The renal safety profile of tenofovir as used in combination antiretroviral therapy

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It all starts here ™

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DEDICATION

I dedicate this research project to my sons, Mihir and Rihir. My dad has always taught me that knowledge is power and I pass this message on to the both of you. Never give up and never give in. Have a positive attitude and work hard.

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LIST OF DEFINITIONS

Acute renal injury: renal injury of sudden onset, such as from physical trauma, infection, inflammation, or toxicity (Medical dictionary, 2016)

Chronic kidney disease: the presence of kidney damage or GFR less than 60 mL/min/1.73 m^2 for three or more months (National kidney foundation, 2011:16).

Creatinine clearance: rate of creatinine excretion in urine to its concentration in serum, a value that reflects the body's ability to excrete creatinine; it is used to diagnose and monitor renal function (Medical dictionary, 2016)

End stage renal disease: irreversible kidney damage; at this stage serum creatinine and blood urine nitrogen (BUN) levels continue to rise and there is uremia with impairment of all body systems (Medical dictionary, 2016).

Fanconi syndrome: a disorder of reabsorption in the proximal convoluted tubules of the kidney characterized especially by the presence of glucose, amino acids, and phosphates in the urine (Medical dictionary, 2016)

Glomerular filtration rate: total of the filtration rates of the functioning nephrons in the kidney (National kidney foundation, 2011:4).

Hematuria: presence of blood in the urine (K/DOQI, 2002:114)

HIV associated nephropahy: kidney disease resulting from infection with the human immunodeficiency virus (Medical dictionary, 2016)

Proteinuria: the presence of excess protein in the urine (Medical dictionary, 2016).

Renal dysfunction: inability of the kidney to maintain normal function so that waste products and metabolites accumulate in the blood (Medical dictionary, 2016)

LIST OF ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ALT	Alanine transaminase
ARF	Acute renal failure
ART	Antiretroviral therapy
ARV	Antiretroviral
AZT	Zidovudine
β2-M	β2 microglobulin
BMI	Body mass index
BSA	Body surface area
CG	Cockcroft-Gault
CKD	Chronic kidney disease
Cr	Creatinine
CrCl	Creatinine clearance
CRP	C-reactive protein
CD_4 count	CD_4 T-lymphocyte cell
d4t	Stavudine
ddI	Didanosine
EFV	Efavirenz
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
FBC	Full blood count

FDA	Food and Drug Administration of the United States of America
FDC	Fixed dose combination
FTC	Emtricitabine
GFR	Glomerular filtration rate
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HIVAN	HIV associated nephropathy
hOAT 1	Anorganic anion transporter 1
HPCSA	Health Professions Council of South Africa
HREC	Human Research Ethics Committee
ICF	Informed consent form
IL-18	Interleukin-18
K/DOQI	Kidney disease outcomes quality initiative
KIM-1	Kidney injury molecule
L-FARP	Liver type fatty acid binding protein
LPV/r	Lopinavir/ritonavir
MDRD	The modification of diet in renal disease
MRP-2	Multi-drug resistance associated protein
MRP-4	Multi-drug resistance associated protein
mtDNA	Mitochondrial DNA
NAG	N-acetyl-β-D-glucosaminidase
NDoH	National Department of Health
NGAL	Human neutrophil gelatinase-associated lipocalin

NHLS	National Health Laboratory Service
NVP	Nevirapine
NWU	North West University
OAT 3	Anion transporter 3
PEM	Protein energy malnutrition
PHC	Primary health care
PI	Primary investigator
RDA	Recommended daily allowance
SANC	South African Nursing Council
SCr	Serum creatinine
SOP	Standard operating procedure
TDF	Tenofovir disoproxil fumarate
VL	Viral load
WHO	World Health Organization
3tc	Lamivudine

ABSTRACT

Background: Tenofovir disoproxil fumarate (TDF) is the prodrug of tenofovir and is currently first-line therapy in adolescents above the age of 15 and adults, according to the consolidated guidelines on the use of antiretroviral therapy (ARV) in South Africa. TDF has proven to be an effective drug in the fight against human immunodeficiency virus (HIV) and is utilised in the treatment of Hepatitis B. Tenofovir disoproxil fumarate is effective and efficient as used in combination for post-exposure prophylaxis and to prevent HIV transmission in heterosexual, serodiscordant couples. However, the renal safety profile of tenofovir remains a contentious issue in the African context. This study investigated the renal function outcome of HIV-positive patients exposed to tenofovir-based antiretroviral therapy.

Method: The study was a retrospective, partial prospective observational cohort analysis of serum creatinine (SCr), CD_4 count, viral load (VL) and body mass index (BMI) data of 66 black patients attending the clinic in Newcastle, KwaZulu-Natal. This study group was subdivided into two age groups (≥20 - <30 years and ≥30 - ≤40 years), and into male and female. The renal function was evaluated by calculating the creatinine clearance (CrCl) by means of the Cockcroft-Gault (CG) equation at baseline (before TDF commencement) and at 12 months post-TDF commencement.

The independent *t*-test was applied to investigate differences in SCr, CrCl, CD_4 count and BMI by comparing female vs male participants at baseline and at 12 months post-TDF commencement. The Mann-Whitney test was utilised as the non-parametric equivalent of the independent *t*-test. The dependent *t*-test was used to investigate the changes on SCr, CrCl, CD_4 count and BMI from baseline to 12 months (female *vs*. male). The Wilcoxon signed-rank test was utilised as the non-parametric equivalent of the dependent *t*-test.

Ethical approval was obtained from The Human Research Ethics Committee of the North-West University on the 18th of August 2015 (NWU 00044-15-A1) and from the KwaZulu-Natal Department of Health (KZ_2015_RP40-426) on the 7th of September 2015 to conduct this study.

Results: Thirty-five female (mean age 30.43 [4.69] years) and 31 males (mean age 30.32 [4.60] years) patients consented to participate in the study. The BMI and CD_4 count improved in all age and gender groups at 12-month follow-up data. There was no statistically significant change in the SCr from baseline to follow-up in any of the age groups but the ≥20 - <30 year age group showed an improvement in CrCl at 12-month follow-up data (p = 0.15 and p = 0.020) for the female and male group respectively. A higher mean SCr value was established for the male group but a higher mean CrCl value was seen in the female group. The ≥30 - ≤40 year age group depicted a minor decline in CrCl at 12-month follow-up (p = 0.176 and p = 0.941) for female and

males respectively. The immunological outcome is depicted by the CD_4 count. The female group stratified according to age ≥20 - <30 and ≥30 -≤40 years, displayed a statistically significant difference in CD_4 count at 12 months post-TDF commencement, with a large practical significance ($p < 0.001$ [0.94], $p = 0.002$ [0.87]). The mean increase in CD_4 count was 174 cells/mm^3 and 208.75 cells/mm^3. The pooled female group (≥20 - ≤40 years) displayed a mean increase in CD_4 count of 196.06 cells/mm^3. The VL was suppressed in majority of the patients at 12-month follow-up data.

Conclusion: Virological suppression was seen in majority of the patients at 12 months post-TDF commencement. The immunological outcome improved in this study population. Selecting a younger age group reduced the risk of age-related degeneration of kidney function. The CrCl in the younger age group (≥20 - <30years) exhibited an increase in CrCl at 12 months post-TDF commencement. The older age group (≥30 - ≤40 years) displayed a decrease in CrCl at 12 months post-TDF commencement for females and males. The BMI improved in all age and gender groups investigated. The renal function exhibited a positive outcome for all patients in this study population. This study supports the use of TDF as first-line therapy in South Africa. This study also supports the existing evidence that age and gender, influence kidney function.

Key words: tenofovir, serum creatinine, creatinine clearance, CD_4 count, BMI

OPSOMMING

Agtergrond: Tenofovir disoproksielfumaraat (TDF) is die voorloper van tenofovir en is tans eerste reël therapie in adolessente bo die ouderdom van 15 jaar en volwassenes volgens die gekonsolideerde riglyne oor die gebruik van antiretrovirale terapie (ARV) in Suid-Afrika. TDF het as 'n doeltreffende middel in die stryd teen menslike immuniteitsgebreksvirus (MIV) bewys en word gebruik in die behandeling van hepatitis B. Tenofovir disoproksielfumaraat is effektief en doeltreffend as gebruik in kombinasie vir na-blootstellingsprofilakse en om MIV-oordrag in heteroseksuele serodiscordant paartjies te voorkom. Maar die veiligheidsprofiel van tenofovir op nierfunksie bly 'n omstrede kwessie in die Afrika-konteks. Hierdie studie ondersoek die nierfunksie van MIV-positiewe pasiënte, blootgestel aan tenofovir gebaseer antiretrovirale terapie.

Metode: Die studie was 'n terugwerkende, gedeeltelike voornemende waarnemings-kohort ontleding van serum kreatinien (SCr), CD_4-telling, viruslading (VL) en liggaamsmassa-indeks (BMI) data, van 66 swart pasiënte wat 'n primêre gesondheidsorgkliniek in Newcastle, KwaZulu-Natal bygewoon het. Hierdie studiegroep is in twee verdeel: ouderdomsgroepe (≥20 - <30 jaar en ≥ 30 - ≤40 jaar) verdeel asook in manlik en vroulik groep. Die nierfunksie was geëvalueer deur die berekening van die kreatinienopruiming (SCr) deur middel van die Cockcroft-Gault vergelyking by basislyn (voor TDF aanvang) en op 12 maande, na TDFgebaseerde terapie.

Die onafhanklike t-toets was toegepas om die verskille in SCr, CrCl, CD_4-telling en BMI te ondersoek en vroulike teen manlike deelnemers by basislyn en op 12 maande na-TDF aanvang, te vergelyk. Die afhanklike t-toets is gebruik om die veranderinge in SCr, CrCl, CD_4-telling en BMI van basislyn tot 12 maande (vroulike teen manlike) te ondersoek. Die Mann-Whitney toets was gebruik as die nie-parametriese ekwivalent van die onafhanklike t-toets. Die Wilcoxon Signed-Rank-toets was gebruik as die nie-parametriese ekwivalent van die afhanklike t-toets.

Resultate: Vyf en dertig vroulike (gemiddelde ouderdom 30.43 [4.69] jaar) en 31 manlike (gemiddelde ouderdom 30.32 [4.60] jaar) pasiënte het ingestem om deel te neem aan hierdie studie. Etiese goedkeuring om hierdie studie uit te voer, was verkry van die Human Research Ethics Committee (HREC) van die Noordwes-Universiteit, op 18 Augustus 2015 (NWU 00044-15-A1) en van die KwaZulu-Natal Departement van Gesondheid (KZ_2015_RP40-426) op 7 September 2015 verkry. Die BMI en CD_4-telling het in alle ouderdomsgroepe en geslag groepe op 12 maande opvolg data verbeter. Daar was geen statisties beduidende verandering in SCr, van die basislyn tot opvolg, in enige van die ouderdomsgroepe, maar die ≥20 - <30 jaar ouderdomsgroep het 'n verbetering in CrCl op 12 maande opvolg data (p = 0.15 en p = 0.020)

vir die vroulike en manlike groep gehad. 'n Hoër SCr-waarde was vir die manlike groep (*p* = 0.343) bevestig, maar daar was hoër CrCl waarde in die vroulike groep (*p* = 0.755). Die ≥30 - ≤40 jaar ouderdomsgroep het 'n klein afname in CrCl op 12 maande opvolg (p = 0.176 en p = 0.941) vir vroulike en manlike onderskeidelik uitgebeeld. Die VL was in meeste van die pasiënte op 12 maande opvolg onderdruk. Die immunologiese uitkoms word deur die CD_4-telling gebaseer. Die vroulike groep gestratifiseerde volgens ouderdom ≥20 - <30en ≥30 - ≤40 jaar) het 'n statisties beduidende verskil in die CD_4-telling 12 maande na TDF aanvang vertoon, met 'n groot praktiese betekenis (*p* < 0.001 (0.94), *p* = 0.002 (0.87)). Die gemiddelde styging in die CD_4-telling was 174 selle/mm^3 en 208.75 selle/mm^3. Die saamgevoegde vroulike groep (≥20 - ≤40 jaar) het 'n gemiddelde toename in die CD_4-telling van 196.06 selle/mm^3 vertoon na 12 maande op TDF gebaseerde behandeling.

Gevolgtrekking: Virologiese onderdrukking na 12 maande opTDF gebaseerde behandeling was teenwoordig in die meerderheid van die pasiënte. Die keuse van 'n jonger ouderdomsgroep het die risiko van ouderdom-verwante degenerasie van nierfunksie verminder. Die CrCl in die jonger ouderdomsgroep (≥20 - <30jaar) het 'n toename in CrCl getoon na 12 maande op TDF gebaseerde behandeling. Die ouer ouderdomsgroep (≥30 - ≤40) het 'n afname in CrCl na 12 maande op TDF gebaseerde behandeling vertoon vir vrouens en mans (*p* = 0.176 en *p* =0.941). Die BMI het in alle ouderdomsgroepe en geslag groepe verbeter. Die nierfunksie stel 'n positiewe uitkoms voor vir alle pasiënte in hierdie studie bevolking. Hierdie studie ondersteun die gebruik van TDF as eerste linie behandeling in Suid-Afrika. Hierdie studie ondersteun ook die reeds bekende bewyse dat ouderdom en geslag 'n invloed het op nierfunksie.

Sleutelterme: tenofovir, serum kreatinien, kreatinienopruiming, CD_4-telling, BMI

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CHAPTER 1: STUDY OVERVIEW AND BACKGROUND

1.1 Introduction

This chapter focuses on the general overview of the study, centring on providing a background to the study, defining the problem, answering questions, aims, specific objectives and methodology that was utilised in the study. This chapter concludes with the division of the chapters.

1.2 Background

HIV/AIDS has been at the helm of clinical research in the 21st century with an estimated 35 million [33.2 million – 37.2 million] people living with HIV as of 2013, affecting approximately78 million people [71 million – 87 million] since the start of the pandemic (UNAIDS, 2014a:1). An estimated 19.1 billion USD was spent globally on HIV programmes in 2013 (UNAIDS, 2014a:9). Funding from governments, private sector and individuals has enabled the fight to decrease HIV/AIDS transmission to continue as is evident in the following statistics:

- New HIV infection has decreased by 38% since 2001 (end 2013),
- Since 2001 new HIV infection has decreased by 58% amongst children,
- HIV/AIDS related death has fallen by 35% since 2005,
- 12.9 Million people living with HIV have access to antiretroviral therapy (ART),
- TB and HIV co-infection deaths have decreased by 33% since 2004 (UNAIDS, 2014a:1).

South Africa boasts the largest HIV/AIDS treatment programme in the world with 2.2 million people accessing ART in 2012 (UNAIDS, 2014b). An estimation of 6.1 million people in South Africa was HIV-positive (UNAIDS, 2014b) and approximately24.7 million people (23.5 million – 26.1 million) in Sub-Saharan Africa were HIV-positive as of 2013 (UNAIDS, 2014a:2).

A number of reasons contribute to the high prevalence of HIV/AIDS in South Africa including poverty, sexual violence, social instability and the high levels of sexually transmitted diseases (AIDS Foundation South Africa, 2013). HIV/AIDS affects the family as a whole and has devastating effects on children. The loss of a parent or both parents is traumatising to any child, leaving the wellbeing of the child to grandparents and orphanage homes. An estimated 2.1 million children were orphaned due to HIV/AIDS in South Africa in 2011 (AIDS Foundation South Africa, 2013).

However, due to increased education, HIV testing and improved access to medical care facilities over recent years, South Africa has played an active role in combatting the HIV/AIDS epidemic (UNICEF, 2012). With more than 4000 clinics offering primary health care services, South Africa aims to achieve an HIV free generation (UNICEF, 2012).

At the forefront of the battle against the HIV/AIDS pandemic is the availability of antiretroviral therapy (ART). ART has not only decreased mortality but has also improved quality of life of patients (CDC, 2013). Antiretroviral (ARV) medicine inhibits the replication of the HIV by different mechanisms of action, thus decreasing the viral load (VL) to undetectable levels in the bloodstream. An HIV-positive person with an undetectable viral load is said to be less infectious (CDC, 2013).

With the beneficial effects, the unwanted adverse effects of ART cannot go unnoticed. Being aware of such side effects and taking the necessary precaution to prevent the occurrence of adverse effects, or progression, can be lifesaving in its own right.

In 2006, tenofovir disoproxil fumarate (TDF)-based first-line regimens were implemented replacing stavudine (d4t). The rationale behind this implementation was to avoid or limit toxicity caused by d4t (Adrieux-Meyer *et al*., 2012:17). This global shift from d4t is important in ensuring that ARV treatment was optimised (WHO, 2010).

Previous arguments about d4t being cheaper than its alternatives have been challenged by the drastic decrease in the TDF price (Adrieux-Meyer *et al*., 2012:19). The price of single drug tenofovir has decreased by 52% from 2008 to 2011 in the international market (Adrieux-Meyer *et al*., 2012:19).

In South Africa, the ART rollout was initiated in the public sector in 2004 with d4t being the primary drug in first-line regimens together with lamivudine and efavirenz or nevirapine (NDoH, 2004). These guidelines were revised in 2010, when d4t was eliminated as the primary drug of choice and replaced with TDF complying with recommendation from the WHO (NDoH, 2010). Tenofovir is expected to add 0.51 quality adjusted life years (QALY's) compared to d4t (Marais *et al.,* 2010:10).

However, the negative effects of the drug cannot be ignored. Tenofovir can cause acute renal failure, Fanconi Syndrome, proteinuria and tubular necrosis (McQueen, 2012). Tenofovir has also been associated with the reduction in mineral bone density (Grigsby *et al*., 2010:44).

1.3 Problem statement

The study emphasises the need to monitor TDF-use closely, to ensure that these drugs do not harm those that need them in order to survive.

The lack of focus pertaining to the nephrotoxic effect of tenofovir can result in debilitating and fatal consequences. Acute renal failure (ARF), chronic kidney disease (CKD), Fanconi Syndrome and proximal tubular dysfunction have been linked to tenofovir use (Kay *et al*., 2013:147). Nephrotoxicity has been seen in 17 - 22% of patients taking tenofovir (Kay *et al*., 2013:147). Due to the potentially life-threatening effect of tenofovir, it is important to monitor therapy.

The purpose of this study was to evaluate the renal safety profile of tenofovir used in combination therapy in patients on ARV treatment in order to assess the effect of tenofovir on kidney function.

1.3.1 Rationale for the study

Kidney disease is a global health care problem with the rates of Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD) increasing. With an estimated 10% of people worldwide suffering from CKD (Renal Care Society of South Africa, 2011) and a growth in the global dialysis market of 7.3% from 2011 – 2015. Early detection and treatment of kidney disease can decrease mortality and improve quality of life (Reportlinker, 2012).

Flandre *et al*. (2011:1700) postulated that CKD and ESRD are significant complications of HIV infection. With more than 70% of patients with ESRD estimated to be living in low-income countries such as those in sub-Saharan Africa, evaluating kidney function is imperative in detecting kidney dysfunction early (Stanifer *et al*., 2014:174). Stanifer *et al.* (2014:178) concluded that CKD is on the increase in sub-Saharan Africa caused by communicable and non-communicable diseases. Stanifer *et al*. (2014:179) advocated that CKD is an important complication of HIV.

In South Africa, the annual incidence of the ESRD is estimated to be between 2 - 4 patients per 100 people (Renal Care Society of South Africa, 2011). These patients will require dialysis and hopefully find a suitable donor for a kidney transplant. There are 4000 people on dialysis in South Africa. This represents the number of people who need kidney transplants, yet only 290 kidneys

were transplanted in 2009 (Renal Care Society of South Africa, 2011). To date, there is little knowledge relating to the development of renal impairment in the black population (Faney *et al*., 9:143). The black population have a different body composition as compared to white people; therefore, studies conducted on other ethnicities will not show true for the black population (K/DOQI, 2002:85). As such it is imperative that we conduct studies on the sub-Saharan black population to ensure that we not only have a better understanding of the disease but also of the efficacy and toxicity of the ARV drugs. With the increased use of TDF, such information is necessary in determining which ARV drug is best suited for a patient.

The nephrotoxic effect of tenofovir can cause irreversible kidney damage in patients (Horn, 2012). The high prevalence of HIV/AIDS in South Africa and the increasing number of patients on highly active antiretroviral treatment (HAART) increases the risk of kidney failure.

1.4 Research aim and specific objectives

The general research aim and specific research objectives of the literature and empirical study will be discussed:

1.4.1 General research objectives

The general research objective of the study was to determine the renal safety profile in adult black HIV-infected patients at a primary health care (PHC) clinic in Newcastle, KwaZulu-Natal, who were on TDF-based treatment for 12 months. The study aims to establish whether tenofovir has an effect on kidney function in HIV-positive patients - meeting certain criteria.

1.4.2 Literature objectives

The literature research objectives of the study include the following:

- To review literature on renal function in black populations on TDF-based treatment nationally and internationally,
- To review literature to assess the prevalence of renal dysfunction after commencing TDF-based treatment,
- To review literature on kidney function; different prediction equations to calculate the creatinine clearance (CrCl).

1.4.3 Empirical study objectives

The specific research objectives of the study include the following:

- To compare the effect of tenofovir on the kidney function in different gender groups as measured with the modified Cockcroft-Gault (CG) equation,
- To determine the CrCl in this black population (≥20 - ≤40 years) at baseline (prior to TDF initiation) and again at 12 months post-TDF commencement,
- To investigate differences in BMI and CD_4 count in the different age and gender groups,
- To investigate the change in CD_4 count and VL over the 12 month period since tenofovir was initiated.

1.5 Empirical investigation

The research methodology utilised in this study incorporated a comprehensive discussion on research design; approach; population and sampling; data collection and analysis; and emphasis was placed on ethical considerations.

1.5.1 Study design

This study adopts a quantitative, observational, cohort study design with a combination of retrospective and partial prospective aspects. All data gathered to answer the research question were previously collected and recorded, with the possibility of certain data being outstanding and needing to be collected after the study had commenced.

Waning and Montagne (2000:46) describes a cohort study as “an incidence study that measures characteristics or attributes in a population free of a disease or drug use problem and relates them to subsequent development of the disease in that population as it is followed over time.”

The advantages of a cohort study are:

- There are precise calculations of risk ratios,
- Imparts information on disease prevalence,
- Explains the relationship between exposure and disease,
- Provides knowledge on various exposures and outcomes,
- Determines cause-effect relationship,

- Takes seasonal changes into account (Waning & Montagne, 2000:56).

The disadvantages of a cohort study are:

- It is time consuming,
- Can be costly,
- Entails a large sample size,
- Validity can be diminished if there are loses during follow-ups,
- Can cause biased results,
- Could be difficult to find participants,
- Disease outbreak of epidemic study has a similar design to a cohort study (Waning & Montagne, 2000:57).

Cohort studies may be retrospective or prospective (Mann, 2003:54).

In order to address the research question, this study design was adopted to examine the renal safety profile of tenofovir as used in combination antiretroviral therapy.

1.5.2 Study site

The study site was a PHC clinic (also known as Lulama clinic) in Newcastle, KwaZulu-Natal which falls under the Amajuba District jurisdiction. The principal investigator (PI) is currently based at Newcastle Provincial Hospital as the ARV pharmacist, which falls under the same district.

This PHC clinic was chosen as the study site for the following reasons:

- The clinic attends to over 3000 HIV-positive patients and the target population can be accessed at this study site,
- This PHC clinic is affiliated to Newcastle Provincial Hospital as the referral hospital,
- The PI does not directly work at this clinic nor does the PI interact directly with the patients at the clinic. Medication and stock are ordered and sent from Newcastle Provincial Hospital to PHC clinics such as Lulama clinic.

1.5.3 Target population and study population

The target population was all adult (male and female) black patients (to exclude any possible genetic variation in renal function) between the ages of ≥20 - ≤40 years on TDF-based ART who

attended the PHC clinic in Newcastle for a consecutive 12-month period. All study participants could be ART-experienced or naïve (not previously on any HAART) patients but must be on TDF-based regimens during the consecutive 12-months period.

1.5.4 Inclusion and exclusion criteria for the study population

The following inclusion and exclusion criteria were set.

Inclusion criteria:

- Black male and female patients who have been on TDF-based ART treatment for at least six months,
- Between the ages of ≥20 - ≤40 years (patient must be at least 20 years old and not older than 40 years when baseline TDF measurement was taken),
- Patients must have had a baseline SCr test before commencement of TDF-based treatment (it may be possible that the 12-month SCr tests result would also be available at time of recruitment for some of the patients).
- The HAART regimens that will be allowed include:

    - ❖ Tenofovir/lamivudine (3tc)/efavirenz (EFV).
    - ❖ Tenofovir/lamivudine/nevirapine (NVP).
    - ❖ Tenofovir/emtricitabine (FTC)/efavirenz.
    - ❖ Tenofovir/emtricitabine/nevirapine.
    - ❖ Tenofovir/lamivudine/lopinovir+ritonavir (LPV/r).
    - ❖ Tenofovir/emtricitabine/lopinovir+ritonavir.

