretroviral therapy initiation	0.31	0.08	968.68	3.85	0.00	0.15	0.46

Table 3-25: Serum creatinine concentration according to treatment duration and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1007.59	381.57	0.00
Body weight	1	962.74	1.10	0.30

The results of **Table 3-25** show that the body weight is independent of change in serum creatinine concentration over treatment duration. As such there was no statistically significant difference in change in serum creatinine concentration relative to body weight over treatment duration ($p=0.30$).

Although not statistically significant, the estimates of fixed effects on **Table 3-26** show 0.07 $\mu\text{mol/l}$ decline in serum creatinine concentration due to body weight over treatment duration ($p=0.30$). According to body weight, patients had declining serum creatinine concentration from baseline on annual basis of treatment. This means that from 86.55 $\mu\text{mol/l}$, serum creatinine concentration declined to $86.55 \mu\text{mol/l} + (-0.07) * 1 \text{ year} = \mathbf{86.48 \mu\text{mol/l}}$.

Table 3-26: Serum creatinine concentration according to treatment duration and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	86.55	4.43	1007.59	19.53	0.00	77.86	95.25
Body weight	-0.07	0.07	962.74	-1.05	0.30	-0.21	0.07

Table 3-27 portrays the combined effect of sex, age at antiretroviral therapy initiation and body weight on change in serum creatinine concentration over treatment duration. The duo sex ($p < 0.05$) and age at antiretroviral therapy initiation ($p = 0.004$) statistically significantly influence the change in serum creatinine concentration over treatment duration, while serum creatinine concentration is independent of body weight over treatment duration ($p = 0.46$) at 95% confidence level.

Table 3-27: Serum creatinine concentration according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1004.66	195.99	0.00
Treatment duration	1	99.50	2.03	0.16
Sex	1	958.31	45.47	0.00
Age at antiretroviral therapy initiation	1	932.59	8.38	0.004
Body weight	1	991.73	0.54	0.46

Table 3-28: Serum creatinine concentration according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	Df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	81.23	5.46	1006.88	14.87	0.000	70.51	91.95
Treatment duration	0.003	0.003	99.50	1.43	0.157	-0.001	0.009
[Sex= female]	-12.14	1.80	958.31	-6.74	0.000	-15.67	-8.61
[Sex= male]	0	0					
Age at antiretroviral therapy initiation	0.23	0.08	932.59	2.89	0.004	0.07	0.39
Body weight	-0.05	0.70	991.73	-0.74	0.462	-0.19	0.09

The results in **Table 3-28** show that while sex is associated with declines in change in serum creatinine concentration over treatment duration, females experience a statistically significant difference and more worsened decline of 12.14 $\mu\text{mol/l}$ in serum creatinine concentration over treatment duration than males ($p < 0.05$). There is also a 0.05 $\mu\text{mol/l}$ decline in serum creatinine over treatment duration relative to body weight, though not statistically significant ($p = 0.46$). In contrast, the age at antiretroviral therapy initiation shows a statistically significant increase of 0.23 $\mu\text{mol/l}$ in serum creatinine concentration over treatment duration ($p = 0.004$). In overall, patients' serum creatinine concentration declined yearly from baseline. That is, $81.23 \mu\text{mol/l} + (-12.14) * 1 + 0.23 * 1 + (-0.05) * 1 \text{ year} + 0.003 * 1 \text{ year} = 69.04 \mu\text{mol/l}$ was observed in females while males' serum creatinine concentration declined to $81.23 \mu\text{mol/l} + (-12.14) * 0 + 0.23 * 1 + (-0.05) * 1 \text{ year} + 0.003 * 1 \text{ year} = 81.18 \mu\text{mol/l}$

The direct determination of glomerular filtration rate by measuring serum creatinine concentration operates on assumptions that may not strictly hold at all times. This measurement increases error chances in estimating glomerular filtration rate as it provides rough indices of glomerular filtration rate (NKF, 2002:84). Therefore, the measurement of serum creatinine concentration alone is not an accurate index of glomerular filtration rate. National Kidney Foundation (2002:81) recommends that the measurement should not be used alone to assess kidney function. Although serum creatinine concentration measurement alone has limitations in determining overall renal function,

in this study some predictor variables have been implicated in changes in serum creatinine concentrations over treatment period. According to National Kidney Foundation (2002:85), factors including age, sex, race, body size, diet and some drugs affect the serum concentration of creatinine.

In contrast to the study findings, Zhao *et al.* (2015:10) conducted a one-year national cohort study on 41 862 patients to identify the predictors and prevalence of abnormal kidney function among Chinese patients prior to antiretroviral therapy and during the treatment with TDF and/or boosted lopinavir regimens. The study found that being female was associated with 1.71 (hazard ratio) greater chance of deterioration renal function than being male ($p<0.0001$). However, in alignment with the current study findings, the same study revealed that being aged >50 years correlates to 3.11 (hazard ratio) risk of deteriorating renal function ($p<0.0001$). A retrospective cohort study done in Toronto by Antoniou *et al.* (2005:287) on 172 patients receiving TDF-containing HAART regimens for median duration of 16 months, neither the body weight, sex nor age were associated with changes in serum creatinine concentration over the treatment duration. This is partially consistent with the results of the current study that found the change in serum creatinine concentration over treatment duration does not depend on the body weight.

A number of equations including the Cockcroft-Gault (for calculating creatinine clearance) and the MDRD study (for calculating estimated glomerular filtration rate) have been developed to consider the factors influencing the level of creatinine in serum so as to attain more accurate and precise estimates of renal function (NKF, 2002:87). These equations provide better estimates of renal function however, each equation has own inherent limitations that can be counteracted by other equations. Therefore, the equations can be used simultaneously.

3.3.3.2 Change in creatinine clearance using the Cockcroft-Gault equation

The results presented from **Table 3-29 to Table 3-38** are the results of change in creatinine clearance as calculated using the Cockcroft-Gault equation relative to the independent variables mentioned.

The creatinine clearance is independent of treatment duration as shown by **Table 3-29**. There is no statistically significant difference in change in creatinine clearance relative to treatment duration over treatment duration at 95% confidence interval ($p=0.39$).

Table 3-29: Creatinine clearance according to treatment duration: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	1028.68	4203.75	0.00
Treatment duration	1	159.46	0.74	0.39

Table 3-30: Creatinine clearance according to treatment duration: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	<i>p</i> value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	87.89	1.36	1028.68	64.84	0.00	85.23	90.55
Treatment duration	0.002	0.003	159.46	0.86	0.39	-0.004	0.009

Table 3-30 reflects 0.002 ml/min increase in creatinine clearance over treatment duration that is not statistically significant at 95% confidence interval ($p=0.39$). In one year of treatment, patients were observed to have elevating creatinine clearance from baseline. In overall, creatinine clearance increased from 87.89 ml/min to **87.892 ml/min** ($87.89 \text{ ml/min} + 0.002 * 1 \text{ year}$).

Table 3-31: Creatinine clearance according to treatment duration and sex: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	<i>p</i> value
Intercept	1	1025.71	4171.25	0.00
Treatment duration	1	157.94	0.72	0.40
Sex	1	980.58	3.74	0.05

Table 3-31 displays the effect of sex on the change in creatinine clearance over treatment duration. Creatinine clearance is independent of sex. The results show a not a statistically significant difference in change in creatinine clearance relative to sex over treatment duration at 95% confidence interval ($p=0.05$).

Although not statistically significant at 95% confidence interval ($p=0.05$), the results in **Table 3-32** reveal an increase in creatinine clearance over treatment duration in both sexes; having additional increase of 4.41ml/min with females over males. There were more elevations in clearance from baseline in females than males. Creatinine clearance increased from 85.50 ml/min to **89.91 ml/min** ($85.50 \text{ ml/min} + 4.41 * 1 + 0.003 * 1 \text{ year}$) over one year of treatment for females while for males only **85.503 ml/min** increase was observed.

Table 3-32: Creatinine clearance according to treatment duration and sex: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	85.50	1.83	1090.42	46.74	0.00	81.91	89.09
Treatment duration	0.003	0.003	157.94	0.85	0.40	-0.004	0.009
[Sex=female]	4.41	2.28	980.58	1.93	0.05	-0.06	8.88
[Sex=male]	0	0					

Table 3-33 results show that creatinine clearance is dependent of age at antiretroviral therapy initiation by a statistically significant difference in change in creatinine clearance over treatment duration ($p<0.05$).