Exclusion criteria:

- Pregnancy,
- Diabetes,
- Hypertension and cardiovascular disease,
- Patients with a baseline CrCl ≤60 ml/min,
- Dementia.

Pregnancy, diabetes, hypertension and other cardiovascular diseases also affect kidney function (K/DOQI, 2002:75-78) and have therefore been included as exclusion criteria. The study involves an investigation involving patients with healthy kidney function with a CrCl ≥60 ml/min. As per the

K/DOQI clinical guidelines, patients with kidney function ≤ 60 ml/min/1.73m^2 (adjusted for body surface area [BSA]) for more than 3 months is indicative of CKD (K/DOQI, 2002:3) and are therefore excluded from this investigation at baseline.

1.5.5 Data collection process and recruitement

Prospective screening of active patient files was performed at the PHC clinic according to inclusion and exclusion criteria by the PI who does not work directly at this PHC clinic but is employed in the same district by the NDoH, KwaZulu-Natal. Screening of patient files only occurred once approval was obtained from the Human Research Ethics Committee (HREC) from North-West University (NWU) and the NDoH, KwaZulu-Natal ethics committee. Majority of the study results (data) were retrospectively gathered from the patient clinic files.

A list of possible research study participants that met the criteria of the study were compiled in a safe and secure room provided to the PI within the same facility to assess patient files. This list was provided to the staff nurse that works directly with the HIV-infected patients in this clinic. The staff nurse acted as mediator between the patient and the PI. The staff nurse was trained and informed by the PI regarding the study details and informed consent procedures. In the absence of the staff nurse, recruitment of patients was delegated to a professional nurse at the clinic who acted as mediator between the patient and PI. Mock training sessions formed part of the training with the staff nurse and the professional nurse to ensure that they were familiar with the required procedures and study information. The trained nurses at the clinic approached the patients (study participants) to recruit and perform consent regarding the study before they could be included into the study. The nurses explained the study and the procedures needed to conduct the study to the patient, as per the approved informed consent form (ICF). Patients were approached during their regular scheduled visits to the clinic and were only consulted with in the consultation rooms at the clinic.

The nurses ensured that the patient (study participant) had decision making capacity by asking the patient (study participant) two questions based on what had been explained about the study. The nurses explained the study in isiZulu to the patient (study participant) if necessary. The ICF was available in English and isiZulu (Annexure 1 and 2). Illiterate patients (study participants) had a literate associate in the form of a friend, family or companion who aided in understanding the study and the role of the study participant in this study. Such associate also signed as witness on the ICF. Study participants were afforded the opportunity to think about participating in the study

and were given the opportunity to take the ICF home to read and bring it back on an unscheduled clinic visit. They were paid a once off transport fee of R30. Participants were informed to return the consent form to the same nurses within a week. The nurses were qualified and registered nurses and it was not to be a power relationship but rather a relationship of trust.

The ICF was handed directly to the nurses who signed the ICF as the person obtaining the actual consent. The signed ICF was placed in a sealed box for the PI to collect on a weekly basis until such time that the required number of participants were achieved (Sept. 2015 – Oct. 2015).

Patients had the right to refuse participation in the study at any time. To ensure confidentiality and to respect the patients' rights to privacy, only the PI; the staff nurse and the registered nurse from the PHC clinic acting as mediators; and the study supervisors were aware of the recruited patients.

1.5.6 Calculation of creatinine clearance

The CrCl values as reflected on the patient information record form (Annexure 3) was at first calculated with a hand held calculator by the PI. All the information including the SCr laboratory values recorded onto Annexure 3 by hand was then transferred onto the electronic Microsoft Excel® spreadsheet (research tool – Annexure 12). The PI used the CG formula and encoded it into the electronic Microsoft Excel® spreadsheet (Annexure 12) the CrCl was calculated automatically once the respective values needed for the calculation was added into the spreadsheet. The values determined by the PI on the hard copy were cross referenced with those on the electronic copy to ensure validity. The modified CG formula that was used is reflected under section 1.6.2.

1.6 Process of obtaining the blood sample by clinic personnel and recording of blood results

The results from the patient files were only recorded once patient consent and permission had been obtained to use these results (Annexure 3). Majority of the blood results were retrospectively recorded from the clinic patient files. Some blood results for the 12-months post-TDF-based treatment initiation were collected prospectively, strictly according to the time schedule and standard guidelines as set out by the NDoH. Blood sampling for SCr values, VL and CD_4 count was only performed if it fell within the standard care and treatment guidelines of the NDoH.

Routine blood samples were obtained by a nurse or doctor at the PHC clinic as part of their day to day health care service that they provide to these patients. All medical staff at the clinic are trained and registered with the various authorities, namely the South African Nursing Council (SANC) and the Health Professions Council of South Africa (HPCSA).

Standard operating procedures (SOP's) for blood collection and medical waste disposal form part of their training and daily routine activities at the clinic.

1.6.1 Data source

Data were collected up and until October 2015 to include baseline and 12-month follow-up data as some results were already available as retrospective data. Data were obtained from patient files at the clinic and entered onto the paper format as reflected in Annexure 3. Data from Annexure 3 were used to complete the electronic Microsoft Excel® spreadsheet which was used as the electronic tool. Data accessed included SCr values, VL, CD_4 cell count, age, weight, gender, body mass index (BMI) and the patient's history pertaining to diagnosis and previous therapy. Data were taken directly from the patient files/records by the PI. The BMI was not routinely calculated and was thus only reflected in the file once the patient was referred to a dietician. The patient data was assessed at the clinic premises during clinic working hours (24 hours a day, 7 days a week). No file was removed from the clinic. A private and secure room was provided to the PI to assess patient files. The original patient files remained at Newcastle PHC clinic. All data gathered from the files remained in the PI's safe possession (locked away and or on password protected personal computer) until they were made available to the NWU for storage once all results had been recorded and analysed.

1.6.1.1 Data collection tool

The PI recorded data from the patient health files onto a pater format (Annexure 3) and then this data was captured on a Microsoft Excel® spreadsheet. Data incorporated into this electronic data collection tool included variables required to assess renal function, immunological outcome, virological outcome and BMI. Data extracted from patient files included diagnosis history, treatment history and laboratory values. History and laboratory valued were incorporated in the Microsoft Excel® spreadsheet. This electronic data collection tool was compressed into a single data collection form as presented under Annexure 12. Cross tabulation and cross checking by the PI was conducted to ascertain internal consistency and accuracy.

All data (paper format and electronic format) was accessible to the study team only and was stored on a password protected personal computer or compact disc. Any hard copies were safely and securely locked at all times. Records will be kept for 5 years after this study report has been completed.

1.6.2 Measuring Instruments

The information gathered from patient files included, history pertaining to diagnosis and previous therapy CD_4 count, VL, date of diagnosis, date of commencement of ART, SCr value, age, gender, weight and study number was incorporated onto a paper format (Annexure 3). A once off summary table was kept by the PI to cross reference the actual name of the study participant (and contact details) with the study number once participants had provided their consent to take part. This summary table was locked away should any further reference, call backs or problems arise.

The routine blood samples were collected according to the standard protocol of the clinic and the analysis of the SCr values were performed by the National Health Laboratory Service (NHLS) according to their standard operating procedures (Annexure 4). The equipment used by NHLS to conduct the tests is the UniCel® DxC 800 System. The creatinine concentration is measured by means of the Jaffe rate method. A precise volume of the sample is injected into a reaction cup containing an alkaline picrate solution. Creatinine in the sample reacts with the picrate to form a red colour complex. The rate of increase of absorbance at 500 nm due to the formation of the complex is directly proportional to the concentration of creatinine in the sample (Annexure 5).

The SCr values were incorporated into the modified CG equation to determine the CrCl in ml/min.

$$CrCl = \frac{[140\text{-age (years)}] \times \text{weight (kg)} \quad *}{\text{Scr } (\mu\text{mol/L})}$$

(* x 0.85 if female) (South African Renal Society, 2006; NDoH 2014)

1.7 Data and statistical analysis

Descriptive statistics as well as inferential statistics were used to present the results.

Zikmund *et al*. (2009:651-653) states that descriptive statistics can be defined as statistics that explain the data in a comprehensible manner whereas inferential statistics refers to applying statistics to show how characteristics from a sample apply to an entire population.

The SCr results of the study patients were obtained from the PHC clinic's patient records. Some of the SCr results were retrospective (baseline – before TDF initiation) and the 12-month follow-up SCr may have been prospectively collected. A quantitative approach was followed based on the results obtained at baseline and 12-month post-TDF initiation.

Dependent variables include:

- SCr value,
- CrCl (continuous variables).

Independent variables include:

- Age,
- Gender (categorical variable).

1.7.1 Independent *t*-test

The independent *t*-test evaluates the difference between the average values taken from two independent groups or samples (Zikmund *et al*., 2009:534). This test was performed to investigate differences in SCr and CrCl by comparing female *vs.* male participants at baseline and at 12-month post-TDF.

Independent *t*-test will be performed to investigate the possible change of the SCr and CrCl within the age groups (age ≥20 to <30 years and ≥30 to ≤40 years) at baseline and at 12 months post-TDF.

The Mann-Whitney test was utilised as the non-parametric equivalent of the independent *t*-test.

1.7.2 Dependent *t*-test

The dependent *t*-test was used to investigate the changes on SCr, CrCl, CD_4 count and BMI from baseline to 12 months. The Wilcoxon signed-rank test was utilised and is the non-parametric equivalent of the dependent *t*-test.

1.8 Sample size justification

The justification of the minimum sample size and effect size was calculated with *t*-Test Power calculation (Statistica®). An effect size of 0.375 was regarded as suitable for this male and female population as it provided a power greater than 0.8 with the sample size at 60 study participants. The power (0.8) of the study was based on data (difference between the CrCl baseline and 12 months) from 17 random results (male and female, aged between ≥20 and ≤40 years) that were available in 2012 from the Newcastle PHC clinic. This sample size justification was calculated in collaboration with Prof Faans (HS) Steyn, NWU Statistical Consultation Services. A minimum of 60 participants were required with a 20% (n = 12) fall out rate due to anticipated withdrawals or non-completers. The total number of study participants to be recruited would be 60 + 12 = 72.

1.9 Data integrity

1.9.1 Validity

The validity of a measure refers to "the degree to which it actually measures what it is designed to measure" (Waning & Montagne, 2000:123). This can be further divided into internal validity and external validity. Internal validity alludes to the extent to which the consequences of this concentrate exactly demonstrate the circumstance in reality (Waning & Montagne, 2000:123). The external validity signifies the extent to which these study results can be used in other populations (Waning & Montagne, 2000:123). The higher the external validity, the more researchers can rely on upon any outcome observed in the study being seen in the present world (Zikmund *et al*., 2009:277).

The validity of the study was established through the utilisation of an appropriate study design and the internal validity was tested while evaluating the random results from 17 anonymous patients conducted before the actual data collection process.

1.9.2 Reliability

Reliability as explained by Waning and Montagne (2000:123) is the extent to which a result can be reproduced.

Retrospective blood results were obtained from the patient health records and the prospective blood samples for the 12-month post-TDF test were obtained as per SOP at the clinic that forms part of the standard treatment of care.

Equipment at the NHLS was calibrated every 72 hours or with each new bottle of reagent as per the systems chemistry information sheet. All procedures pertaining to creatinine testing were according to SOP (Annexure 4).

The results in the patient files were reliable as the original blood result printout was available. The laboratory could be contacted if any values were missing as these results were stored on the electronic data system of the NHLS. All other information (age, weight and gender) required to calculate the CrCl were obtained from the patient files.

In order to ensure that correct information was retrieved from files, only the original printout of the blood results was regarded as reliable. All blood results must have dates corresponding with the individual participant's baseline (when TDF-based HAART was initiated) and 12-month blood results.

All data captured was cross-checked by the PI to ensure accuracy.

1.10 Bias

To limit bias in this study, the PI visited the study site during clinic working hours and data collection was conducted during this time. The ICF was issued to patients via a mediator and was not given to the patients by the PI. The ICF was available in English and isiZulu in order to ensure that the patient understood their involvement in the study in their home language. The mediator is also fluent in English and isiZulu to ensure that accurate information is communicated to the patient. Data was collected from the blood result printouts and information recorded in patient files by professional clinic staff members.

1.11 Ethical consideration

1.11.1 Permission/consent

The Amajuba District Manager, Mrs A.M.E.T. Tshabala acknowledged the intent to conduct the study at the PHC clinic (Annexure 6). Patients were only approached by the staff nurse to participate in the study once approval had been received from the HREC at the NWU (Annexure 7) and the Department of Health KwaZulu-Natal Ethics committee (Annexure 8).

AddItionally, a letter of goodwill has also been submitted (Annexure 9), from the Nursing manager of the clinic (Mrs Gugu Ngema).

Dr Yusuf Moola acted as professional consultant and provided clinical guidance. Dr Moola assisted in interpreting clinical blood results and calculating the CrCl if deemed necessary by the PI as this formed part of his normal clinical duties (standard care and treatment of the patients).

The ICF had to be signed by patients before inclusion into the study. The intermediate person who approached the patient (study participant) for recruitment and consent was a staff nurse at the clinic. In the absence of the staff nurse, the responsibility to recruit the patients was delegated to a professional nurse. Refer to the recruitment process as described above under section 1.5.5 for clarity on this process. The signed ICF was handed directly back to the staff nurse who had to also sign the ICF. The signed ICF was then placed in a sealed box for the PI to collect on a weekly basis until such time that the required number of participants had been achieved (Sept. 2015 – Oct. 2015).

A final report will be provided to the PHC clinic once the research has been completed and the final results have been analysed. No direct feedback was made available to each participant by the PI. The District Manager and the clinic manager will receive feedback once the study is completed. Files that needed to be flagged for further assessment by the doctor were given to the clinic manager.

The ethic forms and procedures of the NWU were completed and submitted. This study is of an observational nature as the treatment regimens and blood tests performed on the patients form part of the standard treatment care guidelines as set out by the NDoH (NDoH, 2015:14-15). The study is conducted in accordance with the Declaration of Helsinki 2008 (World Medical Association Declaration of Helsinki, 2008), the ICH Good Clinical Practice (GCP) guidelines (International Conference on Harmonisation, 1998) and the SA GCP guidelines (NDoH, 2006). The PI completed the GCP introduction course hosted by BCompliant on the 16th of November 2012.

The PI completed the GCP course in November 2012 (Annexure 10).

1.11.2 Anonymity

Only the PI prospectively selected the patients that were recruited by accessing their patient health files at this PHC. Only the mediators had contact with the patient pertaining to the research project. Patients were given individual study numbers and these numbers would be shown on the Microsoft Excel® spreadsheet only (hard copies and electronic database). Only

the PI and NWU staff members have access. Only clinical information will be reflected in any of the reports. All original laboratory results will remain in the patients file and no copy will be made thereof.

A once-off summary table was kept by the PI to cross-reference the actual name of the study participant (and contact details) with the study number, once participants have provided their consent to take part. This summary table will be locked away should any further reference, call backs or problems arise.

1.11.3 Confidentiality

Once the respective ethics approval (Annexure 7) and approval from the Department of Health KwaZulu-Natal (Annexure 8) had been obtained, patients were selected by the PI following the inclusion criteria as stipulated in the protocol. As stated previously the study design is observational, retrospective with some prospective aspects. Study participants are referred to by study numbers only. Results were first recorded onto a paper format (Annexure 3) and then electronically captured onto a Microsoft Excel® spreadsheet where only study numbers were used. Statistical calculations were made from this electronic database. No personal details of any participant was used in any publication, dissertation or congress proceeding.

Only patients who have signed the ICF were able to participate and it was voluntary.

1.11.4 Storage of data

The hard copies (patient files) will remain in the clinic and only the handwritten patient information records (as transferred to Microsoft Excel® spreadsheet) will be in the possession of the researcher (PI). No files left the clinic and no photocopies were made. The hand written patient information record was transferred to the electronic Microsoft Excel® format by the researcher (PI). The handwritten patient information record and the electronic Microsoft Excel® spreadsheets (with study numbers only and not names) are stored and locked away at all times in a safe and secure place. The handwritten transferred copies as well as electronic Microsoft Excel® spreadsheets were made available to the NWU (Medicine Usage of South Africa) for safekeeping and storage after the study had been completed for a period of five years. A private room to work at in the clinic had been secured, so that there would be no interruption or traffic while patient information was being assessed or analysed by the researcher (PI).

All paper data was locked away when in use by the PI or when handed over to the NWU for safekeeping. All electronic data was kept on a password protected personal computer (PC) with the PI and when handed over to NWU it would also be stored on a password protected PC or compact disc. Study supervisors of the PI will ensure that no extra copies (paper or digital) will be kept by the PI after the study has been completed.

The participants had the right to withdraw at any point before data analyses had been performed without providing reason and without accompanying descrimination. After data analyses, the individual data had been anonymised and therefore could not be identified and withdrawn.

1.11.5 Benefit-risk ratio

The blood samples were drawn according to SOP at the clinic and no addItional samples were drawn as part of the routine care and management guidelines. There is a risk involved when blood is drawn. The risk of a needle prick injury when drawing blood forms part of daily risk for trained medical staff drawing blood. For in the event that a staff member was accidently pricked by a needle while drawing blood, standard post exposure prophylaxis (PEP) was available at the clinic. The study participant may have felt discomfort while blood was being drawn; all staff of the clinic are trained and employed by the Department of Health KwaZulu-Natal.

The possible risk of the identity of the participants becoming known and the risk of stigmatisation exists, but it is minimal. Participants could be branded or condemned if their HIV status becomes public knowledge. Hence all precautions were taken to ensure that the identity of the participants remains anonymous. Recruitment and consent took place in a consultation room and not in open public spaces within the clinic.

There is no direct benefit for the participant as most data was obtained retrospectively.

Some direct benefit to the participant was to assess and monitor the effect of tenofovir on their kidney function more closely, by the PI (researcher also a pharmacist) involvement, even in retrospective screening. Although the study is observational, files were flagged for the attending doctor to ensure that the doctor could attend to the failing CrCl or that the CrCl was possibly becoming too low and nearing the cut off point for acceptability to be on TDF-based treatment.

Pharmacists were also encouraged to play a more active role in clinical management by evaluating blood results and flagging patient files for doctors or nurses when encountering deterioration in kidney function.

The indirect benefit is the improvement of knowledge on the effect of tenofovir on kidney function for both the patients and health care professionals working in the field, as a feedback report will be presented to the clinic management at the end of the study to highlight the outcomes, recommendations, challenges and shortcoming of this investigation.

1.11.6 Informed consent forms

Informed consent forms (ICF) were available in English (Annexure 1) and isiZulu (Annexure 2). Each prospective participant was approached by the staff nurse or the delegated registered nurse as described in section 1.5.5 above. The study participant was given time to think about the participation before they signed the ICF. Participation in the study was voluntary and did not affect the quality of service and care they received from the clinic should they have wished to not take part. The staff nurse and registered nurse have direct contact with the patients and were trained to assess if the patient (study participant) had decision making ability to choose to enrol in the study. Once the study participant had decided to take part they would have to bring back their signed ICF to the same staff nurse or registered nurse to be placed in a sealed box and the staff nurse or the registered nurse would also have to sign the ICF declaration. The researcher (PI) collected the content of the sealed box from the staff nurse or registered nurse on a weekly basis until such time that the required number of participants had been achieved (Sept-Oct 2015).

1.11.7 Feedback on study results

A final report on this specific research will be presented to the clinic once the research has been completed and the final results have been analysed. A final report (hard copy and electronic copy) will be made available to the Department of Health, KwaZulu-Natal ethics committee as stipulated in the ethics approval letter (Annexure 8). No direct feedback was provided to the study participants, unless they contacted the researcher (PI) directly and requested for specific information. The findings will be disseminated to the wider research community in the form of the written dissertation, possible national or international conference presentations (poster or podium) and publications in the form of an article in national or international journals. The journal of choice for publication is the SA Health Gesondheid journal.

1.12 Division of chapters

This dissertation is presented in the form of four chapters. The outline of the chapters is presented below:

Chapter 1: Study overview and background

Chapter 1 is an introduction to the study. In this chapter the background of the study is provided. The research aim and specific objectives is discussed in this chapter. The research methodology, study design, study site, target and study population, recruitment and data collection is discussed. Data and statistical analysis are described in this chapter. Focus is also placed on ethical considerations, including consent, anonymity, confidentiality and storage of data.

Chapter 2: Literature review on tenofovir and its impact on renal function

Chapter 2 provides a discussion on the review of literature. A theoretical overview is provided about the effect of tenofovir on kidney function. Physiology of the kidney, discussion on creatinine and various methods of calculating the kidney function are discussed.

Chapter 3: The effect of tenofovir on creatine clearance after 12 months of treatment in a HIV-infected adult cohort in KZN

This chapter contains one full-length manuscript for submission to a peer-review journal (Health SA Gesondheid) and is presented in the manuscript style set out according to the guidelines as stipulated by Health SA Gesondheid journal. The focused results are analysed in this chapter and a brief outline pertaining to the literature review, discussion of results, conclusion and recommendation is elaborated on according to the stated journal requirements.

Chapter 4: AddItional results

This chapter contains addItional data not reflected in the manuscript of chapter 3. Demographic data pertaining to male and female (≥20 – ≤40 years), immunological data and non-parametric data is represented here

Chapter 5: Discussion, conclusion and recommendation

The limitation of the study, conclusion and recommendation is reviewed in detail.

1.13 Chapter summary

This chapter focuses on the scientific research methodology utilised in this study, to investigate the renal safety profile of tenofovir as used in combination antiretroviral therapy. The research design was developed against the background of the research question. A quantitative, observational, cohort retrospective with a partial prospective design was utilised in order to achieve the objectives of the study.

CHAPTER 2: LITERATURE REVIEW ON TENOFOVIR AND ITS IMPACT ON RENAL FUNCTION

2.1 Introduction

The literature review is an evaluative report based on literature related to the topic being discussed and assists in gaining an understanding of the current state of knowledge about the topic. This chapter discusses literature related to the effect of tenofovir on kidney function and provides an overall view on the physiology of the kidney, biomarkers to detect kidney function, methods of calculating kidney function and the effect of drugs on the kidney with special emphasis on tenofovir.

2.2 Background

Tenofovir disoproxil fumarate is the prodrug of tenofovir, a nucleotide reverse transcriptase inhibitor that was approved in 2001 by the Food and Drug Administration (FDA) to be used in combination with other antiretroviral drugs to treat HIV infection in adults (FDA, 2001:6). This recommendation was mainly based on a randomised, controlled study (Study 902), which comprised of 189 patients who were either given TDF in one of three doses (75 mg. 150 mg or 300 mg daily dose) or a placebo to their existing regimen in a double-blinded study (FDA, 2001:21). At week 24 of this study, a decrease in VL of - 0.58 log10 copies/mL ($p < 0.001$) was seen in the group taking 300 mg TDF, compared to the placebo group which reported a change of +0.2 log10 copies/mL (FDA, 2001:26).

The research report provided by Gilead Sciences to the FDA Advisory Committee stated that TDF had an insignificant impact on the development of renal dysfunction (FDA, 2001:16). However, recently a disparity has been observed concerning the nephrotoxic effect of tenofovir (Perazella, 2010:1060).

The beneficial effect of tenofovir was again highlighted in 2008 when the FDA approved its use in the treatment of Hepatitis B (Coffey, 2015). In 2012, this new “wonder drug” was placed in the spotlight again having been approved by the FDA as the first pre-exposure prophylaxis (PrEP) when used in combination with emtricitabine, as taken daily by HIV-negative individuals (Coffey, 2013).

This finding is based on two randomised, double blinded studies; the Partners PrEP study and the TDF2 study (Coffey, 2015).

The Partners PrEP study was conducted in Kenya and Uganda amongst 4758 serodiscordant heterosexual couples where one member in each couple was HIV negative. The results depicted a decrease of up to 67% in HIV transmission in couples on tenofovir only (95% confidence interval, $p < 0.001$) and a reduction of 75% with a tenofovir/emtricitabine combination (95% confidence interval, $p < 0.001$) (Baeten *et al*., 2012:399).

The TDF2 study conducted in Botswana enrolled 1219 men and women. The study showed a reduction of 62.2% *(p* = 0.03) in HIV infection in the study group taking a combination of tenofovir and emtricitabine (Thigpen *et al*., 2012:423).

The Partners PrEP study and the TDF2 study provided evidence of the efficacy of tenofovir as used in combination with emtricitabine to prevent HIV-transmission in heterosexual couples. Prophylactic oral tenofovir/emtricitabine in combination is only intended for those who are confirmed to be HIV negative (CDC, 2012).

In South Africa, the HAART programme commenced in May 2001 with the first pilot site being in Khayelitsha, followed by 18 National Prevention of Mother-to-Child Transmission (PMTCT) pilot sites (NDoH, 2003). This saw the birth of the National ART rollout in the public sector. Accomplishment in the ART system was found in the initial 3 years with an abatement of 25% in HIV/AIDS related mortality (Evan, 2013:229).