Table 3-33: Creatinine clearance according to treatment duration and age at antiretroviral therapy initiation: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	981.51	1000.12	0.00
Treatment duration	1	167.18	4.61	0.03
Age at antiretroviral therapy initiation	1	951.09	124.12	0.00

The observation made in **Table 3-34** is a statistically significant decline in creatinine clearance of 1.06ml/min over the treatment duration relative to age at antiretroviral therapy initiation at 95% confidence interval ($p<0.05$). The table reveals that due to age at ART initiation, decline in creatinine clearance from baseline was observed. Creatinine clearance declined from baseline to **131.02 ml/min** ($132.07 \text{ ml/min} + (-1.06) * 1 + 0.01 * 1 \text{ year}$) over one year of treatment.

Table 3-34: Creatinine clearance according to treatment duration and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	132.07	4.18	981.51	31.63	0.00	123.87	140.26
Treatment duration	0.01	0.003	167.18	2.15	0.03	0.001	0.01
Age at antiretroviral therapy initiation	-1.06	0.10	951.09	-11.14	0.00	-1.25	-0.88

Table 3-35: Creatinine clearance according to treatment duration and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	946.37	0.01	0.93
Body weight	1	889.06	367.67	0.00

Body weight is a determinant of change in creatinine clearance as shown in **Table 3-35**. The results depict a statistically significant change in creatinine clearance relative to body weight over treatment duration ($p < 0.05$). Moreover, the results in **Table-3-36** reveal 1.46 ml/min increase in creatinine clearance relative to body weight over treatment duration at 95% confidence interval ($p < 0.05$).

Table 3-36: Creatinine clearance according to treatment duration and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	-0.43	4.75	946.37	-.09	0.93	-9.74	8.89
Body weight	1.46	0.08	889.06	19.18	0.00	1.31	1.61

Table 3-37: Creatinine clearance according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	893.69	67.26	0.00
Treatment duration	1	151.41	0.004	0.95
Sex	1	868.70	1.06	0.30
Age at antiretroviral therapy initiation	1	856.82	188.41	0.00
Body weight	1	863.63	445.05	0.00

The results presented in **Table 3-37** are a combined effect of the predictors of creatinine clearance namely sex, age and body weight on change in creatinine clearance over treatment duration. There is a statistically significant difference on creatinine clearance over treatment duration relative to age and body weight ($p < 0.05$).

The results in **Table 3-38** that although not statistically significant, females are 1.86 ml/min more at disadvantage to experience reductions in creatinine clearance over treatment duration than males ($p = 0.30$). Again, age at antiretroviral therapy initiation is also associated with a statistically significant decline in 1.10 ml/min decline in creatinine clearance in both sexes over treatment duration ($p < 0.05$). However, the table further shows a statistically significant increment of 1.49 ml/min in creatinine clearance relative to body weight over treatment duration; all at 95% confidence level ($p < 0.05$). In overall, females' creatinine clearance reduced to **43.90 ml/min**

(45.37 ml/min + (-1.86) * 1 + (-1.10) * 1 + 1.49 * 1) in one year of treatment while males' creatinine clearance declined to a lesser extent to **45.76 ml/min**.

Table 3-38: Creatinine clearance according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	45.37	5.51	885.41	8.23	0.00	34.55	56.18
Treatment duration	0.000	0.002	151.41	.06	0.95	-.004	.004
[Sex=female]	-1.86	1.81	868.70	-1.03	0.30	-5.42	1.69
[Sex= male]	0	0					
Age at antiretroviral therapy initiation	-1.10	0.08	856.82	-13.73	0.00	-1.26	-.94
Body weight	1.49	0.07	863.63	21.10	0.00	1.35	1.62

In settings that have adopted the use of TDF-containing HAART regimens as the first-line therapy, renal toxicity has been prevalent (Mulenga *et al.*, 2008:1825). In a retrospective follow-up study conducted on 275 participants on evaluating the risk factors to developing renal impairment in patients taking TDF-containing HAART regimens, Neto *et al.* (2016:15) found that following 36 months of treatment, patients had impaired renal function in a time-dependent manner. Using the Cockcroft-Gault equation to evaluate renal function in the study, the analysis of variance revealed a statistically significant difference in creatinine clearance between sexes whereby the male sex had 3.95 more potential to develop renal impairment ($p=0.048$). These findings are in contrast with the current study findings since the females have been found to have more worsened decline in creatinine clearance than males. However, any specific sex was not associated with the risk of developing renal impairment following one year treatment with TDF-containing HAART regimens in a follow-up study conducted by Chabala *et al.* (2015:102) in Zambia. The study compared creatinine clearance amongst HIV/AIDS patients treated with TDF-containing HAART regimens versus those on non-TDF-containing HAART regimens in 549 participants.

The older age increases the odds of developing renal impairment after 1 year of treatment with TDF-containing HAART regimens ($p<0.001$) (Chabala *et al.*, 2015:101). Neto *et al.* (2016:15)

study also found a 45.81 risk of developing renal impairment in patients taking TDF-containing HAART regimens at ages of over 50 years ($p < 0.001$). For each one year addition to age, Mpondo *et al.* (2014:2) found 1.06 odds of decreasing the glomerular filtration rate as calculated by the Cockcroft-Gault equation. The cohort study was done on 171 adults on at least two-year antiretroviral therapy.

In a single centre cohort study conducted in Japan on 495 HIV infected patients initiated on TDF-containing HAART regimens, Nishijima *et al.* (2011:7) found that low median body weight (63 kg) was an independent risk factor to high incidence of TDF-related renal impairment. This is in contrast to findings of the current study in which patients' median age was 59 kg and the body weight showed a significant increase in creatinine clearance as calculated by the Cockcroft-Gault equation. Mizushima *et al.* (2014:786) also found that the cumulative incidence of renal impairment increased relative to low body weight (mean body weight = 56.5 ± 8.2 kg) in an observational single centre cohort study done on 153 patients who were switched to TDF-containing HAART regimens in Vietnam.

3.3.3.3 Change in the estimated glomerular filtration rate using the MDRD study equation

The results presented from **Table 3-39** to **Table 3-49** show the change in the estimated glomerular filtration rate as calculated by the MDRD study equation relative to the independent variables identified.

The estimated glomerular filtration rate is independent of treatment duration (**Table 3-39**). As such, there was no statistically significant difference in the change in estimated glomerular filtration rate relative to the treatment duration over treatment duration at 95% confidence level.

**Table 3-39: Estimated glomerular filtration rate according to treatment duration:
Type III tests of fixed effects**

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1080.81	5194.08	0.00
Treatment duration	1	139.92	0.65	0.42

**Table 3-40: Estimated glomerular filtration rate according to treatment duration:
Estimates of fixed effects**

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	103.27	1.43	1080.81	72.07	0.00	100.46	106.09
Treatment duration	-0.002	0.003	139.92	-0.81	0.42	-0.01	0.003

The estimates of fixed effects in **Table 3-40** reveal 0.002 ml/min/1.73 m² decline in the estimated glomerular filtration rate that is not statistically significant ($p=0.42$). The estimated glomerular filtration rate declined insignificantly from baseline to 103.268 ml/min/1.73 m² over one year of treatment.

Table 3-41: Estimated glomerular filtration rate according to treatment duration and sex: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	1076.26	5283.38	0.00
Treatment duration	1	135.71	0.62	0.43
Sex	1	898.42	18.65	0.00

The estimated glomerular filtration rate is dependent of sex as shown in **Table 3-41**. This is evidenced by a statistically significant change in the estimated glomerular filtration rate over treatment duration relative to sex ($p<0.05$).

The estimates of fixed effects shown in **Table 3-42** reveal a statistically significant overall decrement in estimated glomerular filtration rate in both sexes over treatment duration ($p<0.05$). The decrement is 10.10 ml/min/1.73m² more exaggerated in females than males at 95% confidence level. There was a worsened decline in estimated glomerular filtration rate observed in females than males; from baseline to **98.66 ml/min/1.73m²** ($108.76 \text{ ml/min/1.73m}^2 + (-10.10) * 1 + (-0.002) * 1 \text{ year}$) for females and **108.76 ml/min/1.73m²** ($108.76 \text{ ml/min/1.73m}^2 + (-10.10) * 0 + (-0.002) * 1 \text{ year}$) for males over one year treatment.

Table 3-42: Estimated glomerular filtration rate according to treatment duration and sex: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	108.76	1.91	1130.76	56.97	0.00	105.01	112.51
Treatment duration	-0.002	0.003	135.71	-0.78	0.43	-0.01	0.004
[Sex=female]	-10.10	2.34	898.42	-4.32	0.00	-14.68	-5.51
[Sex=male]	0	0					

Table 3-43 and **Table 3-44** depict the effect of age at antiretroviral therapy initiation on the estimated glomerular filtration rate. The estimated glomerular filtration rate is dependent upon the age at antiretroviral therapy initiation as shown by a statistically significant difference in the estimated glomerular filtration rate relative to age at antiretroviral therapy initiation over treatment duration ($p < 0.05$). Furthermore, this effect of age at antiretroviral therapy initiation is a 0.73 ml/min/1.73m² decrement in estimated glomerular filtration rate over treatment duration at 95% confidence level ($p < 0.05$). The results infer that at any age of ART initiation, patients developed declining estimated glomerular filtration rate from baseline to **133.00 ml/min/1.73m²** ($133.73 \text{ ml/min/1.73m}^2 + (-0.73) * 1 + (-0.00) * 1 \text{ year}$) over one year of treatment.

Table 3-43: Estimated glomerular filtration rate according to treatment duration and age at antiretroviral therapy initiation: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	963.77	878.06	0.00
Treatment duration	1	144.26	0.01	0.92
Age at antiretroviral therapy initiation	1	924.29	50.36	0.00

Table 3-44: Estimated glomerular filtration rate according to treatment duration and age at antiretroviral therapy initiation: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	133.73	4.51	963.77	29.63	0.00	124.88	142.59
Treatment duration	-0.000	0.003	144.26	-0.10	0.92	-0.006	0.006
Age at antiretroviral therapy initiation	-0.73	0.10	924.29	-7.10	0.00	-0.94	-0.53

Table 3-45: Estimated glomerular filtration rate according to treatment duration and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	p value
Intercept	1	899.75	341.87	0.00
Body weight	1	832.17	0.88	0.35

The estimated glomerular filtration rate is independent of body weight as shown in **Table 3-45** by a not statistically significant difference in the change in estimated glomerular filtration rate owing to body weight over treatment duration at 95% confidence level ($p=0.35$). Thus the 0.09 ml/min/1.73m² decline in estimated glomerular filtration rate due to body weight over treatment duration revealed in **Table 3-46** is not statistically significant at 95% confidence level ($p=0.35$). The table entails that due to body weight, estimated glomerular filtration rate reduced insignificantly to **107.63 ml/min/1.73m²** over one year of treatment.

Table 3-46: Estimated glomerular filtration rate according to treatment duration and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	107.72	5.83	899.75	18.49	0.00	96.28	119.15
Body weight	-0.09	0.09	832.17	-.94	0.35	-0.27	0.10

Table 3-47: Estimated glomerular filtration rate according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Type III tests of fixed effects

Type III Tests of Fixed Effects				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	928.75	380.01	0.00
Treatment duration	1	153.24	0.05	0.82
Sex	1	900.44	30.69	0.00
Age at antiretroviral therapy initiation	1	887.00	56.15	0.00
Body weight	1	903.84	0.07	0.80

The **Table 3-47** and **Table 3-48** are a combined effect of sex, age at antiretroviral therapy initiation and body weight on the estimated glomerular filtration rate over treatment duration. The estimated glomerular filtration rate is independent of sex, age at antiretroviral therapy initiation and body weight over the treatment duration.

This is shown in **Table 3-47** by an insignificant difference in the change in estimated glomerular filtration rate over the treatment duration relative to sex, age at antiretroviral therapy initiation and body weight ($p=0.80$). More specific details in **Table 3-48** show that although there is a decline in the estimated glomerular filtration rate over treatment duration in both sexes, there is 13.05 ml/min worsened decline in females than males ($p<0.05$) and a 0.78 ml/min decline in relation to age at antiretroviral therapy initiation ($p<0.05$). Again, the body weight also shows a 0.02 ml/min/1.73m² decline in estimated glomerular filtration rate over treatment duration at 95% confidence level ($p=0.80$). In overall, at any age of ART initiation, reductions in estimated glomerular filtration rate

following yearly treatment were observed greater in females from baseline to **129.99 ml/min/1.73m²** ($143.84 \text{ ml/min/1.73m}^2 + (-13.05) * 1 + (-0.78) * 1 + (-0.02) * 1 + 0.00 * 1 \text{ year}$) while in males, estimated glomerular filtration rate declined to **143.84 ml/min/1.73m²**.

Table 3-48: Estimated glomerular filtration rate according to treatment duration, sex, age at antiretroviral therapy initiation and body weight: Estimates of fixed effects

Estimates of Fixed Effects							
Parameter	Estimate	Std. Error	df	t	p value	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	143.84	7.17	923.10	20.07	0.00	129.77	157.91
Treatment duration	0.000	0.003	153.24	0.23	0.82	-0.01	0.007
[Sex=female]	-13.05	2.36	900.44	-5.54	0.00	-17.67	-8.43
[Sex=male]	0	0					
Age at antiretroviral therapy initiation	-0.78	0.10	887.00	-7.50	0.00	-0.99	-0.58
Body weight	-0.02	0.09	903.84	-0.25	0.80	-0.20	0.16

Despite its limitations such as overestimating the glomerular filtration rate (Sirwal *et al.*, 2004:122), the creatinine clearance using the Cockcroft-Gault equation is mostly widely and validated used test to evaluate adult renal safety in clinical practice (MOH, 2016a:48). The creatinine clearance has been shown to overestimate the glomerular filtration rate by 10-20% (Sirwal *et al.*, 2004:123). Although not done in Lesotho and less common in clinical practice, the study analysis of the current study adjusted for the overestimation by employing the MDRD study equation which estimates glomerular filtration rate directly. While the eGFR was found to be independent of sex, age at antiretroviral therapy initiation and body weight, declines in eGFR were similarly observed relative to these variables although not statistically significant.

However, Poggio *et al.* (2009:1085) conducted a retrospective cohort study (1974-2005) on 1 481 adults evaluating features associated with glomerular filtration rates in living kidney donors. The study found statistically significant differences in the mean eGFR between males and females ($p=0.015$). Again, the donors aged ≥ 45 years had lower mean eGFR than younger counterparts ($p < 0.001$). The race was not implicated in these changes. In settings of low body weight at baseline a substantial weight gain following antiretroviral therapy initiation is not directly adjusted

by the MDRD study equation (Stohr *et al.*, 2008:769). Generally, the Cockcroft-Gault and the MDRD study equations can give different glomerular filtration rate results and none of the two has been found superior over the other in HIV-infected adults in Africa (Stohr *et al.*, 2008:769).

3.4 Chapter summary

This chapter provided results of the empirical investigation phase of the study. The results were found from the analysis of collected data based on the specific objectives of the empirical investigation. The discussions of the results are provided in alignment with the specific objectives also. Chapter 4 entails the conclusions of both the specific objectives of the literature review and empirical investigation. These conclusions emanate from findings from the literature review (Chapter 2) and results and discussions (Chapter 3). The next chapter also encloses the limitations of the study and recommendations for further study.

CHAPTER 4: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

4.1 Conclusions

The conclusions of the study emanate from the specific objectives of the literature review and the empirical investigation phases.

4.1.1 Conclusions from the literature review

The **first** specific objective of the literature review was to describe therapeutic mechanism of action of TDF in the treatment of HIV/AIDS. The literature findings revealed that TDF is a mono-phosphorylated NtRTI prodrug. In order for the drug to be active, it has to be phosphorylated to a triphosphate (by two phosphate molecules) form which acts as the structural analogue to viral triphosphates (Miller, 2002:17). Since HIV is an RNA virus, the RNA strand firstly has to be copied into a DNA strand for the virus to replicate (Weller & Williams, 2001:1410). The copying process is catalysed by the specific HIV DNA polymerase enzyme called reverse transcriptase. The triphosphate form of TDF is an analogue to viral triphosphates which competitively inhibits the reverse transcriptase since the enzyme cannot distinguish the drug's triphosphate from the viral triphosphates (building blocks of viral DNA).

Once the TDF triphosphate is incorporated into the growing viral DNA chain by the reverse transcriptase, the DNA chain cannot be elongated further. This results in incomplete and non-functional DNA chain. The incomplete DNA chain is susceptible to destruction by host cellular enzymes (Elion & Witt, 2003:2). The end result is failure for HIV RNA to duplicate into DNA, hence the virus cannot multiply into daughter viral copies. The plasma viral content is inhibited from increasing when antiretroviral therapy is in place. This is coupled with better immunological improvements characterised by elevations in CD4 cell count (Smith *et al.*, 2003:969). There are also improvements in clinical outcomes as evidenced by weight gain, betterment feeling, improved energy to perform daily tasks, good recovery from signs and symptoms of HIV infection and reduced incidence of opportunistic diseases (Beard *et al.*, 2008:7; MOH, 2013:37; Mukherjee *et al.*, 2014:6).