The first ARV guidelines released by the NDoH in 2004 (Table 2.1) indicated d4t as the primary drug of choice in first-line therapy (NDoH, 2004:10-23). Reports of d4t related toxicity including peripheral neuropathy, symptomatic hyperlactatemia, lipoatrophy and lactic acidosis supported the WHO move away from d4t based regimens, towards less toxic drugs (Evan, 2013:230). The ARV treatment guidelines were revised in 2006, introducing TDF as second line treatment as used in combination. In 2010, these guidelines (Table 2.2) were re-revised placing TDF as first-line treatment as used in combination in South Africa (NDoH, 2010:9-16). The 2015 consolidated guidelines changed the eligibility criteria for patients. Patients with a CD_4 count < 500 cells/µl were eligible for the commencement of ART compared to < 200 cells/µl previously (NDoH, 2015:14). This revision allowed for more HIV-positive people to receive HAART. The different regimens and the frequency of conducting blood tests are depicted in table 2.3 (NDoH, 2015:74).

Table 2.1: National ART guidelines 2004

Regimen≥	Drugs	Monitoring tests	Frequency
1a (1st line)	d4t/3tc/EFV	CD_4	Staging, 6 monthly
		VL	Baseline, 6 monthly
		Alanine transaminase (Alt)	symptomatic
1b (1st line)	d4t/3tc/NVP	CD_4	Staging, 6 monthly
		VL	Baseline, 6 monthly
		Alt	Baseline, week 2, 4 and 8, thereafter 6-monthly
B2 (2nd line)	AZT / ddI/ LPV/r	CD_4	Staging, 6-monthly
		Full blood count (FBC)	Baseline, then monthly for 3 months, then 6 monthly (with CD_4 and VL)
		Fasting cholesterol and triglyceride Fasting glucose	Baseline, 6 months and thereafter every 12 months Baseline and 12 months
1st line (pregnant women)	d4t+3tc+NVP (EFV can be used after the first trimester	CD_4	Staging, 6 monthly
		VL	Baseline, 6 monthly
		ALT	Monthly if on NVP

d4t – stavudine 3tc – lamivudine NVP – nevirapine AZT – zidovudine LPV/r – lopinivir/ritonavir
EFV – efavirenz ddI - didanosine

Table 2.2: National ART guidelines 2010

Regimen	Drugs	Monitoring tests	Frequency
1a (1st line) + 1b (1st line) All new patients needing treatment.	TDF + 3tc/FTC + EFV/NVP (NVP if 1b)	CD_4	6 months, 12 months and then 12 monthly
		VL	At month 6, 1 year on ART and then every 12 months
		ALT To identify NVP toxicity	If on NVP and develops rash or symptoms of hepatitis
		FBC	At month 1, 2, 3 and 6 if on AZT
		SCr	At month 3 and 6 then every 12 months if on TDF
		Fasting cholesterol and triglycerides	At month 3 if on LPV/r
1c (1st line)	AZT+ 3tc +EFV/NVP		
2a (2nd line) Failing on a d4t or AZT-based 1st line regimen	TDF + 3tc/FTC + LPV/r		
2b (2nd line) Failing on a TDF-based 1st line regimen	AZT + 3tc + LPV/r		
3a (3rd line) (salvage therapy) Failing any 2nd line regimen	Specialist referral		
Pregnant women	TDF + 3tc/FTC + NVP AZT+ 3tc + NVP (if contraindication exists to TDF)		

FTC – emtricitabine FDC – 3 in 1 fixed dose combination

Table 2.3 Consolidated ART guidelines 2015

Population	Regimen	Monitoring test	Frequency
Adolescents > 15 years and weighing > 40kg Adults All TB co-infection All HBV co-infection	TDF + 3tc (or FTC) + EFV FDC	CD_4	Baseline and 1 year post ART commencement
		VL	Month 6, month 12 on ART and then every 12 months
		ALT	If on NVP and develops rash or symptoms of hepatitis
		FBC	Month 3 and 6 if on AZT then every 12 months
		Creatinine (Cr)	Cr at month 3 and 6, month 12, then every 12 months if on TDF
		Fasting cholesterol and triglycerides	Month 3 if on LPV/r
Adults and adolescents on d4t	Change d4t to TDF		
Contraindication to EFV:	TDF + FTC (or 3tc) + NVP or LPV/r		
TDF contraindication: Creatinine clearance (CrCl) of < 50 mL/min	Abacavir (ABC)+ 3tc +EFV (or NVP)		
Pregnant women	FDC		

The fixed dose combination (FDC) rollout in 2013 saw the first three-in-one combination product been introduced as part of the NDoH HIV initiative (Davies, 2013:41). This single, daily dose ARV consisted of a combination of tenofovir, FTC and EFV. The criteria of eligible patients to commence FDC include patients commencing HAART for the first time, pregnant women and breastfeeding mothers (Davies, 2013:41). These showed a change in the treatment of pregnant women from the previously AZT-based regimen to decrease vertical transmission.

As is evident, the increased eligibility criteria for patients to commence with TDF-based regimens have increased the number of people using tenofovir.

A large observational study conducted by EuroSIDA involving more than 16 500 HIV-positive people concluded that tenofovir was associated with and increased risk of CKD (Horn, 2010). The results of the study showed that the risk of developing CKD increased by 16% every year that a person remained on TDF (Horn, 2010).

Risk factors for renal impairment in HIV-positive persons receiving TDF are:

- Older age,
- Low body weight,
- Advanced immune suppression,
- Pre-existing renal insufficiency,
- Concurrent utilisation of nephrotoxic drugs such as digoxin, cyclosporine and aminoglycosides,
- Combination with didanosine or protease inhibitor (Pavie *et al*., 2011:458).

2.3 Physiology of the kidney

This study focuses on the effect of TDF on kidney function as used in combination ART. In order to have a thorough understanding of this relationship, the basic physiology of the kidney needs to be understood.

The kidneys are exceedingly particular bean-moulded organs that lie behind the stomach area, on either side of the spine (Greenberg, 2009:18). Each kidney has about 1 million functional units known as nephron (Field *et al*., 2001:2-3).

Greenberg (2009:14) states that the functions of the kidney are:

- Regulation of the volume of body fluids. This is accomplished by regulating the measures of water and ions eliminated in the urine,
- Elimination of metabolites and foreign substances,
- Production and emission of hormones and enzymes.

The nephrons consist of the following parts:

- The glomerulus: The main responsibility of the glomerulus is to filter blood. It averts the movement of proteins and red blood cells into the urine (Field *et al*., 2001:3).
- The proximal convoluted tubule: This tubule is the site for the maintenance of body fluids. The filtrate from the glomerulus passes into the tubules (Field *et al*., 2001:3). Bulk of the filtered small particles is absorbed by the proximal tubules (Greenberg, 2009:32). It also reabsorbs filtered glucose and amino acids. The proximal tubule plays an active role in phosphate transport. The S_3 segment or the *pars recta* is the site for the removal of a number of drugs and toxins (Greenberg, 2009:32).
- The loop of Henle: Functions in the recovery of water and sodium from the urine (Anon., 2014). This restricts the amount of water eliminated in the urine (Bishop *et al*., 2004:520),
- The distal nephron: Consists of the distal convoluted tubule, the connecting tubule, the cortical collecting duct and medullary collecting duct. The final alterations in the composition of the urine occurs in the distal nephron (Greenberg, 2009:34).

The proximal convoluted tubule, the distal convoluted tubule and the connecting tubule are well endowed with mitochondria (Greenberg, 2009:34). The mitochondria are energy centres which provide energy for sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase) (Greenberg 2009:32). The Na^+K^+-ATPase regulates the movement of Na^+ out of the intracellular space into the blood and moves K^+ into the cell (Greenberg, 2009:37).

2.4 Assessment of nitrogenous waste products.

Nitrogenous waste products are excreted primarily through the kidneys. An increase in plasma nitrogenous product concentration is an indication of decreased kidney function (Porth, 2007:801).

2.4.1 Creatinine

Creatinine (Cr) is the end product of creatine metabolism. Creatinine is produced mainly in the liver and is transported to muscle where it is converted to phosphocreatine (Bishop *et al*., 2004:223). In the muscle, creatinine exists as creatine and creatine phosphate. Creatine loses water and creatine phosphate loses phosphoric acid to form the endogenous product, Cr. Creatinine then passes into the plasma (Wyss & Kaddurah-Daouk, 2000:223).

The rate of Cr formation and excretion depends on muscle mass. Daily, 20% of creatine forms Cr. Creatinine concentration is directly proportional to muscle mass and remains constant in an individual unless muscle mass or renal function changes (Bishop *et al*., 2004:521). Calculation of CrCl is used to estimate the kidney function (Bishop *et al*., 2004:524).

An increase in SCr results from Cr not being filtered by the glomerulus and actively secreted by the tubules. This results in a decrease in the kidney function. SCr is used to determine the kidney function as it is an easier laboratory method compared to the 24 hour urine collection method (Greenberg, 2009:64). The K/DOQI (2002:81) stated that measuring the CrCl over a measured time will not provide a more accurate indication of kidney function. Over collection or under collection of urine over a 24 - hour period can cause mistakes in calculating CrCl, and hence kidney function.

Limitations of using Cr to determine the kidney function include:

- Protein intake influences CrCl. Serum creatinine is increased after eating protein and can cause an overestimation in CrCl.
- Creatinine excretion is increased by physical activity (Kampmann & Hansen, 1981:9).
- Pregnancy affects kidney function and can exaggerate values up to 140% (K/DOQI, 2002:55).
- Geriatric patients may have reduced muscle mass which could affect SCr levels (Nyman *et al*., 2011:1140).
- Obese patients may experience an increase in renal plasma flow and GFR (Nyman *et al*., 2011:1140).
- Certain conditions such as severe liver disease, cachexia and malnutrition may render Cr- based equations inaccurate (Nyman *et al.,* 2011:1132).
- Certain drugs such as trimethoprim and cimetidine increase SCr concentration (K/DOQI, 2002:87).

- Ingestion of cooked meat can also increase SCr concentration (Kampmann & Hansen, 1981:9).
- Serum creatinine may only become evident long after kidney damage has occurred (Wu & Purikh, 2008:1895).

Methods to determine kidney function using SCr concentration are less expensive and less time-consuming (Lewis *et al*., 2004:3182).

2.4.2 Urea

Urea is manufactured in the liver and is formed when protein is broken down by oxidative catabolism to form ammonia. The ammonia is converted to urea and is excreted by the kidneys (Bishop *et al*., 2004:521). The urea is filtered by the glomerulus. Bishop *et al*. (2004:521) explained that 40 - 60% of urea is reabsorbed in the collecting duct. Plasma urea is inversely proportional to kidney function (Bishop *et al*., 2004:69).

Field *et al*. (2001:74) identifies that increased urea in the serum due to decreased glomerular filtration causes uraemia. Uraemia is depicted by drowsiness, confusion, anorexia, nausea, vomiting, asterixes and pericarditis.

Urea is less valuable in determining kidney function for the following reasons:

- The amount of protein absorbed from the gut influences urea production,
- The quantity of urea reabsorbed is variable,
- Conditions such as dehydration increase the quantity of urea reabsorbed (Field *et al*., 2001:69).

2.5 Markers to determine kidney function alternate to creatinine

2.5.1 Proteinuria

Proteinuria refers to the excretion of protein in the urine above the normal levels and is a sensitive marker of kidney damage (K/DOQI, 2002:100). Protein excretion in the urine of 150mg/day is seen as normal (Field *et al*., 2001:40). Protein in the urine can be detected by means of a urine dipstick or by timed urine collection (overnight or 24 hours) (K/DOQI, 2002:101). Protein normally excreted in the urine is smaller and cannot be filtered across the glomerular capillary wall and is not reabsorbed by the tubular cell. Larger proteins such as albumin are found in small quantities

in the urine (Field *et al*., 2001:80). Albumin excretion in the urine, greater than 30 mg/day, is abnormal (Greenberg, 2009:107).

Greenberg (2009:113) deducts that proteinuria greater than 1000 mg/24hours, together with other features such as haematuria, requires further investigation and a kidney biopsy might be required

2.5.2 Haematuria

Haematuria refers to the presence of blood in the urine (K/DOQI, 2002:114). Two types of haematuria exist, namely gross haematuria and microscopic haematuria (NKUDIC, 2012:1). Gross haematuria refers to blood which is visible in the urine with the naked eye and microscopic haematuria refers to blood in the urine which can only be detected under a microscope (NKUDIC, 2012:1).

Presence of haematuria should lead to further clinical examination and is a strong predicator of ESRD (Daugirdas, 2012:309).

2.5.3 Imaging Tests

An imaging test refers to a radiograph (X-ray) of the urinary tract (NKUDIC, 2012:3). Abnormal results on imaging tests are suggestive of kidney disease (K/DOQI, 2002:115). A radiograph of the kidneys can identify the manifestation of CKD (Greenberg, 2009:101). Imaging tests required specialised equipment and is time consuming and expensive as compared to a blood test.

2.5.4 Cystatin C

Due to limitations of using SCr to estimate the CrCl, scientists are trying to determine a more accurate method of determining GFR as an indicator of kidney function. Zerovnik and Jerala (2006:167) advocated that Cystatin C is a low molecular weight protein that acts as a cysteine proteinase inhibitor and can be used as an alternative marker to determine kidney function.

Cystatin C is synthesised at a constant rate and is released into the plasma. Cystatin C levels are not affected by age, gender, diet and muscle mass (Ronco *et al*., 2008:254). However, this statement has been challenged. In a cross sectional study conducted on 8058 patients, several factors influenced Cystatin C levels, namely; older age, male gender, greater height, weight, smoking status and elevated C - reactive protein (CRP) levels (Ronco *et al*., 2008:254).

Ronco *et al*. (2008:254) postulated that more than 97% of Cystatin C is filtered by the glomerulus and is almost completely metabolised by the proximal renal tubular cells, resulting in little or no Cystatin C been excreted into the ultra-filtrate.

Different prediction equations are used to determine the kidney function using Cystatin C, namely the Filler equation, Le Bricon equation, Hoek equation and Larsson equation. These four Cystatin C-based prediction equations were evaluated in adults, paediatrics, and both kidney transplant and non-transplant patients (White *et al*., 2005:3765-3767).

Ronco *et al*. (2008:254) further stated that Cystatin C may be more sensitive in detecting small changes in kidney function as compared to serum creatinine. A meta-analysis based on 46 studies concluded that Cystatin C is superior to SCr in determining kidney function (Chew *et al*., 2008:48). A study conducted on 117 kidney transplant patients demonstrated that the Cystatin C-based equations of Le Bricon and Filler were more accurate than conventional creatinine-based equations in determining the glomerular filtration rate (GFR) (White *et al*., 2005:3766).

The outcome of a study that compared Cystatin C-based prediction equations against MDRD study equation in determining GFR in adults showed that the Cystatin C-based prediction equation assessed GFR better than the MDRD equation (Grubb *et al*., 2005:420).

To date, the FDA has approved a number of devices namely; The ADVIA® Chemistry Cystatin C Method.; the A Dade Behring Inc. and the N Latex Cystatin C method to determine Cystatin C. (Siemens Healthcare Diagnostics, 2009:4).

Using Cystatin C as a biomarker to determine kidney function seems promising and requires further validation.

2.5.5 Human neutrophil gelatinase-associated lipocalin

According to Naicker (2011:118) Human neutrophil gelatinase-associated lipocalin (NGAL) can be detected in the blood and urine of patients and is one of the earlier markers in detecting kidney injury. NGAL is an effective predictor of early kidney injury. Urinary NGAL is detected earlier as compared to NAG and is a sensitive biomarker of post-operative AKI (Rosner & Okusa, 2008:100).

2.5.6 Kidney injury molecule-1

Kidney injury molecule-1 (KIM-1) is detected in the urine after damage to the proximal tubular cells (Naicker, 2011:118). KIM-1 may be an effective biomarker for detecting proximal tubular acute kidney injury (Ronco *et al.*, 2009:258).

2.5.7 N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme and a marker of tubular cell injury and is found in patients with type-1 diabetes, even in the absence of proteinuria (K/DOQI, 2002:118). Elevated levels of NAG in the urine can be indicative of acute and chronic kidney disease, diabetic nephropathy and hypertensive patients with kidney failure (Naicker, 2011:118).

2.5.8 Interleukin-18

Interleukin-18 (IL-18) is a pro-inflammatory cytokine and presence of IL-18 in the urine is an indicator of acute ischaemic proximal tubular damage (Naicker, 2011:118).

2.5.9 Liver-type fatty acid-binding protein

Liver-type fatty acid-binding protein (L-FABP) in the urine is an indicator of AKI and (Naicker, 2011:118). L-FABP is more effective in detecting AKI than SCr (Naicker, 2011:119).

2.5.10 β2-Microglobulin

Bishop *et al*. (2004:525) stated that β2-Microglobulin (β2-M) is a small endogenous peptide molecule that remains at a relatively stable concentration in the serum and is not influenced by muscle mass. Elevated levels of β2-M in the serum can indicate renal failure.

2.5.11 Inulin

Inulin is a small molecule freely filtered by the glomerulus and is not reabsorbed or secreted by the tubules (Greenberg, 2009:63). Inulin is in stable concentrations in the plasma and is not synthesised or metabolised by the kidneys. The clearance rate of inulin is equal to eGFR (K/DOQI, 2002:82).

Inulin clearance is considered as the gold standard in determining eGFR in both adults and children (K/DOQI, 2002:82) but it remains a tedious method requiring intravenous infusion and

timed urine collection. Therefore, it is clinically difficult to conduct eGFR measurements using inulin as a marker. It is not only complex but a costly method in determining eGFR (Greenberg, 2009:63).

2.5.12 ^{125}I-iothalamate

^{125}I-iothalamate is an exogenous radioactive marker which provides an accurate measure of eGFR (K/DOQI, 2002:84). Like inulin, ^{125}I-iothalamate is a time-consuming method requiring multiple blood draws. (Lewis *et al*., 2004:3175).

A study conducted by Lewis *et al*. (2004:3180) on 1094 African American patients that compared the ^{125}I-iothalamte eGFR and the SCr eGFR based outcomes showed that the serum creatinine and the ^{125}I-iothalamate eGFR based measurements had similar outcome results.

2.5.13 Advantages and limitations of using biomarkers

Bonventre *et al*. (2010:439) has indicated the advantages and limitations of utilising biomarkers as indicated in table 2.4

Table 2.4: Advantages and limitation of biomarkers

Advantages of biomarkers	Limitations of biomarkers
Biomarkers have the ability to detect early kidney injury.	Biomarkers used to detect one type of kidney toxicity may not be useful in another.
Biomarkers Identify the degree of toxicity and can be used to adjust dosages and drug therapy.	A biomarker that detects inflammation may not be ableto detect early proximal tubule toxicity in the absence of inflammation.
Biomarkers provide consistent results across multiple species.	A biomarker that detects kidney injury may not be ableto detect a functional defect.
Biomarkers Isolate the site of kidney injury.	Biomarkers used in animal models may not be useful in the same way in human subjects.
Biomarkers can detect drug-induced nephrotoxicity, and AKI, early and prevent its progression to CKD.	Biomarkers suggest different outcomes so the question arises as to whether it is wise to use a single biomarker or multiple biomarkers to detect kidney injury.

2.6 Determination of kidney function

Various methods exist to determine kidney function. The FDA (2010:6), The National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) (2015:1) and the American Society of Nephrology (ASN) endorse the CG and the Modification of diet in renal disease (MDRD) study equations to estimate kidney function in adults (Nyman *et al*., 2011:1131). Both these equations take the SCr concentration into account together with other variables namely; age, gender, race and body size (K/DOQI, 2002:14). Creatinine clearance and eGFR are used as surrogate terms when determining kidney function.

2.6.1 Acute renal failure vs. chronic kidney disease

Greenberg (2009:614) describes acute renal failure (ARF) as a rapid decrease in eGFR and can be seen by an increment in blood urea nitrogen, SCr concentration or a decrease in urine output. The degree of ARF can be categorised according to the RIFLE criteria:

- R – Risk refers to a 25% decrease in eGFR,
- I – Injury is a 50% decrease in eGFR,
- F – Failure refers to a 75% decrease in eGFR,
- L – Loss is a complete loss of kidney function for more than 4 weeks,
- E – End-stage renal disease (ESRD) is a complete loss of kidney function for more than three months (Greenberg, 2009:627).

CKD is defined as:

- "Kidney damage ≥ 3 months, as defined by structural abnormalities of the kidney, with or without decreased GFR manifest by either pathological abnormality or markers of kidney damage including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests." (K/DOQI, 2002:3).
- Kidney function ≤ 60 mL/min/1.73m^2 (adjusted to body surface area) for longer than 3 months with or without kidney damage (K/DOQI, 2002:3). The different staging of kidney disease as recommended by the K/DOQI guidelines is represented in table 2.5 and has been adjusted to BSA.

Table 2.5: Staging of kidney disease

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mild decreased GFR	60 - 89
3	Moderate decrease GFR	30 – 59
4	Severe decrease GFR	15 - 29
5	Kidney failure	< 15 or dialysis

ARF usually resolves and CKD is a progressive disease often leading to ESRD (Greenberg, 2009:627). ESRD is the requirement for dialysis or a kidney transplant and refers to permanent kidney damage (K/DOQI, 2002:35).

Up to 1 in 9 Americans have chronic kidney disease with more than 570 000 Americans having ESRD (American Society of Nephrology, 2012). Racial and ethnic differences influence the development and progression of kidney disease. African-Americans are 6 times more likely to develop kidney failure compared to whites (American Society of Nephrology, 2012).

Due to the absence of functioning registries in most sub-Saharan countries, it is difficult to obtain reliable statistics, however the incidence of CKD seems to be 3 - 4 times more frequent compared to developed countries (Naicker, 2009:1).

2.6.2 The Cockcroft-Gault equation

The CG equation was developed in 1976 with data from 249 males as observed from 24 hour urine collection (Cockcroft & Gault, 1976:31). The CG equation is used clinically and is accepted as an accurate method of determining CrCl. The CG equation is most commonly used to determine drug dosing in patients with kidney failure (Nyman, 2011:1130).

Cockcroft and Gault (1976:31) developed the CG equation using only white patients; therefore, race differences were not taken into account. The equation was multiplied by 0.85 for females in order to take the difference in muscle mass into account. The patient's age, weight and gender must be available in order to calculate the kidney function.

The CG equation is used clinically and is accepted as an accurate method of determining the kidney function (Nyman *et al*., 2011:1130)

The original CG equation is:

$$Scr = \frac{(140 - \text{age in years}) x\ weight\ in\ kilograms}{72 \text{ x Scr } (\frac{\text{mg}}{\text{dl}})}$$

(Cockcroft & Gault, 1976:31).

The South African Renal Society recommendation for early detection and management of CKD is to determine the kidney function from prediction equations and together with the MDRD equation endorses the use of the following modified CG equation (South African Renal Society, 2006).

$$CrCl = \frac{140 - \text{age (years)} x\ weight\ (kg)}{\text{serum creatinine } (\frac{\mu\text{mol}}{\text{L}})}$$

X 0.85 (if female)

Froissart *et al*. (2005:51) indicated that a few studies have tested the CG formula in large cohort groups. The renal function in 1703 African-Americans was evaluated in the African-American Study of Kidney disease (AASK study) (Lewis *et al*., 2004:3180). This study also supported the notion that Scr alone cannot be used to determine kidney function.

The CG equation is easier to calculate compared to MDRD equation, which requires power function.

The Jaffe method is used to determine the CrCl, and does not only measure creatinine but also other substances such as glucose, uric acid, fructose and ascorbic acid. This can cause an overestimation of creatinine (Kampman & Hansen, 1981:8). Discovered in 1886 (Delanghe & Speeckaert, 2011:83), Max Jaffe, a German scientist noticed that creatinine formed a red colour when reacted with picric acid in an alkaline environment (Delanghe & Speeckaert, 2011:84). The Jaffe method was further developed by Otto Folini in 1900. This technique to determine the CrCl remains popular due to its low cost (Delanghe & Speeckaert, 2011:83).

2.6.3 The Modification of Diet in renal disease study equation

The MDRD prediction equation was developed in 1999 using data as obtained from 1628 study participants (Levey *et al*., 1999:461). The equation which takes age, gender and race into account was compared to other eGFR prediction equations. The conclusion of this study showed that the MDRD study equation provided a more accurate estimation of eGFR as compared to other equations. The study also concluded that the CG equation overestimates the kidney function by 16% (Levey *et al*., 1999:461).

The MDRD equation is adjusted for body surface area (BSA). The original equation exists as:

$$\text{GFR} = 186 \text{ x } (\text{SCr})^{-1.154} \text{ x } (\text{age})^{-0.203} \text{ x } (0.742 \text{ if female}) \text{ x } (1.210 \text{ if African-American})$$

(National Kidney Foundation, 2004:5-6)

The equation was re-expressed as (Greenberg, 2009:1272):

$$GFR = 186 \text{ x } SCr\ (mg/dL)^{1.154} \text{ x } (age)^{-0.203} \text{ x } (0.742 \text{ if female}) \text{ x } (1.210 \text{ if black})$$

GFR = ml/min/1.73m^2

Age = years

The reasoning supporting the adjustment of eGFR to body surface area (BSA) was based on the notion that metabolic rate is proportional to BSA (Botev *et al*., 2011:939). BSA was introduced to adjust for different body sizes.