The **second** specific objective of the literature review was to evaluate the response to TDF-containing HAART regimens relating to clinical outcomes and renal safety. In comparison with non-TDF-containing HAART regimens, the literature review indicated that TDF-containing HAART regimens are superior to the non-TDF-containing HAART regimens. The superiority was indicated by excellent efficacy and less toxicity than AZT- and d4T-containing HAART regimens (Louie *et al.*, 2003:1154). The efficacy was shown by greatest decrements in plasma viral load

and elevations in CD4 cell counts (Louie *et al.*, 2003:1154; Pozniak *et al.*, 2006:538). There were more patients who achieved and sustained viral suppression in the TDF treated group than the non-TDF groups (Arribas *et al.*, 2008:77). This was coupled with greater elevations on CD4 cell count in patients treated with TDF-containing HAART regimens. Again, the TDF-containing HAART regimens were found to simplify HIV/AIDS care by reducing single-drug substitution rates (Bygrave *et al.*, 2011a:77).

The **third** specific objective of the literature review was to discuss conventional measures of the clinical outcomes in HIV/AIDS treatment. The clinical response to antiretroviral treatment can be assessed using clinical status, immunologic and virology functions. The clinical indices useful in assessing the response to antiretroviral therapy included betterment of feeling, energy to perform daily tasks, changes in body weight, response of current signs and symptoms and incidence of opportunistic diseases (Beard *et al.*, 2008:7; MOH, 2013:37; Mukherjee *et al.*, 2014:6). Immunologically, the CD4 cell count is the only currently recommended marker of immunologic response to antiretroviral therapy while the plasma viral load is the gold standard for monitoring the success and failure of antiretroviral therapy (Lima *et al.*, 2009:195; Sawe & McIntyre, 2009:463; Smith *et al.*, 2003:969). Therefore, the main goal of antiretroviral therapy is to suppress plasma viral concentration to below detectable limits and to maintain the undetectable levels so that clinical outcomes could improve and CD4 cell count can rise (Pasternak *et al.*, 2013:1).

The **fourth** specific objective of the literature review was to describe renal toxicological mechanisms of action of TDF in the treatment of HIV/AIDS. The literature findings unveiled the existence of specific nephrotoxic site of TDF being the proximal tubules of nephrons in the kidneys (Woodward *et al.*, 2009:483). In attempt to excrete the drug from circulating blood to urine, the drug follows the secretory function of the nephrons (not filtration). The proximal tubular cells have two transporters responsible for the secretion of TDF. The transporters are hOAT-1 that is responsible for the uptake of TDF from blood flow into the proximal tubular cells. The other transporter is MRP-4 that is responsible for the efflux of the drug from the proximal tubular cells into the urine for complete excretion of the drug out of the body (Hagos & Wolf, 2010:2065; Kohler *et al.*, 2009:5).

The renal toxicity manifests due to high affinity of TDF to hOAT-1 that promotes excessive uptake of the drug into the tubular cells, resulting in possible drug accumulation into these cells. The accumulation is further exacerbated by the inhibitory activity of TDF to the MRP-4 (Kohler *et al.*, 2011:7). The drug inside the tubular cell is blocked from being emptied into the urine flow. The net effect is further drug accumulation that results in toxicity to proximal tubular cells owing to excessive drug uptake coupled with inhibited expulsion of the drug from the cells. The nephrotoxicity caused by TDF is characterised by elevated serum creatinine, alkalosis, glycosuria, proteinuria, hypo-phosphataemia and hypokalaemia (Chua *et al.*, 2012:1; Johnson *et*

al., 2012:1; Kalyesubula & Perazella, 2011:3; Leem *et al.*, 2014:246; Pontrelli *et al.*, 2012:1). The glomerular filtration rate is compromised by the toxicity of the proximal tubular cells by TDF.

The **fifth** specific objective of the literature review was to evaluate the renal toxicological response to TDF-containing HAART regimens. The literature findings reveal the evidence established that the use of TDF-containing HAART regimens causes renal toxicity in a time-dependent manner. Clinical trials and short-term studies (for up to 103 weeks) about TDF renal toxicity failed to reveal renal compromise to the drug use (Gallant & Moore, 2009:1975; Schooley *et al.*, 2002:1262). The long-term studies (up to 4 years) on TDF renal safety instead revealed serious renal adverse events to the drug (Gallant *et al.*, 2008:2162; Nelson *et al.*, 2007:1278; Winston *et al.*, 2006:109).

The **sixth** specific objective of the literature review was to discuss conventional measures of renal safety in patients taking TDF-containing HAART regimens. According to the literature, evaluation of renal function through blood tests involves direct measurements of blood urea nitrogen and serum creatinine concentration (Kim & Moon, 2012:268). Since these measurements had inadequacies, equations for renal clearance of ideal biomarkers of renal function were developed (Florkowski & Chew-Harris, 2011:75). The equation for creatinine clearance called the Cockcroft-Gault was developed to estimate glomerular filtration rate indirectly (Florkowski & Chew-Harris, 2011:75; Stevens *et al.*, 2006:2476). The equation also had some limitations such as overestimating the GFR. The limitations were counteracted by the invention of the MDRD study equation that directly estimates glomerular filtration rate. None of the two equations was found superior over one another (Stevens *et al.*, 2006:2476).

The **seventh** specific objective of the literature review was to discuss patient factors influencing both the beneficial and harmful outcomes of TDF-containing HAART regimens. The literature findings availed a wide range of determinants of therapeutic and toxicological outcomes from TDF-containing HAART regimens. These determinants included the likes of age, sex, treatment duration, body weight, CD4 cell count, plasma viral load and serum creatinine concentration which were included as the variables of the study. Clinically, there were compromise in outcomes of antiretroviral therapy relative to being old compared to being younger (Bakanda *et al.*, 2011:704; Laughton *et al.*, 2012:6; Resino *et al.*, 2008:7; Vinikoor *et al.*, 2014:954; Violary *et al.*, 2008:8). Female sex is associated with better improvements in CD4 cell count than males. Long term retention on antiretroviral therapy revealed excellent clinical, immunological and virologic improvements (Sanne *et al.*, 2009:9). Olawumi *et al.* (2008:313) found that over 14 months of antiretroviral therapy body weight increased significantly from baseline.

Toxicologically, being aged ≥ 40 years is associated with renal compromise as kidneys begin to lose significant number of nephrons and decreased tissue repair (AlAhmadi & AbuButain, 2013; Wang *et al.*, 2014:15367). Due to later medical attention seeking by males, there is high mortality

in HIV-infected males on antiretroviral therapy than female counterparts (Chen *et al.*, 2008:517; Taylor-Smith *et al.*, 2010:52). Gallant *et al.* (2008:2162), Nelson *et al.* (2007:1278) and Winston *et al.* (2006:109) found that long term antiretroviral therapy with TDF-containing HAART regimens is associated with progressive renal toxicity. The renal toxicity is characterised by elevated serum creatinine and compromised glomerular filtration rate (Chua *et al.*, 2012:1; Johnson *et al.*, 2012:1; Kalyesubula & Perazella, 2011:3; Leem *et al.*, 2014:246; Pontrelli *et al.*, 2012:1). The other determinants of consideration which were not included as study variables entailed WHO clinical stage, presence of opportunistic infections and co-morbidities, specific baseline regimens, chemoprophylaxis, treatment adherence and plasma haemoglobin levels.

4.1.2 Conclusions from the empirical investigation

The **first** specific objective of the empirical investigation was to determine the change in body weight, CD4 cell count and plasma viral load in patients taking TDF-containing HAART regimens over treatment duration. The results of the study (255 patients enrolled) revealed that yearly over the treatment duration from initiation of antiretroviral therapy at any age, patients gained weight significantly from the baseline levels ($p < 0.05$). The weight gain was estimated to 0.10 kg over the treatment duration in both sexes. Females were found to be the most advantageous to gaining more weight than males, with an estimated 2.64 kg more increase in weight gain ($p < 0.05$) (**refer to section 3.3.1.1 Table 3-10**). In overall, females gained weight from 56.57 kg to **59.30 kg** while males gained weight from 56.57 kg to **56.67 kg** over one year of treatment. The initiation of antiretroviral therapy in HIV/AIDS patients is associated with progressive clinical improvements including significant weight gain from baseline levels over treatment duration (Denué *et al.*, 2013:91; Olawumi *et al.*, 2008:314). While the weight gain is observable in both males and females (Crum-Cianflone *et al.*, 2010:8; Squires *et al.*, 2003:43) initiated on antiretroviral therapy, there is a greater increase in body weight among females than males (Lahey *et al.*, 2013:438).