Not all clinicians support the adjustment of eGFR to BSA. Delanaye *et al.* (2009:3595) provided substantial argument against the adjustment of eGFR to BSA. This study concluded that there are several limitations to the adjustment of eGFR to BSA. Apart from the adjustment being based on poor physiological data, it shows that indexing may be misleading.

Greenberg (2009:67) affirms that the MDRD equation underestimates eGFR in patients with normal eGFR. This deduction was based on the fact that the MDRD equation was derived from a study population with known kidney dysfunction and decreased eGFR. This underestimation of eGFR could misclassify patients having CKD (Greenberg, 2009:67).

The MDRD study equation remains as the most thoroughly validated equation in a population with impaired kidney function and have been evaluated in African Americans, Europeans and Asians (Vasslotti *et al*., 2007:175). It has proven to be an excellent tool to assess kidney function but cannot be used in all patients. It is most accurate to determine kidney function in patients with average body size and muscle mass (National Kidney Foundation, 2004:7).

It is not recommended for use in:

- Children,
- Patients with unstable creatinine concentration,
- Patients with extremes in muscle mass (malnourished and obese) (U.S. Department of Health and Human Services, 2014).
- Acute renal failure, it is only useful in estimating eGFR in stable chronic kidney disease (U.S. Department of Health and Human Services, 2014).

The use of the MDRD equation in the above group of patients may result in errors in estimating the eGFR.

Poggio *et al.* (2004:463) has shown that the MDRD equation has a lower accuracy in a healthy population without kidney disease. It is proven to be accurate at eGFR < 60 ml/min/1.73m^2 and less accurate at eGFR > 60 ml/min/1.73m^2 (National Kidney Foundation, 2004:7).

The accuracy of both the CG and MDRD study equation seems to depend on the population being tested (Poggio *et al*., 2004:463).

2.6.4 The CKD Epidemiology Collaboration (CKD-EPI) equation

The CKD-Epidemiology Collaboration (CKD-EPI) equation was published in 2009 and took variables such as weight, diabetes and kidney transplant into consideration (Rule, 2010:951). The equation was developed using a total of 8 254 study participants, of whom 5 858 patients were high risk patients (Rule, 2010:951). The equation was externally validated using 3 896 patients, of whom 2 810 patients were high risk. In the validation data, the CKD-EPI performed better than the MDRD equation. The studies were limited by the lack of racial and ethnic minorities and the limited number of elderly (Rule, 2010:951).

Although it is as accurate as the MDRD study equation in the group with eGFR < 60 ml/min/1.73m^2 and more accurate in the group with eGFR > 60 ml/min/1.73m^2 (National Kidney Foundation,

2004:6), the CKD-EPI equation has not been recommended to replace the MDRD study equation and is still being validated (U.S Department of Health and Human Sciences, 2014).

The CKD-EPI equation is expressed as:

$eGFR = 141 \times \min(SCr/k, 1)^{\alpha} \times \max(SCr/k, 1)^{-1.209} \times 0.993^{age} \times 1.018(\text{if female}) \times 1.159(\text{if black})$
(U.S Department of Health and Human Sciences, 2014)

Where:

SCr = serum creatinine (mg/dL)

K = 0.7 for females and 0.9 for males

α = -0.329 for females and -0.411 for males

min = minimum of SCr/k or 1

max = maximum of SCr/k or 1

Both the MDRD study equation and the CKD-EPI equation include a constant for African-American race. This takes into account that African-Americans have a higher eGFR than Caucasians at the same level of SCr (National Kidney Foundation, 2004:6).

Although the CG, MDRD and CKD-EPI equations have their limitations and a certain degree of inaccuracy, the calculation of kidney function with the above mentioned equations remains paramount with the modified CG equation and the MDRD study equation been accepted as the most widely used (K/DOQI, 2002:14).

In this quantitative, observational, cohort study with a combination of retrospective and some prospective aspects, only the modified CG equation was incorporated to determine the CrCl (ml/min) of the study participants, to investigate the change in renal function of female and male HIV-infected patients on a TDF-based regimen at baseline (before TDF initiation) and after 12 months on TDF-based treatment.

2.6.5 Limitations of prediction equations

Limitations of prediction equations using SCr concentrations according to the K/DOQI (2002:98) are:

- The SCr levels need to be in a steady state in order to determine the kidney function,
- In cases such as ARF, kidney function will not be accurate due to the change in serum creatinine levels,
- During CKD stage 1 - 2, addItional markers of kidney damage are required to determine the decline in kidney function.

2.7 Drugs and the kidney

2.7.1 Renal drug excretion

The kidney is a key organ for eliminating drugs by means of glomerular filtration, tubular secretion or tubular reabsorption (Field *et al*., 2001:135). Doogue and Polasek (2011:69) indicated that a decrease in the kidney function may result in the accumulation of the drug, increasing the risk of nephrotoxicity. The dose of the drug needs to be adjusted according to the level of kidney function as calculated by prediction equations (Doogue & Palasek, 2011:69).

The excretion of a drug is related to the volume of distribution (Vd) and the half-life time (t½) of the drug. The Vd is calculated as follows:

$$Vd = \frac{\text{Dose of drug administered}}{\text{Plasma concentration}}$$

(Field *et al*., 2001:134)

The t½ can assist in determining drug accumulation. It usually takes 4 - 5 hours for a drug to reach steady-state. Drugs with a long t½ will take longer to reach steady-state. The t½ is inversely proportional to the clearance of the drug. The higher the Vd, the longer it takes for the drug to be eliminated (Field *et al*., 2001:134).

Field *et al*. (2001:134) advocates that during renal impairment, the t½ of the drug is prolonged as is visible in figure 2.1 (adapted from Field *et al*., 2001:134). This occurs because elimination of the drug is reduced; as a result, it takes longer to reach steady state. Estimating the kidney

function is necessary in adjusting the dose of drugs that are excreted by the kidney to prevent toxic accumulation of the drug (Doogue & Palasek, 2011:69).

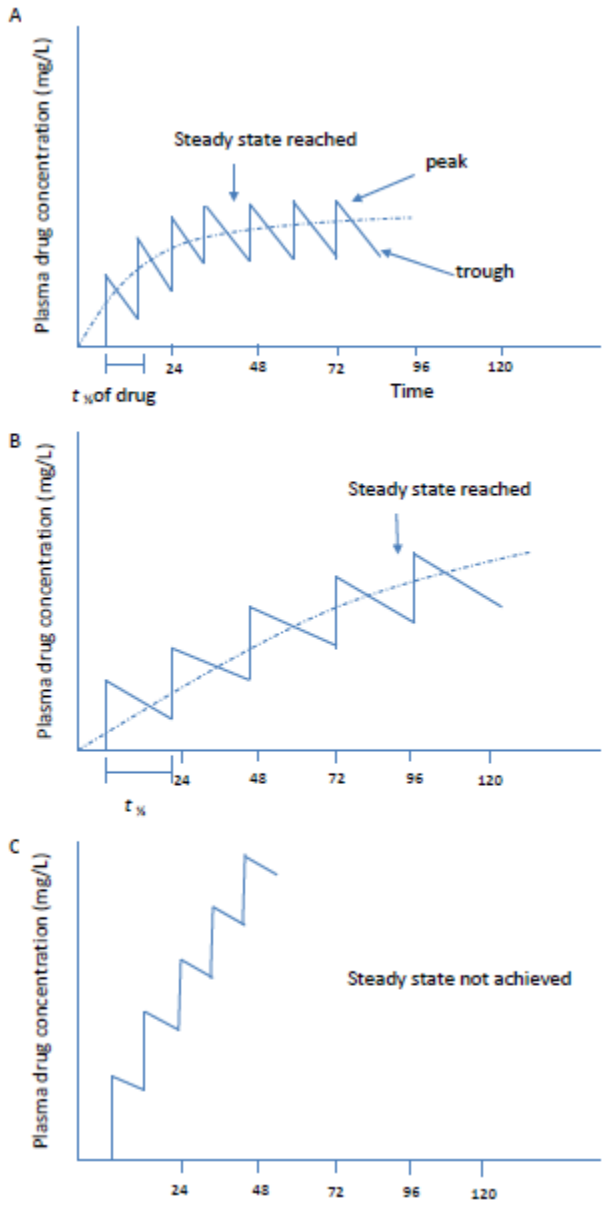

Figure 2.1 Plasma drug concentration after repeated oral administration of drug

Figure. 2.1 refers to the course of plasma drug concentrations after repeated oral administration of the same dose at constant intervals. (A) Drug administered at interval corresponding to its t½. (B) Same drug with lengthened t½ in renal failure caused by reduced renal excretion. Steady state is prolonged. (C) Same drug given in renal failure, resulting in rapid accumulation of the drug to toxic levels (Adapted from Field *et al*., 2001:134)

2.7.2 Dose adjustments in patients with renal impairment

In cases where dose adjustments are not conducted, administration of the usual drug dose may cause accumulation of the drug in the serum at excessively high levels (Doogue & Polasek, 2011:69).

A study conducted at Groote Schuur Hospital in Cape Town concluded that drug dose adjustments in patients with renal impairment were often overlooked (Decloedt *et al*., 2010:304). Failure to make necessary adjustments in renal impairment patients could lead to nephrotoxicity. It is estimated that 29 - 74% of drug prescriptions need dose adjustments in renal impaired patients (Decloedt *et al*., 2010:304).

Factors that influence dose adjustment of a drug include the following:

- If more than 50% of a drug and its metabolite are excreted through the kidney, a dose adjustment is required in renal impairment to prevent accumulation of the drug in the kidneys (Field *et al*., 2001:137).
- Drugs with a narrow therapeutic index can cause toxicity (Doogue & Polasek, 2011:70). An example of drugs with narrow therapeutic indices are; carbamazepine, warfarin, lithium carbonate, phenytoin, levothyroxine and theophylline (FDA, 2011:15).
- During renal impairment, anorganic acids accumulate and compete with drugs for binding onto albumin. Due to a decrease in serum albumin concentration, there is an increased availability of free drug (Field *et al.*, 2001:134). Highly protein bound drugs need dose adjustments (Field *et al*., 2001:137).

2.8 Tenofovir

Tenofovir has poor oral bioavailability and is therefore only available as the prodrug tenofovir disoproxil fumarate (TDF). A single dose contains 300mg TDF, equivalent to 245 mg tenofovir. TDF is hydrolysed to tenofovir and then phosphorylated to the active metabolite tenofovir

diphosphonate. Tenofovir exhibits a serum t½ ranging from 14 - 17 hours and is effective as a once-daily dose and is not significantly (< 8%) bound to plasma proteins. The percentage of unchanged tenofovir after intravenous administration is 70 - 80% (Flexner, 2011:1637). After administration of a single dose of 300 mg tenofovir to HIV-positive patients, in a fasted state, maximum serum concentration is achieved in 1.0 ± 0.4 hours. The Vd at steady-state is 1.3 ± 0.6 L/kg following oral administration of tenofovir (Viread, 2007).

2.8.1 Mechanism of action of tenofovir

Tenofovir disoproxil fumarate, a nucleoside transcriptase inhibitor undergoes diester phosphorylation to form tenofovir diphosphate (active metabolite). Tenofovir diphosphate targets HIV reverse transcriptase and acts as a chain terminator. Tenofovir is effective against HIV-1, HIV-2 and hepatitis B (Viread, 2007).

2.8.2 The rationale for including tenofovir in first-line treatment against HIV infection

The rationale for including tenofovir as the first choice in first-line treatment in HIV-positive patients is based on the following:

- Tolerability - It is well tolerated and does not cause anaemia like AZT,
- Adherence – It is taken as a single daily dose and is now available in a once-daily FDC which is associated with improved patient adherence,
- Hepatitis B – It is used as treatment for chronic hepatitis B and is treatment of choice for HIV/Hepatitis B co-infection,
- Resistance – It is more resistant to the development of mutations,
- Pregnancy – It can be administered during pregnancy. This ability has enabled the use of the FDC in pregnant women (Medecins Sans Frontieres, 2012:1).
- Tenofovir and children – TDF is recommended for use in adolescents ≥ 15years with and eGFR > 80 ml/min with no proteinuria (NDoH, 2015:68).

2.8.3 Efficacy of tenofovir

The initial efficacy of tenofovir was shown in study 901 (USA based) which provided the confirmation of the antiviral activity of tenofovir (FDA, 2001:18). This study showed a median decrease of 1.22 $\log_{10}$ copies/ml viral load in patients who received a single daily dose of 300 mg TDF for 28 days (FDA, 2001:18).

Studies 902 (USA based) and 907 (USA, Europe and South America based) demonstrated the efficacy of TDF as used in combination ART. Both studies were conducted in HIV-positive patients with detectable VL (>400 copies/ml) (FDA, 2001:17). Of the 186 patients who participated in study 902, 138 (74%) were Caucasian, 24 (13%) were black, 21 (11%) were Hispanic and 3 (2%) was other (FDA. 2001:24). The outcome of study 902 showed that TDF 300 mg once daily decreased the VL and improved immunological benefit when used in combination ART (FDA, 2001:30). Of the 552 patients that participated in study 907, 379 (69%) were Caucasian, 92 (17%) were black, 68 (12%) were Hispanic and 11 (2%) represent other (FDA, 2001:33). Majority of the patients in both study 902 and 907 were male Caucasian. Both studies showed that tenofovir has significant anti-HIV1 activity despite 94% of patients in each study showing evidence of nucleoside association resistance mutations.

As is evident from the above, none of the original efficacy studies were conducted in sub-Saharan Africa. Post marketing studies have highlighted the nephrotoxic effect of tenofovir (FDA, 2012a:19; Estrella *et al.,* 2014:1) in the sub-Saharan context.

The above mentioned studies (study 902 and 907) showed minimal effect on SCr values and SCr abnormalities were uncommon (FDA, 2001:74). The safety data from these studies concluded that there was no significant drug related toxicity associated with the use of TDF (FDA, 2001:79).

2.8.4 Tenofovir associated renal toxicity

2.8.4.1 Tenofovir mechanism of nephrotoxicity

Tenofovir mechanism of toxicity involves direct inhibition of mitochondrial function (Greenberg, 2009:653). Tenofovir is excreted unchanged through the kidneys by a combination of glomerular filtration and proximal tubular secretion. Tenofovir enters the proximal tubule cells by organic anion transporters (Fernandez-Fernandez *et al*., 2011:1). It is then transported into the proximal cells by two transporters, anorganic anion transporter 1 (hOAT1) and anion transporter 3 (OAT3). A decrease in renal function can result in an increase in tenofovir uptake by hOAT1. Tenofovir is secreted in the tubular lumen by membrane transporters multi-drug resistance-associated protein, MRP-4 and MRP-2. This increase concentration of intracellular tenofovir boosts tenofovir nephrotoxicity (Fernandez-Fernandez *et al*., 2011:2).

A second possible mechanism of TDF nephrotoxicity is related to the relationship between TDF and mitochondrial DNA (mtDNA) (Perazella, 2010:1060). TDF can cause mtDNA depletion and

mitochondrial toxicity. TDF inhibits DNA-polymerase-γ and this in turn causes a decrease in mtDNA. DNA-polymerase-γ is the only enzyme capable of reproducing mtDNA (Perazella, 2010:1060). This depletion in mtDNA causes a disturbance in mitochondrial function. This can ultimately lead to cell injury and cell death (Perazella, 2010:1060).

Drugs that interact with these transporters can also cause an increased accumulation of intracellular tenofovir, increasing the risk of renal toxicity (Fernandez-Fernandez *et al*., 2011:2). Examples of such drugs interfering with proximal tubular transporters is depicted in table 2.6.

Table 2.6: Drugs interfering with proximal tubular transporters

Transporter	Drug interaction	Effect
hOAT1	Probenecid inhibits hOAT1	Probenecid decreases the occurrence of renal toxicity by tenofovir,
	NSAIDs inhibit hOAT1	NSAIDs can potentiate tenofovir nephrotoxicity
	Acyclovir	Acyclovir increases serum concentrations of tenofovir
	ddI competes with tenofovir	Tenofovir increases ddI levels
MRP-4	Inhibition of MRP-4 by NSAIDs	NSAIDs associated with tenofovir nephrotoxicity
	Cidofovir, acyclovir, valaciclovir, ganciclovir, and valganciclovir inhibits MRP-4	Acyclovir increase serum concentrations of tenofovir
MRP-2	Ritonavir is transported by MRP-2	Ritonavir increases tenofovir concentration and has been associated with tenofovir nephrotoxicity

The clinical presentation of tenofovir nephrotoxicity is:

- Proximal tubular dysfunction with or without diminished renal function,
- A decrease in kidney function as compared to baseline values (Fernandez-Fernandez *et al.*, 2011:2)

2.8.5 Tenofovir safety profile

Tenofovir is structurally similar to antiviral nucleotides adefovir and cidofovir (Perazella, 2010:1060). Both these drugs are nephrotoxic. Data gathered indicates that the affinity of adefovir and cidofovir for hOAT1 may contribute to the accumulation of these drugs in renal proximal tubules (Cihlar *et al*., 1999:570). Cidofovir and adefovir are excreted by the kidneys and accumulation of the drugs in the proximal tubules may cause drug associated nephrotoxicity (Cihlar *et al*., 1999:571). Herlitz *et al*. (2010:1171) postulates that adefovir and cidofovir have been removed as therapy for HIV-1 infection due to the high incidence of renal toxicity.

Due to the structural similarity of tenofovir to adefovir and cidofovir, there was increased concern about the effect of tenofovir on renal function (Perazella, 2010:1060). The structural images of adefovir, tenofovir and cidofovir are depicted in figure 2.2 (adapted from Imaoka *et al*., 2006:620).

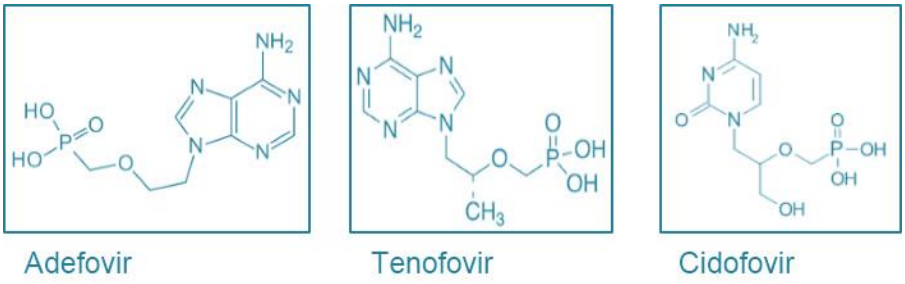

Figure 2.2 Images of adefovir, tenofovir and cidofovir.

The objective of a post marketing study conducted on 10 343 enrolled patients was to characterise the safety profile of tenofovir for the treatment of HIV infection in adults over the first four years of use (Nelson *et al*., 2007:1273). Nelson *et al*. (2007:1273) concluded that renal serious adverse effects (SAE) of any type were observed in 0.5% of patients and elevations in SCr occurred in 2.2% of patients ($p < 0.001$). The data from this study provided evidence to support a favourable

renal safety profile of tenofovir in HIV-positive patients and coincides with data issued to the FDA by Gilead prior to registration of TDF (FDA, 2001:79).

Information released by the FDA (2012:19) identified acute renal failure, renal failure, acute tubular necrosis, Fanconi syndrome, proximal renal tubulopathy, interstitial nephritis, nephrogenic diabetes insipidus, renal insufficiency, increased Cr, proteinuria and polyuria as adverse effects linked to tenofovir use. It is recommended that serum CrCl be calculated at baseline prior to commencing tenofovir. This information contradicts the documentation issued by Gilead that was used during the application for registration of TDF in 2001 in the US (FDA, 2012:3).

Further studies (Calza *et al*., 2011:657; Herlitz *et al*., 2010:1171; Jose *et al*., 2014:363; Scherzer *et al*., 2012:867 & Wever *et al*., 2010:80) have supported the need for renal monitoring in patients taking TDF-based ART and have been discussed here.

A study, Tenofovir-induced renal toxicity in 324 HIV-infected, antiretroviral-naïve patients showed that the tenofovir-exposed group had a significantly greater decline in eGFR and a higher incidence of proximal tubular dysfunction after 24 months of therapy ($p < 0.001$) as compared to the tenofovir-unexposed group. In this study a decrease in eGFR and tubular function was associated with older age, diabetes, hypertension and concomitant therapy with a protease inhibitor (PI) (Calza *et al*., 2011:657).

Herlitz *et al*. (2010:1171) investigated tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological and mitochondrial abnormalities by investigating renal biopsies of 13 patients (7 men and 6 women) who presented with TDF nephrotoxicity. The renal biopsy results revealed toxic acute tubular necrosis. Electron microscopy showed mitochondrial enlargement, mitochondrial depletion and dysmorphic changes. Of the 13 patients investigated, 9 presented with AKI and 4 had mild renal insufficiency. Mean baseline SCr increased from 1.3 ± 0.3 mg/dl to 5.7 ± 4.0 mg/dl at the time of biopsy. Glucosuria was documented in 7 patients. After discontinuation of TDF, renal function improved in all patients that were followed up. This study proved that TDF nephrotoxicity can not only be improved but also reversed once the causative agent is removed.

Jose *et al*. (2014:363) conducted a study to investigate the incomplete reversibility of eGFR decline, following TDF exposure, asserts that TDF use has been linked to an increased rate of decreased eGFR, proximal tubular dysfunction, proteinuria and CKD. The study conducted by Jose *et al.* (2014:366) depicted a decrease in eGFR in 61% of individuals who discontinued TDF

therapy during a median exposure time of 2.6 years. Of the 3088 patients who discontinued TDF therapy, 834 patients had sufficient follow-up Cr data to access recovery (Jose *et al*., 2014:367). Six hundred and one (72%) of the 834 patients showed a decrease in the eGFR whilst on TDF therapy, of which 232 individuals did not experience a recovery in the eGFR upon cessation of TDF (Jose *et al*., 2014:367). This large cohort study of mainly white HIV-positive men demonstrated an "accelerated decline" in eGFR during TDF therapy (Jose *et al*., 2014:368). Although participants experienced a recovery in eGFR within 3 months of TDF cessation, 38% of patients did not experience a recovery of eGFR to within 5% of the baseline eGFR (Jose *et al*., 2014:368). Jose *et al*. (2014:368) revealed that discontinuing TDF therapy at eGFR < 90 ml/min/1.73m^2 was linked to an increased risk of irreversible recovery. This study asserts that a decrease in eGFR during TDF therapy may not be fully reversible and promotes that TDF exposure at low eGFR should be avoided (Jose *et al*., 2014:367).

Scherzer *et al*. (2012:867) evaluated the effect of TDF exposure in 10 841 HIV-infected patients who commenced therapy between 1997 - 2007. This study analysed the association of tenofovir exposure with kidney disease risk and depicted the following results:

- The risk of proteinuria increased by 30% annually after each year of exposure to TDF ($p < 0.0001$),
- The risk of CKD increased by 33% per year of exposure to tenofovir ($p < 0.0001$),
- Patients on tenofovir had repeated measures of proteinuria as compared to non-users,
- Exposure of tenofovir is associated with a 10% increased risk of creatinine doubling ($p = 0.28$) and a 35% increased risk of developing combined CKD and proteinuria ($p = 0.0014$) (Scherzer *et al*., 2012:869-870).

The outcome of this study provided evidence to support the possibility that TDF may cause irreversible kidney toxicity (Scherzer *et al*., 2012:873).

A study conducted on 24 HIV-positive patients assessing the incomplete reversibility of tenofovir-related renal toxicity in HIV-infected, men found that upon cessation of TDF, only 10 (42%) patients achieved their baseline eGFR (Wever *et al*., 2010:78). At the end of follow-up analysis, the most improved eGFR value ($p = 0.028$) and the least improved eGFR value was less than baseline eGFR ($p = 0.0008$) (Wever *et al.,* 2010:79). This study supported Scherzer *et al*. (2012:873) that tenofovir related toxicity is not always reversible. Wever *et al*. (2010:80) postulated that tenofovir cessation is variable and incomplete. TDF discontinuation may be

warranted to avoid permanent renal dysfunction even if the decline in eGFR is gradual and above 60 ml/min/1.73m^2 (Wever *et al*., 2010:80).

New onset and worsening renal impairment caused by tenofovir has been added to the drug information leaflet as approved by the FDA (FDA, 2012:1). This list of precautions includes:

- Lactic acidosis and severe hepatomegaly with steatosis,
- Exacerbations of hepatitis has been reported by patients who discontinued TDF therapy,
- Decrease in bone mass density (BMD). Patients must be monitored for osteoporosis or bone loss,
- Change in body fat composition,
- Immune reconstitution syndrome (IRIS) (FDA, 2012:1).

The nephrotoxic effect of tenofovir is still a contentious subject as there are studies where the occurrence of tenofovir-linked nephrotoxicity was rare. These studies are summarised in the following table 2.7.

Table 2.7: A summary of studies were tenofovir nephrotoxicity was rare

Study	Study design	Results
Gallant *et al*. (2008:2155)	RC, n = 1111 patients. TDF based regimen (n = 556) vs. d4t or AZT -based regimen (Control: n = 555).	Through 144 weeks of the study, less than 1% of patients in both groups showed signs of renal impairment.
Gerard *et al*. (2007:31)	Prospective cohort, n = 53 patients on TDF + Atazanavir/ritonovir	TDF was not associated with any changes in CrCl (p = 0.79). Nephrotoxicity linked to TDF usage was rare. The study showed that the median SCr level increased from baseline to week 48 (p = 0.008) and the median CrCl decreased (p = 0.005).
Antoniou *et al*. (2005:284)	Cohort study, n = 172 patients exposed to TDF.	Significant nephrotoxicity was rare. Slight increase in SCr levels was detected (p = 0.0005)
O Donnell *et al. (*2011:1120)	A retrospective cohort study including 514 patients divided into a TDF exposed group and a TDF unexposed group.	Renal impairment occurred in 14% of patients and was not linked to TDF usage (p = 0.01) but to chronic comorbidity conditions.
Reid *et al*. (2008:1271)	Prospective study, n = 3316 patients (TDF-exposed *vs.* TDF- unexposed).	Patients showed an improvement in renal function after the 96-week study with mild to moderate decreases in function been observed. N = 52 (1.6%) depicted a severe decrease in renal function.
Nyirenda (2012:30-45)	Retrospective descriptive cohort, n = 192 ARV experienced patients (94% black) and n = 60 naïve patients (95% black).	Tenofovir was well tolerated. The CrCl was higher in the experienced group than the naïve group (p = 0.04).
Mugomeri (2013:12)	Phase 1, retrospective case control analysis, n = 312 ART naïve adult patients exposed to TDF, n = 173 unexposed patients. Phase 2 was re-sampling of the study population	Variables such as hypertension, older age, underweight and female gender is a greater contributor of renal dysfunction compared to TDF.

RC = random control

Another prodrug of tenofovir, tenofovir alafenamide (TAF) has a 90% less plasma concentration of tenofovir as compared to TDF and has proven to be effective at lower doses with less side effects and thus less effect on eGFR, bone density and tubular proteinuria. This study promoted the use of TAF based ART whilst maintaining the same level of efficacy as TDF with less adverse effects (Sax *et al*., 2015:2607). Two phase 3 studies; the GS-US-292-0104 and GS-US-292-0111 showed that TAF was effective in VL suppression and was not inferior when compared to the TDF based group (Sax *et al*., 2015:2613). Tenofovir alafenamide is a promising prodrug of tenofovir with higher intracellular drug contentration and less unwanted negative effects as seen with TDF (Sax *et al*., 2015:2613)

2.9 Renal impairment in the black population

Disease patterns vary across different countries and can also be influenced by differences in race or ethnicity. It is estimated that African-Americans are at a higher risk of developing CKD than Caucasians (Tarver-Carr *et al*., 2002:2363). Lower socioeconomic background, lack of healthcare, and higher rates of diabetes mellitus and hypertension amongst African-Americans, are possible explanations for the increased risk potential for developing CKD (Tarver-Carr *et al*., 2002:2363).

A summary of studies conducted in the black population in sub-Saharan Africa is depicted in table 2.8 below:

Table 2.8: Summary of studies conducted in sub-Saharan black population

Study	Study design	Results
Wools-Kaloustian *et al.(*2007:2208)	Cross sectional, n = 373 patients (67.9% female)	Renal impairment (CrCl < 60 ml/min) was observed in n = 43 patients. Lower haemoglobin (p = 0.001) and wasting syndrome *(*p = 0.03) was attributed to the CrCl < 60 ml/min.
Mulenga *et al*. (2008:1821)	Open cohort, n = 25 779 participants between 2004-2007.	This study concluded that renal impairment was present at ART initiation and increased the mortality rate in this sample group. N = 8456 had renal insufficiency (CI = 95%) and baseline SCr was elevated in n = 979 (CI = 95%) participants.
Reid *et al*. (2008:1271)	Observational analysis within a randomised trial, n = 3316 participants (65% female).	Mild to moderate renal impairment improved Once ART was commenced. Severe renal impairment was rare.
Matsha *et al.* (2013:1-10)	Cohort study including 1202 participants of mixed ancestry. Majority of participants were female (75.3%), 32 - 43% Khoisan, 20 – 36% Bantu-speaking African, 21–28% European and 9 – 11% Asian.	CKD stage 3 - 5 is the highest in Africa. There is a higher prevalence of CKD amongst females than males.

Serum creatinine concentration tends to be higher in black people than any other ethnic group (Hsu *et al*., 2008:992). According to Hsu *et al*. (2008:995), a possible explanation for black people generating higher SCr concentration as compared to whites could be attributable to black people having higher muscle mass which results in an increased creatinine production. The higher levels of SCr could result in a reduction in tubular secretion of creatinine.

To date, there is little knowledge regarding the development of renal impairment in the African population (Faney *et al*., 2009:1). With the increased use of TDF in South Africa, such information is vital in planning ARV programmes (Faney *et al*., 2009:1). Naicker (2003:119) states that renal disease is more prevalent in Africa and in a more severe form than Western countries, with CKD 3 - 4 times more frequent in Africa than western countries (Naicker, 2003:119).

Further evidence supporting the increased incidence of Africans developing CKD is the APOL 1 gene variant, present in people of African descent (Limou *et al*., 2014:1). This finding was supported by Feldman and Anderson (2013). According to Feldman and Anderson (2013), APOL 1 was associated with a 40% increased risk of CKD in African-Americans as compared to whites. The presence of the APOL 1 gene could also expedite kidney disease progression. Supporting this statement was the African American Study of Kidney Disease (AASK), a study conducted only on African Americans, which showed that kidney failure occurred in 58% of the participants in the APOL 1 risk group (Feldman & Anderson, 2013). The Chronic Renal Insufficiency Cohort (CRIC) study provided collaborating evidence that African Americans with CKD have a more rapid decline in kidney function and increased rate of developing ESRD (Feldman & Anderson, 2013). Limou *et al*. (2014:7) stated that the APOL 1 gene variants were found in most populations surveyed in sub-Saharan Africa.

Based on these findings, it is evident that renal impairment in black people is more frequent than other ethnicities and extra precaution needs to be taken to ensure that optimum kidney function is maintained and monitored.

2.10 HIV associated nephropathy

HIV-associated nephropathy (HIVAN) is a common risk factor for patients with HIV infection due to the direct effect of the HIV-1 virus that replicates itself in the renal cells (Herman & Klotman, 2003:). According to Greenberg *et al*. (2009:572), half of the acute renal function cases seen in patients on HAART were attributed to infection, of which one third of the cases were medication related.

Wyatt *et al*. (2008:2) characterises the clinical presentation of HIVAN as: “rapidly progressive renal failure, accompanied by moderate to nephrotic range proteinuria, bland urinary sediment and ultrasound findings of large, highly echogenic kidneys.” Diagnosis of HIVAN requires renal biopsy in the HIV-infected patient. HIVAN usually affects patients with a CD_4 count < 200 cells/µl and is a WHO clinical stage 4 defining condition (Regensberg *et al*., 2014:46). Analysing the blood SCr levels at baseline forms part of the initial assessment of HIV-positive patients before ART is commenced. Early detection of HIVAN is important to help prevent increased loss of renal function (Regensberg *et al*., 2014:46).

Kidney disease is on the rise in HIV-positive patients (Greenberg, 2009:574). HIVAN is more common amongst blacks and is shown to be rare among European patients who are on HAART

(Greenberg, 2009:574). HIVAN is the leading cause of ESRD amongst African-Americans (Greenberg, 2009:574). In the United States, 90% of all HIVAN cases occur in African-Americans and is the fourth most common cause of ESRD among 20 - 64 year old HIV-positive black patients (Greenberg, 2009:57; Muller *et al*., 2012:497).

In South Africa, HIVAN is a leading cause of ESRD (Muller *et al*., 2012:497). Muller *et al*. (2012:497) postulated that an estimated 10% of HIV infected South Africans will develop ESRD with an estimated 550 000 people needing dialysis or kidney transplant. Currently, 5000 people in South Africa are receiving dialysis at a cost of R150 000 per person annually in the public sector and R300 000 per person annuallyin the private sector (Anon, 2013). There were 2000 people in the public sector and 3500 people in the private sector requiring a kidney transplant (Anon, 2013). This increase in the number of patients requiring renal replacement therapy will increase the burden on the South African health sector.

A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria, conducted in Durban, demonstrated a prevalence of HIVAN in 83% of the patients who underwent a kidney biopsy (Han *et al*., 2006:2243). This study was conducted on 615 participants, 98% of whom were black adults and 76% comprised of females (Han *et al*. 2006:2244). It is therefore suggested that any patient with persistent proteinuria, hematuria and an eGFR < 60 ml/min/1.73m^2 should be assessed to prevent any further renal deterioration and minimise the risk of developing ESRD (Fabian *et al*., 2007:55).

The nephrotic syndrome in adult black South Africans: HIV-associated nephropathy as the main culprit study conducted on 294 black South Africans in Cape Town over a ten-year period concluded that HIVAN is a major cause of nephrotic syndrome in black South Africans (Okpechi *et al*., 2010:1193). It is also a contributing factor to the increasing incidence of ESRD. This study further showed that HIVAN was a leading cause of nephrotic syndrome in both males and females.

Treatment has helped slow the progression of HIVAN to ESRD and the treatment includes:

- HAART,
- Angiotensin-converting enzyme inhibitor, and
- Corticosteroids (Greenberg, 2009:574).

2.11 Important parameters influencing kidney function

2.11.1 Nutritional status

Patients with kidney function < 60 ml/min/1.73m^2, should be assessed for nutritional status, protein and energy intake (K/DOQI, 2002:145). Decreased protein intake and decreased energy intake could lead to compromised nutritional status and protein-energy malnutrition (PEM). PEM increased the risk of poor clinical outcomes and is related to increased morbidity and mortality (K/DOQI, 2002:146 -147).

The K/DOQI (2002:145) reports that although a decrease in protein intake decreased the accumulation of nitrogenous wastes and may be viewed as beneficial in patients with decreased kidney function, decreased protein intake worsens nutritional status and increases the risk of malnutrition. Decreased protein intake is a cause for malnutrition in CKD. Patients with PEM need to undergo dietary modification, counselling and nutrition therapy.

Markers of PEM include serum albumin concentrations less than 4.0 g/dL, loss of body fat, loss of somatic protein stores and poor performance status and function (K/DOQI, 2002:147). An estimated 50 - 70% of dialysis patients have PEM with PEM being recognised as one of the most significant predicators of adverse outcomes in dialysis patients (K/DOQ, 2002:148). The recommended daily allowance (RDA) of protein for normal adults is 0.75 g/kg/day; therefore a daily protein intake for patients with CKD stage 1 - 3 of 0.75 g/kg/day is reasonable. Age-dependent dietary energy intake of 30 - 35 kcal/kg/day for patients with eGFR < 25 mL/min/1.73m^2 is recommended (K/DOQI, 2002:151).

A cross sectional study conducted on 1785 clinically stable patients with moderate to advanced chronic kidney failure suggests that nutritional status decreased as the eGFR decreased. This decline in nutritional status may be attributed to a decrease in protein and energy intake (Kopple *et al*., 2000:1688). This study also supported the notion that PEM is a common complication of patients undergoing dialysis. Preventing PEM or improving nutritional status should improve clinical outcome. Kopple *et al*. (2000:1700) suggests that, “decreased protein and energy intake are probably the most common causes of PEM in non-dialyzed chronic renal failure and maintenance patients.”

As the eGFR decreases below 50 ml/min/1.73m^2, a decrease in total mass, fat and muscle occurs (K/DOQI, 2002:159). As per K/DOQI (2002:162) guidelines, the nutritional status in CKD patients

should be monitored every 1 - 3 months for patients with eGFR < 30 ml/min/1.73m^2 and every 6 - 12 months for patients with an eGFR of 30 - 59 ml/min/1.73m^2.

Serum bicarbonate concentrations have been linked to protein levels. Serum bicarbonate concentrations provide an indication of the acid-base balance (K/DOQI, 2002:147). Patients with eGFR < 60 ml/min/1.73m^2 experienced a decrease in serum bicarbonate levels. A decrease in serum bicarbonate levels is associated with protein degradation (K/DOQI, 2002:156).

South Africa is a diverse country consisting of many different ethnicities and has a mix of developing and developed areas. Majority of the black population live in rural areas or have migrated to urban areas (Medical Research Council, 2006:33). The rural black population maintains a traditional way of life and consumes a diet high in carbohydrates and low in fats (Medical Research Council, 2006:38). A study conducted in Dikgale in the Limpopo Province asserts that the rural adult blacks consume a diet high in cereal (maize, sorghum and bread), nuts and legumes. Their intake of protein from meat, dairy, fruits, vegetables and vegetable fats was much lower as compared to the black urban diet (Medical Research Council, 2006:38).

The black urban diet however, had a higher intake of protein, sugar and vegetable fat as compared to the rural black population (Medical Research Council, 2006:39) which potentiates the risk of developing diabetes and hypertension, both of which are risk factors for developing CKD (Medical Research Council, 2006:2).

Fanzo (2012:1) advocated that sub-Saharan Africa is home of probably the frailest individuals in the world. Majority of the dietary consumption still consists mainly of cereal or maize. Consumption of fruits, vegetables and proteins are still inadequate (Fanzo, 2012:1). Such an imbalance in diet can result in malnutrition (Fanzo, 2012:1). As indicated previously, PEM is a risk factor for developing CKD (K/DOQI, 2002:145).

As is evident from the above, the sub-Saharan Africans diet is paramount in the development of CKD. With kidney function affected by insufficient nutrition, drugs such as TDF can add extra pressure on kidney function and can lead to ESRD.

2.11.2 Body mass index

Body mass index (BMI) is an indicator of overweight, underweight and obesity in adults (WHO, 2015) and is calculated as follows:

$$BMI = weight\ (kg) / height^2\ (m)$$

The international classification of BMI is stated in table 2.9 below (WHO, 2015):

Table 2.9: The international classification of Body Mass Index

Classification	BMI (kg/m^2)
Underweight	< 18.50
Normal	18.50 - 24.99
Overweight	≥ 25.00
Obese	≥ 30.00

Various studies have supported the possibility of BMI having an influence on the development of CKD (Hsu *et al*., 2006:21; Gelber *et al*., 2005:871; Cohen *et al*., 2013:30). These studies advocated that a higher BMI resulted in a higher risk of developing CKD.

A cross sectional study of HIV-positive patients with normal renal function (≥ 60 ml/min) associated a lower BMI with an increased risk of developing tenofovir-related kidney dysfunction (Calcagno *et al*., 2013:1840). This study concluded that BMI affects the pharmacokinetics of tenofovir and lower BMI resulted in increased tenofovir plasma concentration (p = 0.025) (Calcagno *et al*., 2013:1840).

Our study will assess if BMI has a significant influence on the CrCl value and hence influence kidney function.

2.11.3 Bone disease

Bone disorders and disturbances in calcium and phosphorous metabolism have been linked to CKD (K/DOQI, 2002:163).

The intestine, bone and kidney are the three organs responsible for the regulation of calcium and phosphorous levels (Field *et al*., 2001:282).

A decrease in bone function leads to an increase in phosphorous retention (K/DOQI, 2002:164). This increase in serum phosphorous levels suppresses calcitriol production which ultimately causes a decrease in calcium absorption from the gastrointestinal tract resulting in hypokalemia.

Hypolalemia increases the production of parathyroid hormone (PTH), ultimately leading to bone disease (K/DOQI, 2002:164).

Low calcium and calcitriol levels and elevated PTH and phosphorous levels are abnormalities seen with decreased eGFR (K/DOQI, 2002:165). It is recommended that all patients with an eGFR < 60ml/min/1.73m^2 be evaluated for bone disease and disorders of calcium and phosphorous metabolism (K/DOQI, 2002:163).

A study conducted on 176 patients between the ages of 18 - 81 years with CrCl of 15 - 50 ml/min showed that 132 patients had histological evidence of bone disease (Hamdy *et al*., 1995:358). Ninety-eight patients had osteitis fibrosa and 25 had osteomalacia in combination with osteitis fibrosa (Hamdy *et al*., 1995:360). Hamdy *et al*. (1995:363) concluded that renal disease is common in patients with a CrCl of 15 - 50 ml/min.

Hamdy *et al.* (1995:358) stated that disease of the bone may be detected in patients with a CrCl < 60 ml/min. Renal bone disorders are associated with patients with ESRD and patients with a long history of renal impairment.

As is evident from the above, a decrease in kidney function affects not only the kidney and kidney function but the physiology as a whole. Monitoring of the CrCl is an important facet in preventing the development of bone disorders.

Grigsby *et al*. (2010:41) stipulated that a definite correlation exists between tenofovir use and a loss in bone density in HIV-positive patients. Tenofovir has been associated with a loss in bone density based on three possible mechanisms: increased bone resorption, decreased bone formation or a combination of the two (Grigsby *et al*., 2010:44). Grigsby further stated that a loss in bone density due to tenofovir use could lead to renal insufficiency, resulting in Fanconi syndrome (Grigsby *et al.,* 2010:45). According to Grigsby *et al*. (2010:45), increase output of bicarbonate in the urine due to decreased urine reabsorption by the proximal tubular cells caused phosphate wasting, resulting in bone demineralisation.

A decrease in bone mineral density has been stipulated in the TDF package insert as a precaution and patients who are prone to fractures or osteopenia should be monitored if placed on tenofovir-based therapy (FDA, 2012a:1).

Currently, the FDA has approved the use of tenofovir-based therapy in children older than 2 years (WHO, 2012:1). A concern about this recommendation is that HIV-positive children have lower

than normal bone density and an increased risk of tenofovir-related decrease in bone mineral density exists (WHO, 2012:1).

2.11.4 Neuropathy

Merriam-Webster defines neuropathy as an irregular degenerative condition or malfunction of the sensory system or nerves (Medical dictionary, 2016). Neuropathy is common in patients with kidney failure and is not related to the type of kidney disease but to the level of kidney function (K/DOQI, 2002:180). Neuropathy is detected in up to 65% of patients initiating dialysis (K/DOQI, 2002:181).

Symptoms of peripheral neuropathy present at a kidney function between 12 - 20 ml/min or uremia is detected for at least 6 months. Encephalopathy is seen with an acute decline in GFR. Autonomic neuropathy is present in 66% of patients with severely impaired kidney function or CrCl < 8ml/min. Autonomic neuropathy is also detected in 50% of dialysis patients (K/DOQI, 2002:181).

Symptoms of neuropathy include fatigue; loss of memory and concentration; sleep disturbances; pruritus; muscle cramps; and changes in heart rate and blood pressure (K/DOQI, 2002:180)

Neuropathy is associated with an increase in mortality and a decrease in quality of life (K/DOQI, 2002:182). Symptoms of neuropathy seem to occur at low levels of GFR and this could indicate kidney failure and can be used to determine the need for dialysis (K/DOQI, 2002:185).

2.11.5 Quality of life

The K/DOQI (2002:186) declares that a decrease in the level of kidney function can have a direct influence on a patient's well-being and quality of life. Malnutrition, neuropathy and bone disease have been linked to CKD and negatively affect a patients well-being. It is important to identify stages of CKD early to effectively and efficiently manage this condition and help prevent deterioration of a patient's well-being and quality of life.

Rocco *et al*. (1997:888) provided evidence in a cross-sectional study assessing quality of life and symptoms in chronic renal disease patients using the modification of diet in renal disease equation on 1284 patients with moderate to advanced renal insufficiency. Symptoms associated with decreased kidney function included tiring easily, weakness, lack of energy, difficulty sleeping and

abdominal bloating. The frequency and severity of these symptoms was correlated to the level of eGFR.

Reduced kidney function is also associated with higher levels of depression and anxiety and decreased social activity, social functioning and social interaction (K/DOQI, 2002:189).

It is imperative that patients with decreased kidney function and ESRD be assessed for functioning and well-being to maintain or improves health status and ensures that quality of life is improved or maintained (K/DOQI, 2002:194).

2.11.6 Age

Porth (2007:1685) declares that kidney function changes with age. The kidney matures with age and by the age of fourteen, normal adult eGFR values are achieved. With age comes a number of structural and functional changes to the kidney and this influences the eGFR (Porth, 2007:1685). After 20 to 30 years of age, the mean value for GFR decreased by 1 ml/min/1.73m^2 (K/DOQI, 2002:55).

Carter *et al*. (2011:842), documented the median creatinine (mmol/l) values for the different age groups based on 174 448 individuals, presented in table 2.10. These results were based on the first available SCr results in a large cohort study in the United Kingdom (Carter *et al.,* 2011:842).

The NHLS reference range for SCr values as depicted in the SOP is displayed in table 2.11 (NHLS, 2015:5 - 6).

Table 2.10 Median serum creatinine by age group in the United Kingdom

Age (years)	Number of patients	Median SCr (mmol/l)
18 - 29	11 415	70 (62 to 80)
30 - 39	12 339	72 (63 to 82)
40 - 49	22 269	74 (65 to 85)
50 - 59	28 525	76 (66 to 87)
60 - 69	40 424	79 (68 to 91)
70 - 79	33 938	84 (71 to 99)
80 - 89	21 775	89 (74 to 110)
90 - 107	3 763	94 (76 to 119)

Table 2.11: Serum creatinine reference range in South Africa

Analyte	Age	Gender	Reference Range (µmol/l)
Serum creatinine	0 day		27 - 81
	7 days		10 - 56
	60 days		14 - 31
	12 months		15 - 31
	3 years		23 - 37
	5 years		25 - 42
	7 years		30 - 48
	9 years		28 - 57
	11 years		37 - 63
	13 years		40 - 72
	15 years	Male	36 - 96
		Female	39 - 85
	>18years	Male	64 - 104
		Female	49 - 90

The rate of decline of eGFR accelerates after 65 years of age. Renal blood flow, especially blood flow diminishes with age (Porth, 2007:1685). Urinary albumin excretion increases with age (Porth, 2007:1685).

With increased age comes an increased risk of developing atherosclerosis, hypertension and heart failure. All of which cause a decline in the eGFR (Porth, 2007:1686). Loss of kidney function with increased age seems to be inevitable (Porth, 2007:1686).

The South African consolidated HIV guidelines has stipulated that TDF-based therapy be indicated for adolescents from the age of 15 years, meeting certain requirements (NDOH, 2015:74).

The WHO has advocated that TDF-based therapy is suitable for children from the age of 2 years based on FDA approval of the drug (WHO, 2012:1). The recommended paediatric dose is 8 mg/kg daily, to a maximum of 300 mg daily, based on a favourable pharmacokinetic profile (WHO, 2012:1). The aim is to make the transition from paediatric to adult treatment simpler and to possibly create a fixed-dose combination ARV for paediatrics (WHO, 2012:1).

2.11.7 Gender

The current South African consolidated guidelines have a uniform standard of treatment for males and females (with the exception of pregnant women) (NDOH, 2015:74).

A pilot pharmacokinetic study of HIV-infected patients (n = 7 women and n = 20 men) receiving TDF, revealed that a significant difference in tenofovir concentration was observed at intracellular level between the genders (p = 0.045), indicating that men and women phosphorylate tenofovir at different rates (Pruvost *et al*., 2009:1940). The small sample size in this study was a limitation and further studies need to be conducted to assess if gender has an influence on TDF pharmacokinetics.

2.12 Chapter summary

The SCr cannot be used alone, to determine the level of kidney function. Calculating the GFR is the gold standard in determining kidney function (K/DOQI, 2002:55). The CG equation is endorsed as clinically accepted method of determining the kidney function (Nyman *et al*., 2011:1130).

With an increase in the number of people receiving ART in sub-Saharan Africa (UNAIDS, 2013b:3), there is an increased availability of potentially nephrotoxic TDF. It is imperative that the guidelines as set out by the NDoH are complied with, that is to determine the CrCl at baseline, 3 months, 6 months, 12 months and thereafter annually (NDoH, 2015:75).

There is increasing evidence linking tenofovir use to nephrotoxicity. Studies conducted on sub-Saharan blacks pertaining to the nephrotoxic effect of tenofovir are limited and needs further investigation. The effect of gender on TDF pharmacokinetics also needs further investigation.

This chapter meets all the literature objectives as set about in chapter 1 of this dissertation.

CHAPTER 3: MANUSCRIPT

3.1 Introduction

This chapter contains one full-length manuscript for submission to a peer-review journal (Health SA Gesondheid) and is presented in the manuscript style set out according to the guidelines (http://www.elsevier.com/journals/health-sa-gesondheid/1025-9848?generatepdf=true) as stipulated by Health SA Gesondheid (annexure 11).