The initiation of antiretroviral therapy was also associated with significant immunological improvements characterized by elevations in CD4 cell count from baseline findings ($p < 0.05$). Patients initiated on antiretroviral therapy had an estimated 0.20 cells/mm³ increase in CD4 cell count over the treatment duration. The initiation of antiretroviral therapy at any age predicted an estimated 1.32 cells/mm³ increment in CD4 cell count over the treatment duration in both males and females ($p = 0.02$). In contrast to males, females experienced an estimated 69.13 cells/mm³ added increase in CD4 cell count over the treatment duration (**refer to section 3.3.1.2 Table 3-18**). Generally, females experienced increase in CD4 cell count from 179.67 cells/mm³ to **250.32 cells/mm³** while males had elevations in CD4 cell count over one year of treatment from 179.67 cells/mm³ to **181.19 cells/mm³** over one year of treatment. According to Maskew *et al.* (2013:116) HIV infected patients initiated on antiretroviral therapy had elevations in median CD4 cell count from 100 to 443 cells/mm³ in females and from 81 to 353 cells/mm³ for males over 36-

month antiretroviral therapy treatment duration. Although CD4 cell count elevation was observed in both sexes, females were found to have 2.7 times greater opportunity to attain CD4 cell count to ≥ 350 cells/mm³ than males over the treatment duration (Chow *et al.*, 2015:7). Again, females had the lowest risk of developing immunological failure than males in a systematic review done by Castillo *et al.* (2014:453), with 0.83 pooled risk ratio with reference to males.

The **second** specific objective of the empirical investigation was to determine renal safety in terms of change in serum creatinine concentration, creatinine clearance and eGFR in patients taking TDF-containing HAART regimens over treatment duration. Despite patients gaining weight, which usually leads to higher serum creatinine concentrations since creatinine is the end-product of muscle mass metabolism, the results of serum creatinine measurement revealed improved renal function by 0.05 $\mu\text{mol/l}$ daily decline in serum creatinine concentration over treatment duration ($p=0.16$). However, relative to age at antiretroviral therapy initiation, there was 0.23 $\mu\text{mol/l}$ daily increase in serum creatinine concentration over treatment duration ($p= 0.004$) in both sexes and this outcome is indicative of renal compromise. The analysis relative to sex instead revealed renal improvement by decline in serum creatinine concentration, with 12.14 $\mu\text{mol/l}$ daily decline improvement more in females than males ($p<0.05$) (**refer to section 3.3.2.1 Table 3-28**). Further analysis using equations to counteract limitations associated with direct serum creatinine measurement was done to determine the glomerular filtration rate.

In both sexes at any age, the glomerular filtration rate reduced significantly by 1.10 ml/min over the treatment duration when calculated indirectly as creatinine clearance using the Cockcroft-Gault equation ($p<0.05$). Although not statistically significant, the females had an estimated 1.86 ml/min worsened daily renal compromise than males ($p=0.30$). The decline in glomerular filtration rate was also associated with age at antiretroviral therapy initiation while gain in body weight gave improved glomerular filtration rate. The weight gain reflected 1.49 ml/min daily improvement in glomerular filtrate rate over treatment duration ($p<0.05$) (**refer to section 3.3.2.2 Table 3-38**).

An additional equation for direct calculation of glomerular filtration rate was used in the analysis. This MDRD study equation used for determining eGFR directly still showed a compromised renal function relative to age at antiretroviral therapy initiation, sex and body weight. The equation showed a significant decline of 0.78 ml/min in the eGFR at any age of antiretroviral therapy initiation in both sexes ($p<0.05$) over the treatment duration. When compared to males, females had significant 13.05 ml/min reduction in eGFR over the treatment duration ($p<0.05$). The equation disproved the improved GFR obtained in direct serum creatinine concentration measurement and creatinine clearance analysis above relative to weight gain. Instead, although not statistically significant, the body weight predicted 0.02 ml/min daily decline in estimated glomerular filtration rate over treatment duration ($p=0.80$) (**refer to section 3.3.2.3 Table 3-48**). In summary, reductions in estimated glomerular filtration rate following yearly treatment were

observed greater in females from baseline to **129.99ml/min/1.73m²** than in males whereby estimated glomerular filtration rate declined to **143.84ml/min/1.73m²**.

Despite inherent limitations associated with renal function determination in terms of direct serum creatinine measurement, indirect glomerular filtration rate and direct glomerular filtration rate, these calculations are valuable in determining renal function when done together. The outcomes from these calculations are influenced differently by a variety of factors including sex, body weight and age. According to Poggio *et al.* (2009:1085) and Stohr *et al.* (2008:769), there exist differences in glomerular filtration rate between females and males and between the old and younger aged patients on antiretroviral therapy. The retrospective analysis of change in eGFR at 6, 12 and 24 months following TDF HAART regimen initiation using MDRD study equation revealed progressive renal toxicity (Pujari *et al.*, 2014:4). The analysis showed hazard ratios of developing renal disease of 0.98 annually over treatment duration, 1.85 at every 10-year age increase and 1.63 and 1.00 in females and males respectively (Pujari *et al.*, 2014:4). Therefore, the longer the treatment duration with aging and for females mostly, the more the risk of developing renal disease progressively.

4.2 Limitations of the study

The researcher has identified the following limitations to the study:

The study did not employ any **sampling** technique in drawing up the sample of the study as it was anticipated that the sample size would be smaller. Therefore, the sample size was obtained from convenient sample of patients who complied with the inclusions of the study. Unfortunately, the convenient sample had inequalities in characteristics such as sex and age at antiretroviral therapy initiation. Since these two features were implicated in the study as independent variables, the female dominance as well as greater composition of younger aged (≤ 50 years) participants has contributed to some unequal and difficult comparative analysis. Generally, analysis relative to age gave slight outcomes across clinical and renal safety outcomes although the outcomes show promises and consistency with other studies' findings.

The **sample size** of the study was small relative to the variables investigated in which some had missing data although they were adjusted by the mixed modelling. The studies of similar nature as the current one were multicentre studies and had bigger samples which allowed for in-depth analysis beyond statistical significance and generation of practically (clinically) significant correlations in which the current study only managed to attain analysis at statistical significance level.

There were some **determinants of outcomes** which were not indicated as excluded by the study sampling criteria but had implications on the outcomes of antiretroviral therapy. Following the

literature findings, the researcher observed that it was worth noting that such variables including WHO clinical stage, treatment adherence and chemoprophylaxis should have been included in the study's independent variables.

The unavailability of **plasma viral load** measurements brought incompleteness of the efficacy (therapeutic) outcomes of TDF-containing HAART regimens. This measurement is the gold standard for evaluating treatment outcomes from antiretroviral therapy and for detection of antiretroviral therapy treatment failure. Its absence in the patients' records denied the evaluation of the true picture of ART fate in Lesotho.

4.3 Recommendations for further study

For researchers who wish to venture into the study related to the present one, the researcher has made the following recommendations:

The **stratified random sampling technique** would best fit the study of this nature. This is because patients' characteristics such as sex and age will be taken into account in forming sample strata. The strata will be formed in order to balance for these implicated predictor variables so as to avoid unnecessarily skewed data.

Since this single centre study obtained a small convenience sample, a **multicentre approach** is highly recommended for studies of this nature in Lesotho. The approach will be done as to enhance the sample size that will improve the power of the analysis and allowance for obtaining correlations of practical significance.

The empirical investigation should include **more independent variables** such as WHO clinical stage, treatment adherence and chemoprophylaxis as they play an important contribution on the outcomes of antiretroviral therapy.

The unavailability of the plasma viral load was due to resource limitations in getting patients tested virologically. The limitations have been dealt with by now. As of the 2016 treatment guidelines for HIV/AIDS, the plasma viral load test is highly advocated and the implementation of viral load test begun in **June 2016** as the measure of antiretroviral therapy success (MOH, 2016a:2).