A brief description pertaining to the introduction, background and methodology is provided. Data was analysed using Statistica® to determine the independent and dependent *t*-tests for males and females in the different age groups (≥20 - <30 and ≥30 - ≤40 years). An explanation of the results, conclusion, recommendation and limitations are provided.

The effect of tenofovir on creatine clearance after 12 months of treatment in a HIV-infected adult cohort in KZN

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ABSTRACT

Background: The renal safety profile of tenofovir disoproxil fumarate (TDF) remains a contentious issue in the African context. This study investigated the renal function outcome of healthy HIV-positive patients exposed to tenofovir-based antiretroviral therapy.

Method: The study was a retrospective, partial prospective observational cohort analysis of serum creatinine (SCr) data of 66 black patients in Newcastle, KwaZulu-Natal. The study population was stratified according to age and gender. The renal function was evaluated by calculating the creatinine clearance (CrCl) by means of the Cockcroft-Gault (CG) equation at baseline (before TDF commencement) and at 12 months post-TDF commencement. The viral load (VL), CD_4 count and body mass index (BMI) was assessed to determine the virological and immunological outcome. The independent *t*-test and the dependent *t*-test were incorporated for the parametric values. The Mann-Whitney test and the Wilcoxon signed rank test were used to determine the non-parametric values.

Results: The mean BMI and CD_4 count improved in all age and gender groups at 12-month follow-up data. The ≥20 - <30year age group showed an improvement in CrCl at 12-month follow-up data ($p = 0.15$ (female) and $p = 0.020$ (male). The ≥30 - ≤40 year age group depicted a minor decline in CrCl at 12-month follow-up ($p = 0.176$ (female) and $p = 0.941$ (male).

Conclusion: No change in kidney function was observed after 12 months of TDF-based treatment when CrCl was calculated using the modified CG equation.

Key words: tenofovir, serum creatinine, creatinine clearance, CD_4 count, BMI.

1 Introduction

HIV/AIDS has been at the helm of clinical research in the 21st century with an estimated 25.8 million people living with HIV as of 2014 in sub-Saharan Africa (UNAIDS, 2014).

In South Africa, the antiretroviral therapy (ART) rollout was initiated in the public sector in 2004 with stavudine (d4t) being the primary drug in first-line therapy, together with lamivudine and efavirenz (NDoH, 2004). These guidelines were revised in 2010, replacing d4t with tenofovir disoproxil fumarate (TDF) as the primary drug in ART (NDoH, 2010). The rationale behind this implementation was to avoid or limit toxicity cause by d4t (Adrieux-Meyer *et al*., 2012). Efficacy data provided to the Food and Drug Administration (FDA) by Gilead Sciences in 2001 provided substantial evidence to support the renal safety characteristic of TDF (FDA, 2001). However, recent studies have reported that TDF can cause acute renal failure, Fanconi syndrome and irreversible renal damage (Herlitz *et al*., 2010; Jose *et al*., 2014).

1.1 Background

Tenofovir mechanism of toxicity involves direct inhibition of mitochondrial function (Greenberg, 2009). Tenofovir is mainly excreted unchanged through the kidneys by a combination of glomerular filtration and active proximal tubular secretion. Tenofovir enters the proximal tubule cells via organic anion transporters 1 and 3 (OAT1 and OAT3). A decrease in renal function can result in an increase in tenofovir uptake by OAT1. Tenofovir is secreted in the tubular lumen by multidrug resistance-associated protein 4 (MRP-4) and MRP-2. This increase in intracellular tenofovir concentration boosts tenofovir nephrotoxicity (Fernandez-Fernandez *et al*., 2011). Perazella and co-workers (2010) postulated that tenofovir nephrotoxicity is linked to the interaction between tenofovir and mitochondrial DNA (mtDNA), resulting in DNA depletion, mitochondrial toxicity and disturbance of mitochondrial function. This can ultimately lead to cell injury and cell death (Perazella, 2010).

In order to assess renal function, renal markers can be used to determine the degree of toxicity. These markers include calculating the estimated glomerular filtration rate (eGFR),

creatinine clearance (CrCl) or Cystatin C utilising the serum creatinine (SCr) level (Naicker, 2011).

Calza *et al.* (2011) assessed the tenofovir-induced renal toxicity in 324 HIV-infected, antiretroviral (ARV)-naïve patients and showed that the tenofovir-exposed group had a significantly greater decline in eGFR and a higher incidence of proximal tubular dysfunction after 24 months of therapy ($p < 0.001$) as compared to the tenofovir-unexposed group. In this study a decrease in eGFR and tubular function was associated with older age, diabetes, hypertension and concomitant therapy with a protease inhibitor (PI).

Scherzer *et al*. (2012) evaluated the effect of TDF exposure in 10 841 HIV-infected patients who commenced therapy between 1997-2007. This study investigated the association of tenofovir exposure with kidney disease risk and depicted the following results:

- The risk of proteinuria increased by 30% annually after each year of exposure to TDF ($p < 0.0001$),
- The risk of CKD increased by 33% per year of exposure to tenofovir ($p < 0.0001$),
- Patients on tenofovir had repeated measures of proteinuria as compared to non-users,
- Exposure of tenofovir is associated with a 10% increased risk of creatinine doubling ($p = 0.28$) and a 35% increased risk of developing combined CKD and proteinuria ($p = .0014$) (Scherzer *et al*., 2012).

The outcome of this study provided evidence to support the possibility that TDF may cause irreversible kidney toxicity (Scherzer *et al*., 2012). A subsequent study in 153 patients on TDF-based treatment also observed that 27% of their patients had proteinuria after 12 months of treatment (Kelly *et al* 2013).

The nephrotoxic effect of tenofovir is still a contentious subject as there is evidence where the occurrence of tenofovir-linked nephrotoxicity was rare. As was evident in a retrospective cohort study comprising of 192 ARV-experienced patients (94% black

patients), at Themba Lethu Clinic in Johannesburg, conducted over a median duration of 15 months (Nyirenda, 2012). They concluded that TDF was well tolerated and nephrotoxicity was rare (Nyirenda, 2012). Mugomeri *et al.* (2014) reported that TDF is a weak contributing factor to renal impairment in Lesotho where 312 TDF-exposed patients and 173 non-TDF-exposed patients over 24 months were retrospectively investigated (Mugomeri *et al*., 2014).

These disparities pertaining to TDF-based therapy can cause uncertainty regarding its safety profile amongst the clinicians, health care professionals and patients.

1.2 Purpose of the study

This study demonstrated the effect of TDF on kidney function in the black population and provided further insight on the effect of TDF on different parameters in black patients over a 12-month period post-TDF initiation within ≥20 - ≤40 year old group. This study rendered a clinical review of black patients commencing TDF-based ART therapy at a PHC clinic in Newcastle, KwaZulu-Natal. The kidney function was determined by utilising the SCr to calculate the CrCl (ml/min) by means of the modified Cockcroft Gault (CG) equation. Variances between the male and female gender was evaluated and documented.

1.3 Problem statement

Disease patterns vary across different countries and can also be influenced by differences in race or ethnicity. The SCr concentration tends to be higher in black people than any other ethnic group (Hsu *et al.,* 2008). According to Hsu *et al*. (2008), a possible explanation for black people generating higher SCr concentration as compared to white people could be attributable to black people having higher muscle mass which results in an increased creatinine production. The higher levels of SCr could indicate a decrease in tubular secretion of creatinine.

To date, there is little knowledge regarding the development of renal impairment in the Black population (Faney *et al*., 2009). With the increased use of TDF in South Africa, such information is vital in planning ARV programmes (Faney *et al*., 2009). Naicker (2003) states that renal disease is more prevalent in Africa and is in a more severe form than

western countries, with chronic kidney disease (CKD) 3 - 4 times more frequent in Africa than western countries (Naicker, 2003).

With an estimated 10% of people worldwide suffering from CKD (Renal Care Society of South Africa, 2011) and a growth in the global dialysis market of 7.3% from 2011-2015 (Reportlinker, 2012), early detection and treatment of kidney disease can decrease mortality and improve quality of life.

Black people have a different body composition as compared to white people (K/DOQI, 2002), therefore studies conducted on other ethnicities will not show true for black people. As such, it is imperative that we conduct studies on the South African black population to ensure that we not only have a better understanding of the disease but also of the efficacy and toxicity of the ARV drugs.

The South African 2015 national consolidated guidelines for the prevention of mother-to-child transmission of HIV and the management of HIV in children, adolescents and adults changed the eligibility criteria for patients commencing HAART (NDoH, 2015). Patients with a CD_4 count < 500 cells/µl, adolescents older than 15 years with a weight greater than 40kg, pregnant women and adults with a CrCl ≥ 50 mL/min were eligible for the commencement of ART (NDoH, 2015). This revision allowed for more HIV-positive people to receive HAART (NDoH, 2015). With the increased use of tenofovir, such information is necessary in determining which ARV drug is best suited for a patient.

2 Research method and design

The study was a quantitative, observational, cohort study design with a combination of retrospective and partial prospective aspects. The study site was a primary health care clinic in Newcastle KwaZulu-Natal. The patient files housed at this clinic were assessed between September 2015 - October 2015 during normal clinic working hours (open 24 hours a day, 7 days a week) to gather the following data; previous ART history, date of diagnosis, date of commencement of TDF-based therapy and clinical data (age, weight, height and SCr). The routine blood samples were collected according to the standard

protocol of the clinic and the analysis of the SCr was conducted by the National Health Laboratory Service (NHLS) according to their standard operating procedures.

2.1 Target population

The target population were all adult (male and female) black patients (to exclude any possible genetic variation in renal function) between the ages of ≥20 - ≤40 years on TDF-based ART who attended the PHC clinic in Newcastle for a consecutive 12-month period. Selecting a younger age group reduced the risk of age-related degeneration of kidney function.

All patients had a baseline SCr test conducted before commencement of TDF-based treatment. All study participants could have been ART-experienced or naïve (not previously on any HAART). Pregnancy, diabetes, hypertension and other cardiovascular disease that affect kidney function were included as exclusion criteria. Patients who had a baseline CrCl ≤ 60ml/min were excluded due to possible existing renal insufficiency.

2.2 Measuring instrument

The primary investigator gathered clinical data from patient health files onto a paper copy which was transferred to an electronic data collection tool (Microsoft Excel® spreadsheet). The equipment used by NHLS to conduct the SCr tests was the UniCel® DxC 800 System. The creatinine concentration was measured by means of the Jaffe rate method (NHLS, 2012). The SCr values were incorporated into the modified CG equation to determine the creatinine clearance (CrCl) (South African Renal Society, 2006; NDoH, 2015)

$$\text{Creatinine clearance} = \frac{[140 - \text{age (years)}] \times \text{weight (kg)}}{\text{SCr } (\mu\text{mol/L})} \quad (* \times 0.85 \text{ if female})$$

2.3 Data and statistical analysis

Descriptive statistics as well as inferential statistics were used to present the results. Data were entered into Microsoft Excel® and were analysed using Statistica® analysing programme. The dependent variable includes the SCr, CrCl, CD_4 count and BMI (continuous variables) and the Independent variables include the age and gender (categorical variable).

The independent *t*-test was performed to investigate differences in SCr, CrCl, CD_4 count and BMI by comparing female *vs*. male participants at baseline and at 12 months post-TDF. The Mann-Whitney test was utilised as the non-parametric equivalent of the independent *t*-test.

The dependent *t*-test was used to investigate the changes on SCr, CrCl, CD_4 count and BMI from baseline to 12 months (female *vs.* male and within the 2 different age categories). The Wilcoxon signed-rank test was utilised as the non-parametric equivalent of the dependent *t*-test.

The sample size consisted of 72 study participants allowing for a 20% (n = 12) fall out rate due to withdrawals or non-completers. Based on this deduction, the total number of study participants recruited was a minimum of 60. The justification of the sample size and effect size was calculated with *t*-Test Power calculation (Statistica®). An effect size of 0.375 was regarded as suitable for this male and female population as it provided a power greater than 0.8 with the sample size of 60 study participants. The power (0.8) of the study was based on data (difference between the CrCl at baseline and 12 months) from 17 random results (male and female, aged between ≥20 and ≤40 years) that was available in 2012 from the Newcastle PHC clinic.

3 Ethical consideration

Ethics approval was obtained from the HREC at the NWU (NWU-00044-15-A1) and the Department of Health, KwaZulu-Natal Ethics committee (7/9/2015). This study was of an observational nature as the treatment regimens and blood tests performed on the patients form part of the standard treatment care guidelines as set out by the National Department of Health (NDoH, 2015) and the SA GCP guidelines (NDoH, 2006).

Informed consent was obtained by the trained and registered nurses at the clinic although majority of the study results (data) were retrospectively gathered from the clinic files by the primary investigator. The informed consent forms were available in English and isiZulu. Patients had the right to refuse participation in the study at any time. To ensure

confidentiality and to respect the patients' rights to privacy study numbers were allocated when data was captured on the research tool form.

3.1 Data protection

The hard copies (patient health files) remained in the clinic and only the anonymised data were recorded on handwritten spreadsheets (research tool) which was in the possession of the researcher and kept locked away at all times. Study numbers had been allocated to these individual spreadsheets to ensure confidentiality and anonymity. All data collected (hard copies and electronic) will be locked and stored for a period of five years as per the NWU guidelines.

3.2 Validity and reliability

The validity of the study was established through the utilisation of an appropriate study design. The primary investigator and all supervisors were qualified to analyse and evaluate data obtained. The internal validity was determined from the justification of sample size. Equipment at the NHLS is calibrated every 72 hours or with each new bottle of reagent as per the systems chemistry information sheet. The results in the patient files were reliable as the original blood result printout was available. All other information required to calculate the CrCl was obtained from the patient files.

4 Results

Sixty-six patients attending the PHC clinic in Newcastle met the inclusion criteria and were included in the study. The age of the study population ranged from ≥20 - ≤40 years at baseline. This study population was further sub-divided into the ≥20 - <30 year age group and the ≥30 - ≤40-year age group. The number of females who participated in the study was 53% compared to 47% of males. Table 1 provides the demographic characteristics of the study participants prior to the TDF-based treatment.

Table 1. Demographic characteristic of study participants at baseline (prior to TDF initiation)

	≥20 - <30 year age group		≥30 - ≤40 year age group	
Gender	**Female N = 18**	**Male N = 15**	**Female N = 17**	**Male n = 16**
Age (years) Mean (SD)	26.39 (1.61)	26.47 (2.39)	34.71 (2.47)	33.94 (2.86)
Weight (kg) Mean (SD)	66.98 (23.12)	60.96 (11.78)	73.22 (15.29)	62.91 (9.23)
Length (m) Mean (SD)	(n = 13) 1.61 (0.04)	(n = 9) 1.70 (0.08)	(n = 11) 1.61 (0.05)	(n = 14) 1.73 (0.07)
BMI (kg/m^2) Mean (SD)	(n = 13) 23.94 (6.69)	(n = 9) 21.14 (2.39)	(n = 11) 28.38 (6.66)	(n = 14) 21.54 (3.81)
Months since HIV was diagnosed to baseline Mean (SD)	(n=18) 58 (33.68)	(n=14) 32.14 (19.46)	(n=16) 78.76 (29.58)	(n=14) 50.07 (33.63)
Duration (months) on current TDF based therapy (FDC) Mean (SD)	(n=17) 19.53 (4.43)	(n=14) 19.64 (4.2)	(n=16) 22.38 (3.26)	(n=16) 21.63 (4.75)
CD_4 count ($cells/mm^3$) Mean (SD)	(n = 17) 196.12 (161.10)	(n = 13) 213.62 (167.83)	(n = 12) (254.42 (194.65)	(n = 15) 261.93 (168.51)

n-values will differ as not all information was available to record and are thus missing data
FDC = fixed dose combination

Table 2 presents results on BMI, CD_4 count, SCr and CrCl within the different age groups. The CrCl was calculated using the modified CG equation. Data were extrapolated using the dependent *t*-test and portrays the clinical measurements at baseline and 12-month follow-up stratified according to age and gender.

Table 2: Clinical measurements at baseline and 12-month follow-up stratified according to age and gender

Age group	Gender	Clinical measurement	Baseline		12-month follow-up		Dependent *t*-test		Wilcoxon test	
			Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	*p*-value	Effect size	*p*-value	Effect size
Group 1 ≥20-<30	Female (n = 18)	BMI (kg/m^2) n = 13$^{\#}$	23.94 (6.69)	21.05 (19.82;27.23)	26.37 (6.51)	23.53 (21.98;28.36)	**0.004***	0.36	**0.006**	**0.54**
		CD_4 count (cells/mm^3) n = 17$^{\#}$	196.12 (161.1)	151.00 (32.75;312.50)	370.12 (185.04)	321.00 (278.00;500.50)	**<0.001***	**0.94**	<**0.001**	**0.62**
		Serum Creatinine (µmol/L) n = 18$^{\#}$	57.5 (10.19)	55.00 (49.75;68.50)	58.33 (8.08)	57.00 (51.75;65.50)	0.728	0.08	0.605	0.09
		CrCl (mL/min) n = 18$^{\#}$	113.55 (33.13)	103.49 (88.38;140.25)	121.32 (34.02)	122.02 (89.60;142.73)	0.150	0.23	0.286	0.18
	Male (n = 15)	BMI (kg/m^2) n = 9$^{\#}$	21.14 (2.39)	20.82 (19.10;22.13)	22.52 (2.83)	21.60 (20.62;25.62)	0.068	0.49	0.036	0.49
		CD_4 count (cells/mm^3) n = 13$^{\#}$	213.62 (167.83)	219.00 (39.00;278.00)	343.77 (166.13)	362.00 (212.00;438.50)	<**0.001***	**0.78**	**0.002**	**0.61**
		Serum Creatinine (µmol/L) n = 15$^{\#}$	76.67 (8.49)	75.00 (71.00;86.00)	68.47 (12.88)	69.00 (62.00;80.00)	0.053	0.64	0.068	0.33
		CrCl (mL/min) n = 15$^{\#}$	91.02 (18.76)	91.08 (78.78;101.95)	110.93 (30.75)	102.67 (84.40;139.59)	**0.020***	**0.65**	**0.008**	**0.49**
Group 2 ≥30-≤40	Female (n = 17)	BMI kg/m^2 n = 11$^{\#}$)	28.38 (6.66)	31.25 (21.63;32.81)	29.95 (5.55)	31.63 (25.47;32.47)	0.152	0.24	0.248	0.25
		CD_4 count (cells/mm^3) n = 12$^{\#}$	254.42 (194.65)	187.50 (114.75;346.75)	463.17 (239.35)	434.00 (262.25;643.00)	**0.002***	**0.87**	**0.005**	**0.58**
		Serum Creatinine (µmol/L) n = 17$^{\#}$	56.41 (9.94)	57.00 (50.00;62.50)	62.35 (9.56)	59.00 (55.00;70.00)	**0.013***	**0.60**	**0.027**	**0.38**
		CrCl (mL/min) n = 17$^{\#}$	117.98 (26.61)	125.90 (98.03;136.59)	112.02 (26.59)	108.68 (84.18;139.37)	0.176	0.22	0.163	0.24
	Male (n = 16)	BMI (kg/m^2) n=14$^{\#}$	21.54 (3.81)	21.29 (19.62;22.91)	22.22 (4.63)	20.70 (19.48;24.96)	0.209	0.15	0.173	0.26
		CD_4 count (cells/mm^3) n = 15$^{\#}$	261.93 (168.51)	261.50 (121.00;339.00)	395.80 (175.21)	336.00 (308.00;590.00)	<**0.001***	**0.76**	**0.001**	**0.62**
		Serum Creatinine (µmol/L) n = 16$^{\#}$	70.38 (14.03)	71.00 (60.00;78.50)	72.00 (11.76)	74.00 (63.50;81.75)	0.747	0.12	0.569	0.10
		CrCl (mL/min) n = 16$^{\#}$	97.67 (19.75)	103.07 (81.43;110.26)	97.14 (24.16)	90.99 (79.06;115.43	0.941	0.02	0.756	0.05

the n-values will differ as the dependent t-test only incorporates values from variables that are both available at baseline and at 12 months

Based on the results reflected in table 2, there was a statistically significant ($p < 0.001$, $p < 0.001$, $p = 0.002$ and $p < 0.001$) increase in CD_4 count in all of the age groups from baseline to follow-up (12 months since TDF-based initiation). This was further supported by a medium to high practical significance in the effect size values (Cohen's d-value) which ranged from 0.76 - 0.94. The more stringent nonparametric Wilcoxon test also supported the same statistical significant increase in the CD_4 cell count values in all the age and gender groups from baseline to 12-month follow-up.

The BMI also increased in all of the groups but was only statistically significant in the female (≥20 - <30) from baseline to follow-up ($p = 0.004$).

A statistically significant increase in SCr was seen in females (≥30 - ≤40), (56.4[9.94] *vs.* 62.35[9.56], $p = 0.013$) from baseline to 12 months with no statistical significant effect on CrCl ($p = 0.176$). There was no statistically significant change in SCr from baseline to follow-up data in any of the other age groups. However only the male group (≥20 - <30) depicted a statistically significant increase in CrCl (mL/min) from baseline to 12-month follow-up data (91.02 (18.76) *vs*. 110.93 (30.75), $p = 0.020$). The SCr and the CrCl effect size for the male group (≥20 - <30) portrayed a medium practical significance (0.64 and 0.65). The female group (≥30 - ≤40) illustrated an effect size of 0.60 for SCr also suggesting a medium practical significance. The more stringent nonparametric Wilcoxon test also confirmed a medium practical effect.

Tables 3 and 4 depict the results obtained after comparing the BMI, CD_4 cell count, SCr and CrCl for the different genders (male *vs*. female) stratified according to age. Table 3 depicts the results as obtained by the independent *t*-test (parametric) and Mann-Whitney (non-parametric) test at baseline. Table 4 depicts the results obtained by the independent *t*-test and Mann-Whitney test at 12-month follow-up data stratified according to age group and gender.

Table 3: Comparing male and female clinical measurements at baseline stratified according to age

Age group	Clinical measurement	Male		Female		Independent t-test		Mann-Whitney test	
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	*p*-value	Effect size	*p*-value	Effect size
≥20-<30 (n=15; n=18)	BMI (kg/m^2) (n=9) (n=13)	21.14 (2.39)	20.82 (19.10;22.13)	23.94 (6.69)	21.05 (19.82;27.23)	0.185	0.42	0.443	0.16
	CD_4 count (cells/mm^3) (n=15) (n=18)	205.00 (162.45)	219.00 (39.00;278.00)	185.94 (162.14)	151.00 (32.75;312.50)	0.739	0.12	0.759	0.05
	Serum Creatinine (µmol/L) (n=15) (n=18)	76.67 (8.49)	75.00 (71.00;86.00)	57.50 (10.19)	55.00 (49.75;68.50)	**<0.001***	**1.88**	**<0.001***	**0.72**
	CrCl (mL/min) (n=15) (n=18)	91.02 (18.76)	91.08 (78.78;101.95)	113.55 (33.13)	103.49 (88.38;140.25)	**0.021***	0.68	**0.043***	**0.35**
≥30-≤40 (n=16; n=17)	BMI (kg/m^2) (n=14) (n=11)	21.54 (3.81)	21.29 (19.62;22.91)	28.38 (6.66)	31.25 (21.63;32.81)	**0.008***	**1.03**	**0.009***	**0.53**
	CD_4 count (cells/mm^3) (n=16) (n=14)	263.63 (162.94)	261.50 (121.00;339.00)	245.00 (181.90)	187.50 (114.75;346.75)	0.770	0.10	0.533	0.11
	Serum Creatinine (µmol/L) (n=16) (n=17)	70.38 (14.03)	71.00 (60.00;78.50)	56.41 (9.94)	57.00 (50.00;62.50)	**0.002***	**1.00**	**0.003***	**0.51**
	CrCl (mL/min) (n=16) (n=17)	97.67 (19.75)	103.07 (81.43;110.26)	117.98 (26.61)	125.90 (98.03;136.59)	**0.019***	**0.76**	0.061	0.33

Table 4: Comparing male and female clinical measurements at <u>12-month follow-up</u> stratified according to age

Age group	Clinical measurement	Male		Female		Independent t-test		Mann-Whitney test	
		Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**p-value**	**Effect size**	**p-value**	**Effect size**
≥20-<30 ((n=15; n=18)	BMI (kg/m^2) (n=9) (n=13)	22.52 (2.83)	21.60 (20.62;25.62)	26.37 (6.51)	23.53 (21.98;28.36)	0.112	0.59	0.089	0.36
	CD_4 count (cells/mm^3) (n=13) (n=17)	343.77 (166.13)	362.00 (212.00;438.50)	370.12 (185.04)	321.00 (278.00;500.50)	0.690	0.14	0.738	0.06
	Serum Creatinine (µmol/L) (n=15) (n=18)	68.47 (12.88)	69.00 (62.00;80.00)	58.33 (8.08)	57.00 (51.75;65.50)	**0.010***	0.79	**0.020***	**0.40**
	CrCl (mL/min) (n=15) (n=18)	110.93 (30.75)	102.67 (84.40;139.59)	121.32 (34.02)	122.02 (89.60;142.73)	0.369	0.31	0.406	0.14
≥30-≤40 (n=16; n=17)	BMI (kg/m^2) (n=14) (n=11)	22.22 (4.63)	20.70 (19.48;24.96)	29.95 (5.55)	31.63 (25.47;32.47)	**0.001***	1.39	**0.004***	**0.58**
	CD_4 count (cells/mm^3) (n=15) (n=14)	395.80 (175.21)	336.00 (308.00;590.00)	453.64 (238.94)	434.00 (262.25;643.00)	0.461	0.24	0.760	0.06
	Serum Creatinine (µmol/L) (n=16) (n=17)	72.00 (11.76)	74.00 (63.50;81.75)	62.35 (9.56)	59.00 (55.00;70.00)	**0.014***	**0.82**	**0.027***	**0.39**
	CrCl (mL/min) (n=16) (n=17)	97.14 (24.16)	90.99 (79.06;115.43)	112.02 (26.59)	108.68 (84.18;139.37)	0.103	0.56	0.078	0.31

The BMI and the CD_4 cell count in the ≥20 - <30 age groups did not show a statistically significant difference between the males and females at baseline and follow-up data. The ≥30 -≤40 age group showed a statistically significant difference (p = 0.008 and p = 0.001) in BMI and a high practical significance (Cohen's d-value of 1.03 & 1.39) at baseline and follow-up data.