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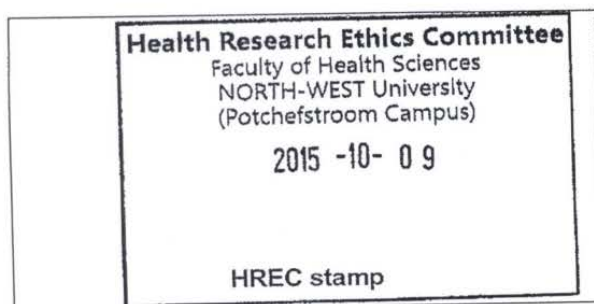
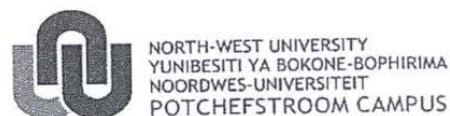
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APPENDIX I: PARTICIPANT INFORMED CONSENT (ENGLISH)



ENGLISH VERSION

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR

TITLE OF THE RESEARCH PROJECT:

Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing regimens in Lesotho

REFERENCE NUMBERS: NWU-00084-15-A1

PRINCIPAL INVESTIGATOR:

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THE INFORMED CONSENT TO BE COMMUNICATED TO PATIENTS BY THE
MEDIATOR

You are being invited to take part in a research project that forms part of the researcher's studies. I am the mediator and I am going to discuss the information he provided with you. Please listen carefully as I read the information presented here, which will explain the details of this project. Please ask me any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Furthermore, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Lesotho Ministry of Health Research and Ethics Committee (ID43-2014) and the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00084-15-A1) and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

- This study will be conducted in this health care facility and will involve the data collection tool to capture relevant data from your medical records that are kept in this facility.
- The general aim of the research project is to evaluate the clinical outcomes and the prevalence of renal toxicity in patients taking TDF-containing regimens in Lesotho. There is a need for the periodical assessment and evaluation of each patient's response to these regimens so that appropriate adjustments can be done where needed, with the aim of improving individuals' quality of life. He intends to do the research to evaluate the clinical outcomes in relation to your CD4 cell count, viral load and body weight and renal safety of these drugs in relation to the patient's age, body weight, gender and the duration of treatment with the drugs.
- TDF is an antiviral drug used in the treatment of HIV/AIDS to arrest the multiplication of HIV in the body.

Why have you been invited to participate?

- You have been invited to participate because your health care facility has been selected and you are on a TDF-containing regimen. The researcher wants to evaluate the clinical outcome and renal safety of your regimen.

- You have also complied with the following inclusion criteria:
 - You have been on a TDF-containing regimen for at least six (6) months
 - Your body weight is $\geq 35\text{kg}$,
 - Your age is ≥ 18 years;
- In total, 250 participants are invited.

What will your responsibilities be?

- You will not be expected to participate physically in this study. The researcher kindly requests your permission to have access to your medical records kept in this facility. As you come for the next medical review and/or medication refills, I will appreciate it if you sign on the signatures columns given below if you are willing to participate in the study. You can drop this form in the sealed box placed in the receptionist's office.

What are the researchers' responsibilities?

- The researcher is responsible for the capturing of data from the medical records using the data collection tool. He will be visiting the facility daily on working days for a period of one to two months.

Will you benefit from taking part in this research?

- There are no direct benefits for you as a participant since you will not be participating physically.
- The indirect benefit will be a positive influence on the treatment approach towards using TDF-containing regimens with the aim of minimising inherent adverse effects of the drug and optimising the outcome of the regimens.

Are there risks involved in your taking part in this research?

- The risks in this study are that you may hesitate not to participate in the study for a variety of reasons including your understanding of precautions of participation and believing that the study will have impact on services offered to you.
- The benefits outweigh the risk.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

- The research promises no physical harm to your health; as the research will involve neither your intact body part nor your current opinions about the disease and the

treatment. He is only going to extract the information from past medical history on your files.

Who will have access to the data?

- Anonymity will be ensured by assigning codes that hide your real identity to your information. Confidentiality will therefore be ensured and the researcher will be the only who has access to your records and shall keep confidentiality of information found in your records. Only the researchers, study leaders and statistician will have access to stored data. Data will be kept safe and secure by locking hard copies in locked cupboards in the research entity's office, and for electronic data, it will be password protected. Data will be stored for a minimum of seven years.

Will you be paid to take part in this study and are there any costs involved?

- There are no added costs to you in carrying out the study; therefore the researcher will not offer any monetary items or gifts in this research.

Who are the members of the research team and what are their qualifications?

- Project leader: Dr DM Rakumakoe (PharmD)
- Researcher/ post-graduate student: M Sello (BPharm (Hons))
- Co-supervisor: Prof MS Lubbe (PhD)
- Co-supervisor: Mrs M Ramathebane (MPharm)

Is there anything else that you should know or do?

- You can contact Mr Molungoa Sello at (+266) 58409977 if you have any further queries or encounter any problems.
- You can contact the **Lesotho Ministry of Health Research and Ethics Committee at (+266) 223 144 04** and the **NWU Health Research Ethics Committee** via Mrs Carolien van Zyl at 018 299 2089 or carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.
- You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

- The findings of the research will be shared with you by the researcher. Specific information relating to a patient will not be shared. General results and knowledge gathered in this research will be shared with you through announced meetings in the facility. The researcher will also make presentations at conferences and national and

international research forums. The results will also be published internationally, so that other interested people may learn from his research.

Declaration by participant

By signing below, I agree to take part in a research study entitled:

Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing regimens in Lesotho

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (place) on (date) 20....

.....
Signature of participant

.....
Signature of witness

Declaration by mediator

I (name) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did not use an interpreter.

Signed at (place) on (date) 20....

.....
Signature of mediator

.....
Signature of witness

Declaration by researcher

I (name) declare that:

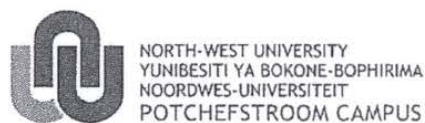
- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (place) on (date) 20....

.....
Signature of researcher

.....
Signature of witness

APPENDIX II: PARTICIPANT INFORMED CONSENT (SESOTHO)



KAROLO EA SESOTHO

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR

TITLE OF THE RESEARCH PROJECT:

Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing regimens in Lesotho

REFERENCE NUMBERS: NWU-00084-15-A1

RALIPHUPUTSO:

Mr M Sello

O FUMANEHA MONA:

National University of Lesotho

Faculty of Health Sciences

Department of Pharmacy

P.O.Roma 180

Maseru 100

Lesotho

NOMORO TSA MOHALA:

(+266) 584 099 77

TOKOMANE E BALLOA BAKULI KE MORUMUA (MOOKI)

Lebitsong la raliphuputso ea boletsoeng ka holimo, keu memela ho nka karolo liphuputsong tsena eleng karolo ea lithuto tsa hae. 'Na ke morumuoa fela 'me ke tlo qoqa le wena fela litaba tsa tokomane eena, tse hlalosang likateng the liphuputso tse tlo etsoa. Ke kopa u botse lipotso moo osa utloisisng. Ho bohlokoa ho ikhotsofatsa hore o fela o utloisisa sohle se boleloang ka liphuputso tsena le hore na o ka nka karolo joang. Hape, ho nka karolo ha hau mona ke boithaopi ha ho se tlamang 'me o ka hana ho nka karolo kapa ho ikhula hau no ile oa lumela pele. Boemong boo u lumelang ho nka karolo, u bolokolohing ba ho ikhula khabareng.

Liphuputso tsena li chaetsoe monoana ke lekala la lesotho la bophelo karolong ea liphuputso (ID43-2014) le komiti ea liphuputso lekaleng la bophelo la North West University (NWU-00084-15-A1). Liphuputso tsena li tlo etsoa ho latela tataiso ea tsa liphuputso e ikarabellang ho komiti ea ketsa-molao ea naha ea liphuputso. Ho kaba bohlokoa hore komiti tse ling tse amehang li nyakurele likateng tsa liphuputso tsena.

Se boleloang ke liphuputso tsena

- Liphuputso li tlo etsoa setsing sena 'me ho tlo sebelisoa foromo feela ho bokella litaba tse amanang le liphuputso tsena feela. Ho tlo sebelisoa libukana tsa lona tse bolokiloena setsing feela.
- Sepheo sa liphuputso tsena ke ho hlaloba litlamorao le sekhahla sa lefu la liphieo ho bakuli ba sebelisang sethlare sa tdf naheng eena. ho bonahetse bohlokoa ba ts'alo morao ho bakuli ba sebelisang TDF ele hore liphetohe tse reretsoeng ho phahamisa boleng ba bophelo. Liphuputso ka kotloloho li tsepame ho litlamorao boemong ba sesole sa 'mele, boemo ba ts'oaetso ka bo eona, boima ba 'mele ha sekhahla sa lefu la liphieo lona le tsepamisitsoe khahlanong le lilemo tsa mokuli, boima ba 'mele, boleng ba botona kapa bots'ehali le nako eo oso e nkile o sebelisa sethlare sena.
- TDF ke sethlare se fokotsa sekhahla sa ts'oaetso ea HIV.

Mabaka a ho memeloa ho nka karolo

- O memeloa ho nka karolo hobane setsi sena se khethuoe ho tla etsetsa liphuputso ho sona 'me o emong ea sebelisang sethlare sa TDF. Raliphuputso otlo hlaloba litlamorao le sekhahla sa lefu la liphieo ho bakuli ba sebelisang sethlare sa TDF.
- U emong oa bakuli ba fihletse litekanyetso tse latelang
 - U so sebelisitse sethlare sa tdf bonyane likhoeli tse ts'eletseng.
 - Boima ba hau ba 'mele ke 35 kg kapa ho feta.
 - Lilemo tsa hau li 18 kapa ho feta.