In the ≥20 - <30-year age group the SCr was statistically significant (p < 0.001 and p = 0.010) and high practical significance (1.88 & 0.79). The SCr was higher in the male group compared to the female group at baseline (76.67 [8.49] *vs.* 57.50 [10.19] and at the 12-month follow-up (68.47[12.88] *vs*. 58.33 (8.08). A similar deduction is made in the ≥30 - ≤40 age group which also showed a statistically significant and practical higher SCr at baseline (70.38 [14.03] *vs*. 56.41[9.94], p = 0.010) and 12 months (72.00[11.76] *vs.* 62.35[9.56], p = 0.014) for the male group. This was also supported by the more stringent nonparametric Mann-Whitney test.

The CrCl in both age groups illustrated statistically significant higher CrCl values at baseline (p = 0.021 for ≥20 - <30 and p = 0.019 for the ≥30 - ≤40) in the female groups compared to the male groups but not after 12 months of treatment. This was also moderate practically significant at baseline only (0.68 and 0.76 respectively).

5 Discussion

Based on the results obtained during the 12-month study period, the follow argument is proposed taking the different variables (BMI, CD_4 count SCr and CrCl) into account.

The following analysis can be deduced from the dependent *t*-test; the mean BMI at baseline was within the normal range according to the international BMI classification system, except for the ≥30 - ≤40-year age group (female) that saw a baseline BMI ≥ 25kg/m^2 and is classified as overweight (WHO, 2015). At 12-month follow-up, an increase in BMI was noted for all age groups and genders investigated, with the BMI of the females in both age groups been classified as overweight. The increase in weight seen at 12 months post-TDF commencement, corresponded with the finding of Madec *et al*. (2009) who concluded that weight gain improved after one year of ART.

The findings of Gelber *et al.* (2005) and Cohen *et al.* (2013) postulated that a higher BMI was linked to a higher incidence of CKD. In our study, the higher BMI did not did not point toward an increased risk of developing CKD.

Improvement in all groups was observed with statistical and practical significance concerning the CD_4 count from baseline to 12 months. This was expected from a clinical point of view that the CD_4 count values would have increased after 12 months, this was indicative of the immunological improvement of the patients after being on TDF-based treatment. This provides evidence to support the clinical efficacy of TDF-based treatment as was shown in Barditch-Crovo *et al*. (2001).

Nelson *et al*. (2007) postulated that lower CD_4 counts and lower weight are risk factors for acquiring increased SCr concentration. Our study contradicts this statement as patients with low CD_4 counts < 100 cells/mm^3 still had CrCl above 60 ml/min. Bygrave *et al*. (2011) advocated that people with a lower CD_4 count were at an elevated risk of developing kidney disease. Bygrave *et al*. (2011) further stated that a CD_4 count < 50 cells/µl increased the risk of developing renal insufficiency by 2.35 times as compared to having a CD_4 count > 200 cells/µL. At baseline our study consisted of 11 patients who had a CD_4 cell count <50 cells/mm^3 and 19 who had a CD_4 cell count between 50-200 cells/mm^3. These patients all displayed CrCl > 60 mL/min at baseline.

In this investigation the renal function as determined by CrCl exhibited a positive outcome for all patients (n = 66) of this study. A study conducted in 26 HIV-infected young adults, also provided evidence to support the lack of renal insufficiency in the TDF-based patients after 60-month study duration (Vigano *et al.,* 2011). Brennan *et al*. (2011) conducted a study on 890 patients who commenced TDF-based therapy at Themba Lethu Clinic in Johannesburg and established that tenofovir-linked renal insufficiency were likely to have been linked to a prior existing renal disorder. In this study 28.7% (n=19) of the patients presented with baseline CrCl (60-89 mL/min) classified as stage 2 renal failure according to K/DOQI (2002:3). There were no other tests conducted to confirm renal damage in our study. The remaining 47 patients had CrCl values > 90 ml/min. In total six patients presented with CrCl > 150 mL/min of which four were females, and this is classified as hyperfiltration (Palatini, 2012). Palatini (2012) postulated that hyperfiltration is indicative of nephropathy in diabetes and hypertension

At the 12-month follow-up investigation, no CrCl value fell below 60 mL/min. Majority of patients had an increase in CrCl value at 12-month follow-up, although only statistically significant in the male group (≥20 and < 30 years). Our study support results from Mugomeri *et al*. (2014) that TDF did not contribute to renal dysfunction after 12 months when using SCr and CrCl as measures.

The results of our study differed from other studies that linked TDF exposure to the development of renal insufficiency (Herlitz *et al*., 2010; Jose *et al*., 2014 & Scherzer *et al*., 2012).

The ≥20 - <30 female group portrayed a lower mean CrCl at baseline compared to the older female group but showed a higher mean CrCl at 12-month follow-up. This female group (≥20 - <30years) displayed an improved mean CrCl value from baseline to 12-month follow-up data. The older female group showed a decline in mean CrCl at 12-month follow-up. Similar results can be extrapolated from the male mean CrCl value for the ≥20 - <30-year age group and the ≥30 - ≤40-year age group. This finding is supported by Porth (2007) who indicated that kidney function changes with age.

The effect of gender on the development of renal disease is a controversial subject. Our study showed a higher mean SCr value for males at baseline and 12-month follow-up ($p < 0.001$ and $p = 0.01$ for the ≥20 - <30 age group; $p = 0.002$ and $p = 0.014$ for the ≥30 - ≤40 age group) compared to the female groups. This supports the National Kidney Foundation (NKF) who advocated that males have a higher SCr compared to females due to increased muscle mass (NKF, 2004). The mean CrCl value was higher in the female group throughout the study ($p = 0.021$ and $p = 0.369$ in the ≥20 - <30 age group; $p = 0.019$ and $p = 0.103$ in the ≥30 - ≤40 age group). The ≥30 - ≤40 age group showed a minor decline in CrCl at 12-month follow-up as compared to baseline values.

It can be deduced from the above that gender and age have had an influence on kidney function during the 12-month study duration in this study population.

6 Limitations of the study

The limitations of the research method used were:

- The NDoH guidelines state that SCr tests be conducted at baseline, 3, 6 and 12 months and thereafter every 12 months (NDoH, 2015). However, due to financial constraint experienced by the NHLS, only baseline and estimations around 12-month blood results could be evaluated
- Viral suppression from baseline to 12-month post-TDF-based initiation could not be investigated due to a lack of baseline VL results.
- The study was restricted to the Amajuba district, KwaZulu-Natal.

- Only SCr was used as a marker for kidney function as proteinuria and hematuria was not available.
- No proteinuria data was available.

7 Conclusion

The FDA (FDA, 2010), The National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) (NIDDK, 2015) and the South African Renal Society (2006) endorse the CG equation as an efficient and effective method of determining the level of kidney function. Supporting the statement from the NKF (2004), the SCr alone cannot be utilised to determine renal degeneration as an increase in SCr concentration only occurs after substantial damage to the kidneys has transpired. Males and females will have different kidney function values at the same SCr concentration (NKF, 2004) supporting the fact that SCr alone cannot be used to assess kidney function. It is imperative that CrCl calculation is conducted to prevent any decline in kidney function being overlooked. Our study provides further evidence to support the fact that the SCr levels are higher in males compared to females.

There was no deterioration in the kidney function after 12 months of TDF-based therapy when the CrCl was calculated using the modified CG equation. All patients maintained a CrCl ≥ 60 ml/min at the 12-month follow-up stage.

The CD_4 count increased at 12-month follow-up data, this is indicative of the immunological improvement of the patients on TDF-based therapy.

The BMI (based on weight gain as height was constant) increased for all age and gender groups investigated. Weight gain can be used as an important tool in resource-limited settings to assess a HIV-positive patient's progress. The increase in weight is an important indication that the ARV combination therapy is effective.

Our study supports the use of TDF as first-line ART in South Africa and champions the TDF-based ART rollout as effective and efficient.

8 Recommendation

- Blood tests must be strictly conducted according to the NDoH 2015 consolidated guidelines and at the intervals as suggested.
- Additional tests such as urine dipstick to detect proteinuria and hematuria must be conducted for patients presenting with stage 2 kidney failure
- Further studies should also include investigations into genetic variations in proximal tubule transporters as it can also affect tubular toxicity in certain patients taking tenofovir.

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3.2 Chapter summary

Chapter 3 represents the manuscript that will be published in the peer-reviewed journal Health SA Gesondheid. The style of the manuscript is set out according to the requirements of the journal. The dependent *t*-test was used to investigate the changes on SCr, CrCl, CD_4 count and BMI from baseline to 12 months (female *vs*. male and within the two different age categories). The Wilcoxon signed-rank test is the non-parametric equivalent of the dependent *t*-test. The independent *t*-test was utilised to assess the effect of gender and age on kidney function. The non-parametric equivalent of the independent *t*-test is the Mann-Whitney test.

As is expected, the SCr levels were higher in the males than the females but the females presented with higher CrCl values at the 12 months post-TDF commencement in both age groups. The BMI and CD_4 count improved in the female groups (≥20 - <30year and ≥30 - ≤40 year) as compared to the male groups. The immunological outcome of TDF-based therapy was enhanced in the female groups.

The results show that age and gender influence kidney function. The older age group (≥30 - ≤40 years) showed a slight decline in CrCl post-TDF commencement as compared to baseline values. Overall, the results illustrate a positive outcome for TDF-based HAART in this study population with no kidney function deterioration, after 12 months on TDF.

CHAPTER 4: ADDITIONAL RESULTS

4.1 Introduction

Chapter 4 presents with addItional results not reflected in the manuscript (chapter 3). Data pertaining to the combined age groups (≥20 – ≤40 years) for male and female is reflected in this chapter. The analysis of data commences with probing the demographic data of the combined age group for the female and male gender at baseline and 12-month follow-up data.

4.2 Demographic data

Table 4.1 reflects the demographic data of the different gender groups (≥20 – ≤40 years) at baseline and 12-month follow-up. Thirty-five females and thirty-one males consented to participate in the study. The mean age is similar for males and females at baseline and 12-month follow-up data. Both genders portrayed an increase in mean weight from baseline to 12-month follow-up. The females were diagnosed as HIV-positive for a longer period (27.97 [29.37] months) as compared to the males (13.68 [23.84] months). The females have also been on TDF based therapy for a longer duration (21.97 [7.51] months) compared to the males (20.19 [5.28] months) at baseline.

Table 4.1: Demographic information of study population (≥20 – ≤40 years) at baseline and 12-month follow-up

	Female (≥20 - ≤40 yrs.) baseline Mean (SD) (n = 35)	**Females (≥20 - ≤40 yrs.) 12 months post-TDF commencement Mean (SD) (n = 35)**	**Male (≥20 - ≤40 yrs.) baseline Mean (SD) (n = 31)**	**Males (≥20 - ≤40 yrs.) 12 months post-TDF commencement Mean (SD) (n = 35)**
Age (year)	30.43 (4.69)	31.34 (4.65)	30.32 (4.60)	31.26 (4.64)
Weight (kg)	70.01 (19.68)	74.99 (18.38)	61.97 (10.41)	64.77 (10.73)
Length (m)	1.61 (0.048) n = 24	1.61 (0.048) n = 24	1.72 (0.074) n = 23	1.72 (0.074) n = 23
Time (months) since HIV diagnosis(mean) to baseline	27.97 (29.37) n = 34		13.68 (23.84) n = 28	
Mean duration on TDF (months)	21.97 (7.51) n = 33		20.19 (5.28) n = 31	

The length was only measured once at baseline and the same length was utilised to calculate the BMI at baseline and 12-month post-TDF commencement. Only 24 females and 23 males had length measurements taken, which was utilised to calculate the BMI.

At baseline only one patient was diagnosed with a co-morbid disease, Hepatitis B. All other patients had no other documented chronic illness at baseline. All patients had a CrCl ≥ 60ml/min at baseline complying with the inclusion criteria.

Majority of the study population (n = 65) were taking the 3-in-1 fixed dose combination (FDC) containing TDF 300mg, FTC 200mg and EFV 600mg. Study participants commenced with TDF-based HAART from 2010 - 2014. Thirteen patients were changed from a d4t containing regimen to a TDF-based regimen according to their treatment history.

4.3 Clinical outcome

Certain clinical results at baseline and at 12 months follow-up for the combined age group (≥20 - ≤40 years) for the respective genders are reflected in table 4.2.

There was a statistically significant increase in the BMI (p = 0.002 with small effect size of 0.29) and CD_4 count (p < 0.001 with large effect size of 0.90) from baseline to follow-up for the female group. The mean CD_4 count increased significantly from a mean 220.24 (174.84) cells/mm^3 to 408.62 (210.35) cells/mm^3 in the female group.

The male group also showed a statistically significant change in CD_4 count (p < 0.001 with large effect size of 0.78). The CD_4 count increased from a mean of 239.5 (166.87) cells/mm^3 to 371.64 (169.94) cells/mm^3 in this group. The BMI also increased statistically significant (p = 0.027) although the practical significance was small (0.24).

As is expected from a clinical point of view, this increase in CD_4 count is indicative of the immunological improvement of the patients after being on ARV treatment. The same is true for the BMI as weight improved in both males and females at 12-month follow-up.

The serum creatinine (NHLS values) showed a borderline (p = 0.05) significant increase with only a small practical effect size (0.33) after 12 months for the females, but when CrCl was calculated, based on the modified CG formula, no significant change (115.70 [29.78] *vs.* 116.80 [30.55], p = 0.755) was seen after 12 months. For the male group there was no significant change in the SCr and the CrCl after 12 months, although the CrCl value did increase slightly (94.45[19.25] *vs*. 103.81 [27.97], p = 0.096).

Although a slight increase in CrCl can be seen as an improvement in kidney function it was insignificant in both genders. No kidney function deterioration was noted in both gender groups after 12 months of commencing TDF-based regimen.

Table 4.2: Immunological outcome at baseline and 12-month follow-up

Age group	Gender	Clinical measurement	Baseline	12-month follow-up	Dependent t-test	
			Mean (SD)	Mean (SD)	p-value	Effect size
≥20 - ≤40	Female (n=35)	BMI (kg/m^2) n=24$^{\#}$	25.98 (6.91)	28.01 (6.23)	**0.002***	0.29
		CD_4 count (cells/mm^3) n=29$^{\#}$	220.24 (174.84)	408.62 (210.35)	**<0.001***	**0.90**
		Serum Creatinine (µmol/L) n=35$^{\#}$	56.97 (9.94)	60.29 (8.93)	0.05	0.33
		CrCl (mL/min) n=35$^{\#}$	115.70 (29.78)	116.80 (30.55)	0.755	0.04
≥20 - ≤40	Male (n=31)	BMI (kg/m^2) n=23$^{\#}$	21.38 (3.27)	22.34 (3.95)	**0.027**	0.24
		CD_4 count (cells/mm^3) n=28$^{\#}$	239.50 (166.87)	371.64 (169.94)	**<0.001***	**0.78**
		Serum Creatinine (µmol/L) n=31$^{\#}$	73.42 (11.93)	70.29 (12.24)	0.343	0.26
		CrCl (mL/min) n=31$^{\#}$	94.45 (19.25)	103.81 (27.97)	0.096	0.33

The n value indicated will differ as the dependent t-test only incorporated values for the variable that are both available at baseline and 12-month follow-up.

To investigate gender differences, the combined age group were compared at baseline (table 4.3) and after 12-month follow-up (table 4.4).

Table 4.3: Comparing male and female clinical measurements at baseline

Age group	Clinical measurement	Male (n=31)	Female (n=35)	Independent t-test	
		Mean (SD)	Mean (SD)	p-value	Effect size
≥20 - ≤40	BMI (kg/m^2) (n = 23) (n = 24)	21.38 (3.27)	25.98 (6.91)	0.006	0.66
	CD_4 count (cells/mm^3) (n = 31) (n = 32)	235.26 (162.72)	211.78 (170.82)	0.579	0.14
	Serum Creatinine (µmol/L) (n = 31) (n = 35)	73.42 (11.93)	56.97 (9.94)	**<0.001***	**1.38**
	CrCl (mL/min) (n = 31) (n = 35)	94.45 (19.25)	115.70 (29.78)	**0.001***	**0.71**

At baseline, the females had a statistically significant higher BMI compared to the males (p = 0.006) with a medium practical effect size (0.66). The mean (sd) CD_4 count was higher in the males (235.26 [162.72]) as compared to the females (211.78 [170.820]), although this difference was insignificant (p = 0.579) with a small practical effect (0.14). The female group presented with a statistically higher kidney function at baseline if the calculated CrCl is used as a marker (115.7 [29.78] *vs*. 94.45[19.25], p = 0.001) with a medium (0.71) practical effect. The SCr was statistically higher (73.42[11.93] *vs.* 56.97[9.94], p < 0.001) in the male group compared to the female group with an even larger practical significance (1.38).

Table 4.4: Comparing male and female clinical measurements at 12-month follow-up

Age group	Clinical measurement	Male (n=31)	Female (n=35)	Independent t-test	
		Mean (SD)	Mean (SD)	p-value	Effect size
≥20 - ≤40	BMI (kg/m^3) (n = 23) (n = 24)	22.34 (3.95)	28.01 (6.23)	0.001	0.91
	CD_4 count (cells/mm^3) (n = 28) (n = 31)	371.64 (169.94)	407.84 (211.63)	0.475	0.17
	Serum Creatinine(µmol/L) (n = 31) (n = 35)	70.29 (12.24)	60.29 (8.93)	<0.001*	0.82
	CrCl (mL/min) (n = 31) (n = 35)	103.81 (27.97)	116.80 (30.55)	0.078	0.43

At 12-month follow-up, the females showed a significantly improved BMI (p = 0.001) with a large practical significance (0.91). The CD_4 count was higher in the females compared to the males although it was not statistically significant (p = 0.475). Although there is a statistically significant difference in SCr (p < 0.001) with a large practical significance (0.82), there is an insignificant difference in CrCl between the males and females (p = 0.078) with a medium practical significance (0.43). At 12-month follow-up data, the females presented with improved BMI (28.01 [6.23] *vs*. 22.34 [3.95]) kg/m^3; CD_4 count (407.84 [211.63] *vs.* 371.64 [169.94]) cells/mm^3 and CrCl (16.80 [30.55] *vs.*103.81 [27.97]) mL/min.

Due to the lack of baseline viral load (VL) results, a comparison could not be made between baseline VL and follow-up VL. Baseline viral load results were only available for 16.4% (n = 11) of this study population. This lack of viral load results could be attributable to various challenges experienced by the NHLS.

Based on the 12-month follow-up VL data depicted in table 4.5, it can be concluded that viral suppression was being maintained by the TDF-based treated, as majority (n=59) of the participants had undetected VL.

Table 4.5: Viral load 12 months post TDF commencement

	Females (20-40 yrs.) n = 33	**Males (20-40 yrs.) n = 28**
VL (copies/ml)	All VL was undetectable .	n = 26 reflects undetectable VL. n = 2 depicted VL values ≥ 1000 copies/ml.

4.4 Chapter summary

This chapter provides addItional data for the pooled age group (≥20 – ≤40 years) for males and females. The results were statistically analysed by incorporating the dependent *t*-test and independent *t*-test to assess the influence of time and gender on kidney function. The BMI and CD_4 count at baseline and at 12 months post-TDF commencement was assessed to provide an indication of immunological outcome.

The results show that gender has had an influence in this study population. The SCr at baseline and 12-month follow-up data in the males was higher than that of the females. This is attributed to the greater muscle composition in males. The CrCl in females portrayed a higher kidney function value at baseline and at 12-month follow-up compared to the males, although it was not statistically significant.

The BMI and CD_4 count improved at 12-month post-TDF commencement in both genders, indicating that TDF-based treatment was effective and improved the immunological outcome for this population group.

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Introduction

The final chapter provides a discussion of the results presented in the previous 2 chapters. The conclusion and recommendation based on the outcome of this study is provided at this time. Limitations that could have affected the study are also elaborated upon.

5.2 Immunological outcome

The immunological response of the study participants to TDF-based therapy is reflected by the CD_4 count.

Nelson *et al.* (2007:1278) postulated that lower CD_4 counts and lower weight are risk factors for acquiring increased SCr concentration. This study contradicts this statement as patients with low CD_4 counts < 100 cells/µL still had SCr values within normal range (Male 64 – 104 µmol/l and female 49 - 90 µmol/l). Bygrave *et al*. (2011:3) advocated that people with a lower CD_4 count are at an elevated risk of developing kidney disease. In our study, the CrCl improved in 20 (66.7%) patients with CD_4 count less than 200 cells/mm^3 at baseline. Ten patients (33.3%) reported a slight decline in the CrCl at 12-month post-TDF commencement. These patients still maintained a CrCl > 60 mL/min.

Bygrave *et al*. (2011:3) further postulated that a CD_4 count <50 cells/µl increased the risk of developing renal insufficiency by 2.35 times as compared to having a CD_4 count >200 cells/µl. At baseline our study consisted of 11 patients who had a CD_4 count <50 cells/µl and 19 patients who had a CD_4 count between 50 - 200 cells/mm^3.

Our study showed an increase in the CD_4 count from baseline to 12-month follow-up in 27 (16 female and 11 male) patients, who had baseline CD_4 counts below 200 cells/mm^3. Three patients did not have CD_4 counts available at 12 months post-TDF commencement to form a comparison.

The female group stratified according to age (≥20 - < 30 and ≥30 - ≤40 years) displayed a statistically significant difference in CD_4 count at 12 months post-TDF commencement, with a large practical significance (p < 0.001 (0.94), p = 0.002 [0.87]). The mean increase in CD_4 count was 174 cells/mm^3 (≥20 - <30 year age group) and 208.75 cells/mm^3 (≥30 - ≤40year age group). The pooled female group (≥20 – ≤40 years) displayed a mean increase in CD_4 count of 196.06 cells/mm^3. These results correspond with Esser *et al*. (2011 431) who conducted a multicentre study (n = 67) in which the mean CD_4 count increased by 176 cells/mm^3 at the end of a 48-week study.

The male group stratified according to age (≥20 - < 30 and ≥30 - ≤40) portrayed a statistically significant difference in mean CD_4 count at 12 months post-TDF commencement, with a large practical significance ($p < 0.001$ [0.78] and $p < 0.001$[(0.76]). The mean increase in CD_4 count was 143 cells/mm^3 (≥20 - <30 year age group) and 74.5 cells/mm^3 (≥30 - ≤40 year age group). The pooled male group (≥20 – ≤40) showed a mean increase in CD_4 count of 136.38 cells/mm^3 that was statistically significant.

Our result correlates with Theuring *et al.* (2015:3) who determined that the female gender showed an improved CD_4 count ($p = 0.035$) after 12 months of ARV therapy and thus have an improved immunological outcome as compared to males.

Hawkings *et al*. (2011:1195) revealed gender differ outcomes among HIV-infected adults in an urban Tanzanian setting cohort study involving 4383 men and 8459 women that the CD_4 count statistically improved in women after one year of ART ($p < 0.001$). This difference in CD_4 count could be attributed to physiological differences between men and women including differences in weight, fat distribution, protein binding and differences in ARV drug metabolism and distribution (Hawkings et al., 2011:1195).

The CD_4 count improved statistically and practically in all age groups and is an indication of immunological response to the TDF-based ARV therapy. Our study supports Ford *et al*. (2014: 2003) who deduced that monitoring the CD_4 count in resource limited settings is imperative in assessing the effectiveness of ART. Due to this improvement in clinical response to ARV combination, it can be deduced that the TDF-based therapy was clinically positive and has had a positive impact on this group of patients.