- Kakaretso ea bakuli ba mengoang ke 250.

Boikarabello ba hau

- Haua lebelloa ho nka karolo ka kotlolo liphuputsong tse. raliphuputso o kopa ka boikokobetso tumello ea hau feela hore a fumants'oe monyetla oa ho nyakurela bukana ea hau e bolokiloeng setsing mona. Ke tlo u fa foromo eena 'me ke kopa o ee hae ka eona, raliphuputso o tla thabela hau ka saena ka tlase oa e ts'ela ka lebokoseng le ka officing ea lipotso.

Boikarabello ba raliphuputso

- Ke boikarabello ba raliphuputso ho bokella litaba tse amang liphuputso tsa hae a sebelisa foromo ea hae. O tla ba teng setsing mona nako ea khoeli ho isa ho tse peli.

Melemo ea ho nka karolo

- Kaha haua lebelloa ho nka karolo ka kotloloho, ha ho na melemo e lebeletsoeng ka kotloloho
- Melemo e tla ba teng ho bohle ba sebelisang sethlare, 'me e kenyeletsa liphetofo tse reretsoeng ho phahamisa maemo a bophelo ka kakaretso a tsamaea le ho fokotsa litla-morao tse sa hlokeheng.

Litla-morao tsa amanang le ho nka karolo

- Litla-morao li kenyeletsa ho tsoafa ho se kenye letsoho ka kutlouisiso ea hore hose kenye letsoho ho ka ba le ts'usumetso lits'ebeletsong tse fanoang ke setsi sena
- Litla-morao tsa ho nka karolo li bonahala li le tlaase haholo papisong le melemo ea ho nka karolo.

Seka etsoang boemong boo u sa khotsofalang

- Raliphuputso o ts'episa hore kaha hau tlo nka karolo ka kotloloho, bophelo ba hau kapa maikutlo a hau a ha joale ha li tsietsing ho hang.

Ba amehang polokelong ea litaba tsa liphuputso tsena hase li phethetsoe

- Raliphuputso o netefatsa hore seo a tla se fumana liphuputsong tsena e tla ba lekunutu la hae le lona fela ebile haana ho sebelisa mabitso la batho ba nkileng karolo. Ho ka etsahala batho ba sa nkang karolo mona ba hlokomela hore le nkile karolo mona ebe ba le botsa lipotso, se ts'abe ho ba araba.
- Seo a tla se fumana ho uena ka liphuputso ha hona motho a tla se tseba ntle le eena le baetapele baka liphuputsong tsena. Seo a tla fumana ho uena se tla ts'oauoa ka mokhoa oa linomoro eseng lebitso la hau. Ke eena feela ea tla tseba nomoro ea hau

ebile o tla boloka seo ke ase fumaneng ka lebokoseng leo ke tla le notlela le tla tsejoa ke eena le baetapele bahae fela.

- Litaba tsa liphuputso tsena li tla bolokoa ka thata sebakeng sesa fihleloeng ke mang kapa mang ntle le ba amehang 'me li tla bolokoa nako ea bonyane lilemo tse 7.

Meropotso

Ha hona phapanyetsano ya limpho, leha ele ka mokhoa oa chelete, e tlo etsahala malebana le liphuputso tsena.

Ba amehang liphuputsong tsena

- Moetapele: Dr DM Rakumakoe (PharmD)
- Raliphuputso: M Sello (BPharm (Hons))
- Motlatsi ba moetapele: Prof MS Lubbe (PhD)
- Motlatsi ba moetapele: Mrs M Ramathebane (MPharm)

Hauna le seo u ka ratang ho se tseba ho feta mona

- O ka fumana lithlakisetso ho raliphuputso eleng Mr Molungoa Sello ho (+266) 584 099 77.
- Lithlakisetso tse ling li ka fumaneha ho lekala la lesotho la bophelo karolong ea liphuputso (id43-2014) ho (+266) 223 144 04 le komiti ea liphuputso lekaleng la bophelo la North West University ho Mrs Carolein Van Zyl ho (018) 299 2089 kapa carolein.vanzyl@nwu.ac.za
- Ketlo u fa kopi ea litaba tsena bakeng sa polokelo ea hau.

Ho fana ka sephetho

- Litaba tsa lekunutu li pakeng tsa hae le uena haana li bolella mang kapa mang. sephetho se tla fumaneha le tla se tsebisoa hona mona setsing ka likopano tse tla etsoa tseo eleng hore o tla fana ka sona kamora phutheho ele hore batho bao ba ka bang le thahasello ba ithute phuputsong ena eaka. Sephetho setla boetse se phatlalatsoa boemong ba naha libokeng tsa liphuputso, boithuto le karolelano ea litsebo. Sephetho seka phatlalatsoa marang-rang a likhokahano a chaeletsoeng monoana kamora likopano le lona ele hore ba nang le kob'a bohali baka una molemo ho sona.

Phatlalatso ka moithaopi

Ka ho saena mona, 'na.....ke lumela ho nka karolo
liphuputsong tsa:

**Clinical outcomes and renal safety in HIV/AIDS patients on tenofovir-containing
regimens in Lesotho**

Ke phatlalatsa hore:

- Ke balloetse litaba tsena ka puo eo ke e utluisisang 'me ke khotsofetse
- Ke fuoe monyetla oa ho botsa lipotso 'me ke fumane likarabo tse khotsofatsang
- Ke utluisisa hore ho nka karolo ke boithaopi fela 'me hakea qobelloa
- Ke ka khetho ea ho ikhula nako eohle
- Nka kopuo oa ho tlohela ho nka karolo boemong boo eleng molemong oa ka, kapa
ke sa nke karolo ho latela litekanyetso tsa liphuputso hore ka tumellano

Ke saenetse (sebaka)ka la 20....

.....
Motekeno oa moithaopi

.....
Motekeno oa paki

Phatlalatso ka morumua

'Na (lebitso) ke phatlalatsa hore:

- Ke hlalositse litaba tsa tokomane ena ho.....
- Ke mo file monyetla oa ho botsa lipotso 'me ka nka nako ea ka ho mo araba
- Ke ikhotsofalitse hore o utluisisa likateng tsa liphuputsena ka ho lekana
- Ha kea sebelisa toloko

Ke saenetse (sebaka) ka la 20...

.....
Motekeno oa morumua

.....
Motekeno oa paki

Phatlalatso ka Raliphuputso

'Na (lebitso) ke phatlalatsa hore:

- Ke hlalositse litaba tsa tokomane ena ho.....
- Ke mo file monyetla oa ho botsa lipotso 'me ka nka nako ea ka ho mo araba
- Ke ikhotsofalitse hore o utluisisa likateng tsa liphuputsena ka ho lekana
- Ha kea sebelisa toloko

Ke saenetse (sebaka) ka la 20...

.....
Motekeno oa morumua

.....
Motekeno oa paki

APPENDIX III: CONFIDENTIALITY AGREEMENT



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

CONFIDENTIALITY UNDERTAKING

entered into between:

I, the undersigned

Prof / Dr / Mr / Ms [✓] TEBESI MABOTE

Identity Number: RC 002072

Address: HA-LEQELE MASERY LESOTHO

hereby undertake in favor of the **NORTH-WEST UNIVERSITY**, a public higher education institution established in terms of the Higher Education Act No. 101 of 1997

Address: Office of the Institutional Registrar, Building C1, 53 Borchard Street, Potchefstroom, 2520

(hereinafter the "NWU")

1 Interpretation and definitions

1.1 In this undertaking, unless inconsistent with, or otherwise indicated by the context:

1.1.1 "Confidential Information" shall include all information that is confidential in its nature or marked as confidential and shall include any existing and new information obtained by me after the Commencement Date, including but not be limited in its interpretation to, research data, information concerning research participants, all secret knowledge, technical information and specifications, manufacturing techniques, designs, diagrams, instruction manuals, blueprints, electronic artwork, samples, devices, demonstrations, formulae, know-how, intellectual property, information concerning materials, marketing and business information generally, financial information that may include remuneration detail, pay slips, information relating to human capital and employment contract, employment conditions, ledgers, income and expenditures and other materials of whatever description in which the NWU has an interest in being kept confidential; and

1.1.2 "Commencement Date" means the date of signature of this undertaking by myself.

1.2 The headings of clauses are intended for convenience only and shall not affect the interpretation of this undertaking.

2 Preamble

2.1 In performing certain duties requested by the NWU, I will have access to certain Confidential Information provided by the NWU in order to perform the said duties and I agree that it must be kept confidential.

2.2 The NWU has agreed to disclose certain of this Confidential Information and other information to me subject to me agreeing to the terms of confidentiality set out herein.

3 Title to the Confidential Information

I hereby acknowledge that all right, title and interest in and to the Confidential Information vests in the NWU and that I will have no claim of any nature in and to the Confidential Information.

4 Period of confidentiality

The provisions of this undertaking shall begin on the Commencement Date and remain in force indefinitely.

5 Non-disclosure and undertakings

I undertake:

5.1 to maintain the confidentiality of any Confidential Information to which I shall be allowed access by the NWU, whether before or after the Commencement Date of this undertaking. I will not divulge or permit to be divulged to any person any aspect of such Confidential Information otherwise than may be allowed in terms of this undertaking;

5.2 to take all such steps as may be necessary to prevent the Confidential Information falling into the hands of an unauthorised third party;

5.3 not to make use of any of the Confidential Information in the development, manufacture, marketing and/or sale of any goods;

5.4 not to use any research data for publication purposes;

5.5 not to use or disclose or attempt to use or disclose the Confidential Information for any purpose other than performing research purposes only and includes questionnaires, interviews with participants, data gathering, data analysis and personal information of participants/research subjects;

5.6 not to use or attempt to use the Confidential Information in any manner which will cause or be likely to cause injury or loss to a research participant or the NWU; and

5.7 that all documentation furnished to me by the NWU pursuant to this undertaking will remain the property of the NWU and upon the request of the NWU will be returned to the NWU. I shall not make copies of any such documentation without the prior written consent of the NWU.

6 Exception

The above undertakings by myself shall not apply to Confidential Information which I am compelled to disclose in terms of a court order.

7 Jurisdiction

This undertaking shall be governed by South African law be subject to the jurisdiction of South African courts in respect of any dispute flowing from this undertaking.

8 Whole agreement

8.1 This document constitutes the whole of this undertaking to the exclusion of all else.

8.2 No amendment, alteration, addition, variation or consensual cancellation of this undertaking will be valid unless in writing and signed by me and the NWU.

Dated at Potchefstroom this 21 / 08 2015

Witnesses:

1

2

(Signatures of witnesses)

(Signature)

APPENDIX IV: PATIENT DATA COLLECTION FORM

PATIENT DATA COLLECTION FORM

Date / /2014 Patient code /

GENDER: M ☐ F ☐ Date of birth: / age

BASELINE INFORMATION

Date of initiation:	/ / /	Dates taken	HIV/AIDS stage
CD4 Count:	cells/mm ³	/ / /	I ☐
Viral load:	copies/ml	/ / /	II ☐
Serum creatinine:	µmol/L	/ / /	III ☐
Body weight:	Kg	/ / /	IV ☐

ARV REGIMEN [✓]: TDF/3TC/NVP ☐ TDF/3TC/EFV ☐
 AZT/3TC/EFV ☐ AZT/3TC/NVP ☐
 D4T/3TC/EFV ☐ D4T/3TC/NVP ☐

REGIMEN DURATION:

	Year I	Year II	Year III
6-12 ☐ months	18-24 ☐ months	30-36 ☐ months	
12-18 ☐ months	24-30 ☐ months	36-40 ☐ months	
> 42 ☐ months			

DURING THERAPY

CHANGE OF REGIMEN: Y ☐ date / to TDF/3TC/NVP ☐ TDF/3TC/EFV ☐

Reason for change.....

N ☐

Serum creatinine measurements & dates

Serum creatinine ($\mu\text{mol/L}$)	Date (dd-mm-yyyy)

CD4 count measurements & dates

CD4 COUNT (cells/mm ³)	Date (dd-mm-yyyy)

Body weights measurements & dates

Body weight (Kg)	Date (dd-mm-yyyy)

APPENDIX V: MINISTRY OF HEALTH AUTHORISATION/ETHIC APPROVAL



Ministry of Health
P.O. Box 514
Maseru 100

02nd April, 2014

Mr. Molungoa Sello
Masters Degree in Pharmacy Practice
National University of Lesotho
Department of Pharmacy
Faculty of Health Sciences
P.O. Roma 180

Dear Mr. Molungoa Sello

Re: The Renal Safety Profile of Tenofovir as Used in Antiretroviral Therapy in Lesotho (ID43-2014)

Thank you for resubmitting the above mentioned proposal. The Ministry of Health, Research and Ethics Committee having reviewed your protocol hereby authorizes you to conduct this study among the specified population. The study is authorized with the understanding that the protocol will be followed as stated. Departure from the stipulated protocol will constitute a breach of the permission.

We are looking forward to have a progress report and final report at the end of your study.

Sincerely,


Dr. Piet McPherson
Director General Health Services (acting)


Dr. Jill Sanders
Co-Chairperson
National Health Research and Ethics
Committee (NH-REC)

APPENDIX V: ETHICS CERTIFICATE



NORTH-WEST UNIVERSITY
YUNIBESITHI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

Institutional Research Ethics Regulatory Committee

Tel: +27 18 299 4849
Email: Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by Health Research Ethics Committee (HREC), the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: Clinical outcomes and renal safety in HIV/AIDS patients on Tenofovir-containing regimens in Lesotho																																												
Project Leader: Dr DM Rakumkoe																																												
Ethics number:	<table border="1"><tr><td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>0</td><td>8</td><td>4</td><td>-</td><td>1</td><td>5</td><td>-</td><td>A</td><td>1</td></tr><tr><td colspan="3">Institution</td><td colspan="6">Project Number</td><td colspan="2">Year</td><td colspan="2">Status</td></tr><tr><td colspan="15">Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation</td></tr></table>	N	W	U	-	0	0	0	8	4	-	1	5	-	A	1	Institution			Project Number						Year		Status		Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation														
N	W	U	-	0	0	0	8	4	-	1	5	-	A	1																														
Institution			Project Number						Year		Status																																	
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation																																												
Approval date: 2015-10-02	Expiry date: 2017-06-30																																											
Risk	Minimal																																											

Special conditions of the approval (if any): None

General conditions: While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following: - The project leader (principal investigator) must report in the prescribed format to the NWU-IRERC: - annually (or as otherwise requested) on the progress of the project; - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project. - The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-IRERC. Would there be deviation from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited. - The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date. - In the interest of ethical responsibility the NWU-IRERC retains the right to: - request access to any information or data at any time during the course or after completion of the project; - withdraw or postpone approval if: - any unethical principles or practices of the project are revealed or suspected; - it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented; - the required annual report and reporting of adverse events was not done timely and accurately; - new institutional rules, national legislation or international conventions deem it necessary.
--

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Linda du
Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU,
ou=Vaal Triangle Campus,
email=Linda.duplessis@nwu.ac.za,
c=ZA
Date: 2015.10.09 12:11:49 +0200

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

APPENDIX VI: PERMISSION FROM PABALLONG CENTRE



PO Box 1839

Maseru 100

Lesotho

21/08/2015

Dear Mr. M Sello,

**Re: CLINICAL OUTCOMES AND RENAL SAFETY ON HIV/AIDS
PATIENTS ON TENOFOVIR – CONTAINING REGIMENS IN LESOTHO**

This letter serves to inform you that your request to conduct a study at Paballong has been approved and you can continue at your convenience.

The study is titled: CLINICAL OUTCOMES AND RENAL SAFETY ON HIV/AIDS PATIENTS ON TENOFOVIR – CONTAINING REGIMENS IN LESOTHO.

The study has also been approved by the Ministry of Health (ID43-2014).

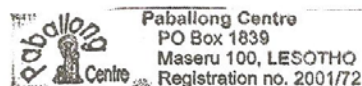
Wishing you all the success and looking forward to receiving a copy of your final report once released and which help with improvements where applicable.

For more information please contact Paballong at tel. 5250700

Regards

Yours sincerely

Mrs. M. Malunga-Nthakong
Manager - Paballong



20 AUG 2015

APPENDIX VII: LANGUAGE EDITING

THE NATIONAL UNIVERSITY OF LESOTHO

Telephone: +266 22340601

+266 52217166

+266 52213922



P.O. Roma 180,
Lesotho.

FACULTY OF HUMANITIES

DEPARTMENT OF ENGLISH

4th November 2016

Subject: Editorship of Sello Molungoa's Thesis

I hereby confirm that I, Taelo Qhala, have edited Sello Molungoa's thesis entitled "Title: Clinical Outcomes and Renal Safety in HIV/AIDS Patients on Tenofovir-Containing Regimens in Lesotho". I have read through the document and edited it only for language.

A handwritten signature in black ink, appearing to read 'Taelo Qhala'.

Taelo Qhala