5.3 Virological response

Baseline viral load results were only available for 16.7% (n = 11) of this study population. This lack of viral load results could be attributable to various challenges experienced by the NHLS such as high sample volumes, difficulty in transporting the blood samples from clinics to the NHLS location and correct storage of blood samples during transit (Stevens & Marshall, 2010:78). The financial crisis experienced in 2011 (Tomlinson, 2011) and the deficit of R152 million experienced by the NHLS in the 2013 - 2014 financial year (NHLS, 2015b:11) resulted in less blood tests being conducted.

There are currently only 17 NHLS facilities that conduct VL testing nationally (NHLS, 2015b:68). At 12-month follow-up, 92.4% (n = 61) patients presented with VL results, of which 89.4% (n = 59) was undetectable (VL < 400 copies/ml). Two patients exhibited with VL > 1000 copies/ml. One patient was changed from a TDF based regimen to Alluvia/AZT/3tc and the second patient

remained on the original TDF therapy. Both of these male patients had a CD4 count < 100 cell/mm^3. The CrCl did not reflect any risk of renal insufficiency for either of these patients.

Based on the 12-month follow-up VL data, it can be concluded that TDF was effective in maintaining viral suppression as the VL was undetected in the majority (89%) of the study participants.

5.4 The effect of tenofovir on renal function

The renal function as determined through using the Scr to calculate the CrCl with the modified CG equation, exhibited a positive outcome for all patients (n = 66). This outcome is supported by a study conducted on 26 HIV-infected young adults, which provided evidence to support the lack of renal insufficiency in the TDF study group after a 60-month study duration (Vigano *et al*., 2011:407-411). Brennan *et al*. (2011:1603) conducted a study on 890 patients who commenced TDF-based therapy at Themba Lethu Clinic in Johannesburg and established that TDF-linked renal insufficiency were likely to have been linked to a prior existing renal disorder. In our study, 19 patients had a baseline CrCl value between 60 - 89 ml/min which is classified as stage 2 renal failure according to K/DOQI (2002:3). There were no other tests conducted to confirm renal damage in this group of patients. At the 12-month follow-up investigation, no CrCl value fell below 60 ml/min. Thirty-nine (59.1%) of patients had an increase in CrCl value at 12-month follow-up.

The results of this study differed from that of other studies that linked TDF to the development of renal insufficiency (Herlitz *et al*., 2010:1171; Jose *et al*., 2014:363; Scherzer *et al*., 2012:867). These studies determined that TDF-exposure was linked to the development of renal insufficiency. Jose *et al*. (2014:367) postulated that TDF-nephrotoxicity might not be reversible.

The findings of our study supported the conclusion of Mugomeri (2013:136) that TDF was a weak contributor to renal insufficiency when calculating the CrCl using the Scr as a marker.

5.5 Gender and age as a predicator of kidney disease

According to the NDoH guidelines (2015:73) HAART for males and females without complications remains the same. TDF is seen as 1st line therapy as used in combination ART for males and females, including pregnant women.

The effect of gender on the development of renal disease is a controversial subject. The National Kidney Foundation (2004:6) indicated that males have a higher SCr than females which may be attributed to the increased muscle mass in men. Our study also confirmed this finding and the males had statistically higher SCr levels at baseline and 12-month follow-up compared to the females. The mean CrCl values were higher in the female group throughout the study. At baseline,

the CrCl for the female group was statistically significantly higher but not at 12-month follow-up. This deduction contradicts the statement by the NKF that female gender is a risk factor for developing renal insufficiency. The difference in mean CrCl between the genders was not statistically significant but it can be deduced from these results that gender influences renal function differently.

The younger female age group portrayed a lower mean CrCl at baseline as compared to the older female group but showed a higher mean CrCl at 12-month follow-up. Similar results can be extrapolated from the male mean CrCl value for the younger group compared to the older male group. The mean CrCl for the ≥20 - <30 year male group improved at 12-month follow-up and the mean CrCl for the older age group decreased slightly.

Although these differences in CrCl were not statistically significant except for the 12-month follow-up data in the ≥20 - <30 year male age group (p = 0.020) with medium practical significance (0.65), it can be deduced that age has an influence on kidney function. The K/DOQI (2002:55) guidelines purports that the kidney function decreases by 1 ml/min after 20 - 30 years of age. This finding is supported by Porth (2007:1685) who indicated that kidney function changes with age. This is a possible explanation for a decline in mean CrCl value for the ≥30 - ≤40-year male and female age groups.

5.6 Body mass index and kidney function

The mean BMI at baseline was within the normal range according to the international BMI classification system, except for the ≥30 - ≤40-year female age group that saw a baseline BMI ≥ 25kg/m^2 and is classified as overweight (WHO, 2015). At 12-month follow-up, an increase in BMI is noted for all age groups and genders investigated. A statistically significant increase in BMI was noticed in the ≥20 - <30-year female age group (p = 0.004) with small practical significance (0.36) at 12-month follow-up data. In this study group, an increase in BMI has had a positive influence on the CrCl. An improvement in BMI was seen in all age and gender groups investigated.

The participants who depicted BMI values as overweight or obese reflected CrCl ≥ 60 ml/min. Patients with a BMI ≥ 25kg/m^2 presented with CrCl ≥ 90 ml/min. At baseline, 7 patients had a BMI ≥ 30kg/m^2. These 7 patients reflected CrCl values ≥ 90 ml/min. At 12-month follow-up BMI, 11 patients presented with a BMI ≥ 30kg/m^2. All of these patients displayed a CrCl value ≥ 90ml/min.

These results contradict the findings of Gelber *et al*. (2005:871) and Cohen *et al*. (2013:133) who postulated that higher BMI has been linked to a higher incidence of CKD.

This increase in BMI concurs with Madec *et al*. (2009:856) who concluded that BMI at baseline was associated with an increase in weight during the first year of therapy. The increase in BMI at 12 months post-TDF commencement is attributed to an increase in weight. Weight gain can be used as an important tool in resource-limited settings to assess a HIV-positive patient's progress (Madec *et al*., 2009:859). Poor weight gain can be related to the lack of adherence, insufficient nutritional intake and opportunistic infections (Madec *et al*., 2009:860). Madec *et al.* (2009:853) has provided evidence to support the concept that ART is associated with an improved rate of survival.

5.7 Conclusion

In our study, the objectives as laid out in chapter 1 (1.4.1 – 1.4.3) have been achieved. These objectives include:

- To review literature on renal function in black populations on TDF-based treatment nationally and internationally,
- To review literature to assess the prevalence of renal dysfunction after commencing TDF-based treatment,
- To review literature on kidney function, different prediction equations to calculate the creatinine clearance (CrCl),
- To compare the effect of tenofovir on the kidney function in different gender groups as measured with the modified Cockcroft-Gault (CG) equation,
- To determine the CrCl in this black population (≥20 - ≤40 years) at baseline (prior to TDF-initiation) and again at 12 months post-TDF commencement,
- To investigate differences in BMI and CD_4 count in the different age and gender groups.
- To investigate the change in the CD_4 count and VL over the 12 month period since tenofovir treatment was initiated.

The information gathered from patient files located at the PHC clinic in Newcastle, KwaZulu Natal included, history pertaining to diagnosis and previous therapy CD_4 count, VL, date of diagnosis, date of commencement of ART, SCr value, age, gender and weight. Data was gathered retrospectively with some prospective aspects.

The SCr concentration was the only marker employed to determine the kidney function in this study population. The kidney function was represented by the calculated CrCl value determined by means of the modified Cockcroft-Gault equation.

The overall renal function exhibited a positive outcome for patients on TDF-based therapy in this study population based at the PHC clinic. All patients maintained a CrCl ≥ 60 ml/min at the 12-month follow-up investigation. This study supports the use of TDF as first-line therapy in South Africa.

Selecting a younger age group reduced the risk of age-related degeneration of kidney function. The CrCl in the younger age group (≥20 - <30 years) exhibited an increase in CrCl at 12 months post-TDF commencement. The older age group (≥30 - ≤40) displayed a decrease in CrCl at 12 months post-TDF commencement for females and males. This supported evidence by the K/DOQI (2002:55) that kidney function decreased annually after the age of 20 – 30 years.

Although there was a difference in CrCl values between the males and females, this difference was not statistically significant. The females in the pooled age group (≥20 – ≤40 years) displayed an improved renal function at the end of 12 months of TDF-based ART compared to the males. This contradicts the perception that female gender has a risk factor for developing renal insufficiency (NKF, 2004:6).

As is also apparent from the results of our study, age and gender do influence kidney function.

The undetectable VL visible in 59 (89.4%) patients at 12-month follow-up provided a positive indication pertaining to the efficacy of TDF-based treatment in this group of patients. The undetectable VL also provided evidence to support the use of TDF. The VL results were only available for 11 patients at baseline and a comparison between baseline and 12 months could not be conducted.

The immunological outcome as determined by the CD_4 count showed a statistical and large practical significance increase in all age and gender groups investigated. This outcome provided evidence to support the use of TDF-based ART.

The CD_4 count was higher in females than males at 12-month post-TDF commencement, although insignificant. Our study supports the findings of Theuring *et al*. (2015:6) and Hawkings *et al*. (2011:1195) that females have an improved immunological outcome after being on ARV therapy.

The non-parametric tests were also performed due to the small sample size of the study population. The same trends were shown in the parametric and non-parametric statistical analysis.

This study investigated the renal safety profile in adult black HIV-infected patients at a Primary Health Care (PHC) Clinic in Newcastle, KwaZulu-Natal who were on TDF-based treatment for 12 months to establish whether tenofovir has an effect on kidney function in HIV-positive patients, meeting certain criteria.

Based on these findings, TDF-based therapy is a critical component of ART and has been proven to be beneficial in this study group.

5.8 Limitations of the study

The limitations of the research method used were:

- Only baseline and 12-month blood results were evaluated due to financial constraints experienced by the NHLS blood tests stipulated in the NDoH guidelines was inconsistent.
- Blood tests to determine SCr levels were not drawn at exactly 12 months post TDF commencement. The blood test closest to the expected 12 month date was utilised.
- Viral load could not be compared from baseline to 12 month follow-up due to lack of data.
- Only SCr as a marker for kidney function was used.
- The study was restricted to black patients in the Amajuba district, KwaZulu-Natal.

5.9 Recommendation

- Patients need to be informed and given a reminder of when they need to attend the clinic to have laboratory tests conducted.
- Monitoring of SCr levels and CrCl should be conducted according to NDoH guidelines to determine any renal dysfunction early.
- Further studies need to be conducted to assess glomerular filtration as a marker of nephropathy, diabetes mellitus, hypertension and patietns taking other drugs that may impair renal funciton.
- Future studies should also include additional genotyping of specific single nucleotide polymorphisms to investigate the effect thereof on possible tenofovir induced tubular toxicity.

5.10 Chapter summary

Chapter 5 provides an in depth discussion on the results presented in chapters 3 and 4. The overall finding of this study portrays the female gender as immunologically advanced in terms of CD_4 count. The BMI improved in all gender and age groups investigated. The SCr was higher in males throughout the study. The females in both age groups displayed a greater CrCl value as compared to the males. After 12 months of TDF-based therapy, the females flaunted an improved CD_4 count, BMI and CrCl compared to the males, with the females in the younger age group parading a superior CrCl as compared to the older age group. Our study also supports the already known evidence that gender and age influence kidney function.

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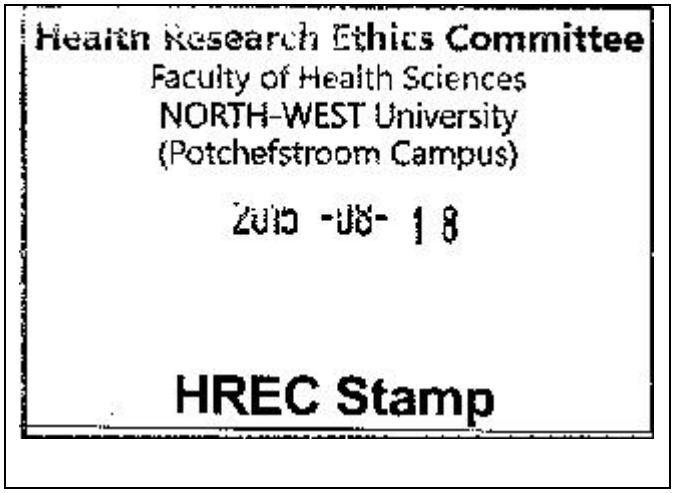
ANNEXURE 1: INFORMED CONSENT FORM (ENGLISH)

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS



PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR PARTICIPANT

TITLE OF THE RESEARCH PROJECT: *The renal safety profile of tenofovir as used in combination antiretroviral therapy*.

REFERENCE NUMBERS:

PRINCIPAL INVESTIGATOR: Me Arthi Gajee

ADDRESS: Primary Health Care clinic (also known as Lulama in the Amajuba District), Newcastle, KZN 2940 (where the research study will take place)
The principal investigator is working at Newcastle Provincial Hospital.

CONTACT NUMBER: 072 966 8457 / 034 32 80000

You are being invited to take part in a research project that forms part of my Master's degree. Please take some time to read the information given here, which will explain the details of this project. Please ask the researcher or the staff nurse 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 is about and how you could be involved. Also, 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 **Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU.....)** 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.

6 What is this research study all about?

- *Tenofovir (TDF) may have negative effects on the kidney. We want to see if TDF has any side effects on the kidney within the South African population who started on tenofovir treatment with healthy kidney function.*
- *We also want to see if the effects in men and women are any different. This information will help us to improve our quality of treatment and care.*
- *We also want to see if any kidney damage occurs in this black population (20-40 years) by comparing your kidney function before starting with TDF to your kidney function after 12 months of TDF treatment. This can be measured by using blood to test for serum creatinine levels that will give an indication if any kidney damage is present or not.*
- *This study will be conducted at Newcastle Primary Health Care (PHC) clinic, which is also known as Lulama clinic and will involve the Principal investigator (PI) looking at your patient files for blood results under the supervision of experienced and registered health professionals (registered with the South African Nurses Council and the Health Profession Council of South Africa).*
- *In this study we want to stress the importance of having your kidney function checked by having routine blood tests (serum creatinine levels).*

7 Why have you been invited to participate?

➢ *You have been invited to participate because you are:*

- *attending the Newcastle Primary Health Care clinic (also known as Lulama clinic).*
- *a black male or female patient who has been on tenofovir-based treatment for at least six months,*
- *between the ages of 20 – 40 years,*
- *required to have had a baseline kidney function test (serum creatinine test result that indicates how healthy your kidneys are) before you started on your TDF-based treatment.*

➢ *You will be **excluded** if the following is applicable to you:*

- *Pregnant*
- *Sugar problems (Diabetes)*
- *High blood pressure and heart diseases*
- *A baseline kidney function less than 60 ml/min/1.73m^2 (this will be calculated by the PI or doctor) at start of TDF treatment*
- *If you are not mentally able to make decisions (dementia) or are not able to make the right choice for yourself or to speak up for yourself*

A total of 72 adult patients (men and women) will be included in the study.

8 What will your responsibilities be?

- *You will have to sign this form in order for me (the PI) to use your file information e.g. when you started TDF treatment, other antiretroviral treatment, dosages, weight, height, blood results relating to your kidney function and TDF treatment. Information from your file just before you started tenofovir treatment and also at around 12 months after you had started tenofovir treatment will be recorded and used in this study.*
- *Information will be obtained from your patient files. This information includes weight, age, viral load, CD4 count, serum creatinine blood value (kidney function test), HIV diagnosis date, medical history on current and previous antiretroviral treatments.*
- *No addItional blood tests or follow-up visits are needed. The blood that may be drawn will form part of normal routine blood tests that would have been drawn anyway even if you do not take part in this research study.*
- *All your data and information will be collected anonymously (no name or surname) and will not be able to be traced back to you. We will use a study number that is specific for you.*

9 Will you benefit from taking part in this research?

- *The direct benefit to you in participating in this study will be to have a better understanding of how TDF affects your kidney function. The study will provide us with knowledge on the 12-month effect of TDF on kidney function. Although this study is observational, files will be shown to the attending doctor to ensure that the doctor sees any abnormalities if they are observed and of any concern.*
- *The indirect benefit will be that future generations are likely to benefit from this study as we may learn more information that we can use in the standard care and management.*

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

- *There is always a risk involved when blood is drawn. Blood will be drawn as per the clinic standard care and management guidelines as listed by the National Department of Health. You may feel a burning sensation when blood is drawn but the nurse and doctor are qualified and well trained to do this.*
- *The possible risk of your identity becoming known and the risk of stigmatisation does exist. However all precautions will be taken to ensure that your identity remains anonymous (no names, surnames, ID numbers will be ever be used).*
- *The benefit outweighs the risk.*

11 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?

- *Should you have the need for further discussions after the blood tests are carried out an opportunity will be arranged for you to speak to the staff nurse Sr Sabelo Nkosi who has received training about the project and can answer your questions.*
- *Sr Sabelo Nkosi will have direct contact with the participants and will be trained to know when a participant has decision making ability to enrol in the study and when not.*
- *The PI can also be contacted at any time (see contact details on page 4 of 6).*

12 Who will have access to the data?

- *Anonymity (no names or surnames) will be maintained. Only the PI and the staff nurse will know who takes part in this study. NWU staff members will have access to data however it will only reflect the study number of the participants on the electronic database. No copies of laboratory results will be kept by any investigator.*
- *Confidentiality will be ensured by the PI. Reporting of findings will be anonymous and study numbers will be used by researchers and the study team.*
- *Data will be kept safe and secure by locking hard copies (with study numbers) in locked cupboards and for electronic data it will be password protected.*
- *Data will be stored for a period of five years after this research study had been completed.*

What will happen with the data/samples?

- *This is a once off collection or results. All paper data will be locked away when in use by the PI or when handed over to the NWU for safekeeping. Study supervisors of the PI will ensure that no extra copies (paper or digital) will be kept by the PI after the study has been completed.*
- *No blood samples will be taken and stored for any later analysis.*
- *The participants have the right to withdraw at any point before data analyses have been performed without providing reason and without accompanying discrimination. After data analyses the individual data have been anonymised and therefore cannot be identified and withdrawn.*

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

*Yes. Participants will be approached to participate in the study during their routine scheduled visits to the clinic but not all will consent to participate immediately. Participants will receive time to think about taking part and if they would like to participate and complete the consent form during an unscheduled clinic visit, their transport to the clinic will be paid for as a **once off** payment of R30. Patients must return all the consent forms within a week to Sr Sabelo Nkosi who will place it in a sealed box for the PI to come and collect on a weekly basis.*

Is there anything else that you should know or do?

- *You can contact the PI at 072 9668457 if you have any further queries or encounter any problems.*
- *You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2089; 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?

- *All routine blood results will be placed in you file for your attending health care worker to make specific decisions regarding your treatment as this is part of the standard treatment and care guidelines.*
- *A final report on this specific research will be presented to the clinic once the research has been completed and the final results have been studied.*

- *You are free to contact the PI should you need any further feedback regarding the outcome of this research study directly.*

Declaration by participant:

By signing below, I ……..……. agree to take part in this research study titled: *The renal safety profile of tenofovir as used in combination antiretroviral therapy*.

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.
- The information has been explained to me in a language which I understand and is comfortable with.
- 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 understand that certain medical results will be used from my clinic file.
- 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**

13 Declaration by person obtaining consent:

I *(name)* ……..…… declare that:

- The information in this document has been explained to ……..
- The participant was encouraged to ask questions and I to take time to answer them.
- I am satisfied that he/she was adequately informed and understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

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

....

Signature of person obtaining consent **Signature of witness**

14
15
16
17
18 Declaration by researcher:

I *(name)* ……..…… declare that:

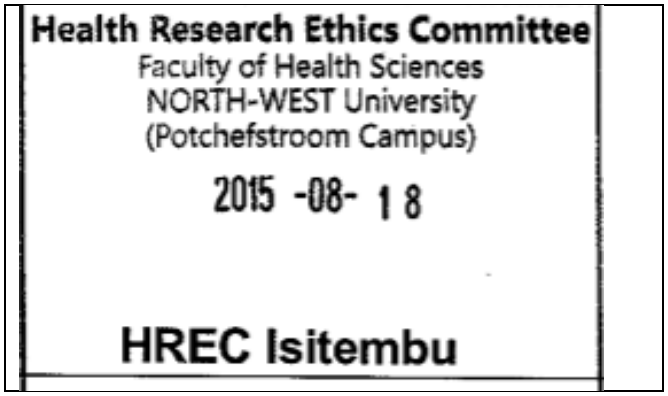
- The information in this document has been explained to ...……..
- It was encouraged to ask questions and to take adequate time to answer them.
- I am satisfied that he/she was adequately informed and understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

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

....

Signature of researcher **Signature of witness**

ANNEXURE 2: INFORMED CONSENT FORM (ZULU)



NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

IPHEPHA NOLWAZI LWESIVUMELWANO

Isihloko wocwaningo: *ukucilongwa ngokusebenza kwezinso nokuphepha kwe-tenofovir njengoba lisetshenziswe inhlanganisela ukwelashwa ngezidambisi gciwane.*

REFERENCE IZINOMBOLO:

Umphenyi EMAYELANA : Yimi uArthi Gajee

IKHELI: *Umtholampilo Newcastle Primary Health care (owaziwa ngokuthi iLulama okuSifunda Amajuba),KwaZulu-Natal 2940 (lapho ucwaningo luzokwenzelwa khona) Umseshi ophethe lolucwaningo usebenza Newcastle Provincial Hospital*.

Inombolo: 072 966 8457/034 32 80000

Uyamenywa ukuba uzibandakanye ngocwaningo yokwakha ingxenye yeMaster Degree yami okuwufundo oluphezulu eNorth West University. Sicela uthathe isikhathi esithile ufunde ulwazi olunikezwe lapha,okuzobe kuchazwa imininingwane yale phrojekthi. Sicela ubuze umcwaningi noma umhlengikazi uma unemibuzo mayelana ngengxenye yale phrojekthi uma ungaqondi ngokuphelele. Kubalulekile kakhulu ukuthi kucacile ukuthi lolucwaningo lumayelana nani nangokuthi iyiphi indlela ekuxhumanisa nayo. iqhaza lakho ***lingokuzithandela akuphoqiwe*** *futhi sikhululekile ukuba unqabe ukubamba iqhaza. Uma uthi cha, lokhu ngeke kube nomthelela omubi kuwe nangaba iyiphi indlela.Ukhululekile ukuba uhoxe kulolucwaningo nangasiphi isikhathi noma wawuvumile ekuqaleni.*

*Lolu cwaningo lugunyazwe i****Health Research Ethics Committee of the Faculty of Health Sciences of the University North-West (NWU ....)*** *lizoqhubeka ngesivumelwano neziqondiso kanye nezimiso nemihlahlandlela ngokumukelekayo eNational Health Research Ethics Council. Kungase kudingeke ucwaningo*

luzohlwengwa amalunga ekomidi noma iziphathimandla ezifanele ukuhlola iminigwane namabhuku ocwaningo.

Ngabe lolucwaningo lizokhuluma mayelana nani?

- *Tenofovir (TDF) kungenzeka kube nemiphumela engemihle ezinsweni. Sifuna ukubona ukuhi kungenzeka Itdf ibenemiphumela emibi ezinsweni zabantu baseNingizimu Afrika abaqala umshanguzo itenofovir benizinso ezinempilo*
- *Sifuna ukubona umehluko kubantu besilisa nabesifazane ukuthi lomshanguzo ubaphatha ngokuhlukana. Lolu lwazi luyosisiza ukuba sithuthukise izinga lethu lokwelashwa kanye nokunakekelwa.*
- *Sifuna nokubona uma kukhona ukulimala kwezinso okwenzeka kubantu abamnyama (abaphakathi kweminyaka 20-40) ngokuqhathanisa ukusebenza kwezinso ngaphambi kokuba uthole uTDF,siphinde siqhathanise ukusebenza kwezinso emva kwezinyanga 12 zokwelashwa ngeTDF. Lokhu kuzokwenziwa ngokusebenzisa igazi ukuhlola iserum creatinine kubhekwe amazinga ukuthi ayosinika inkomba uma kukhona ukulimala kwezinso okukhona noma cha.*
- *Lolu cwaningo oluzobanjelwa eNewcastle Primary Health Care (PHC) emtholampilo,eyaziwa ngokuthi iLulama clinic elifaka umphenyiukuba ebuke ifayela lakho abheke imiphumela yegazi ngaphansi kweso labanolwazi futhi ababhalisiwe abasebenzela ezempilo (olubhalisiwe kanye Abahlengikazi South African Council kanye Health Profession Council of South Africa).*
- *Kulolu cwaningo sifuna ukugcizelela ukubaluleka beye umsebenzi wakho kwezinso uhlolwa kokuba nesimiso kwegazi (serum creatinine amazinga).*

Kungani ucelwa ukuba ahlanganyele?

- *Umenyiwe ukuba bahlanganyele ngoba wena:*
- *uhamba eLULAMA (owaziwa ngokuthi Newcastle Primary Health Care Clinic).*
- *yingoba ungowesilisa/owesifazane osebenzisa lomshanguzo itenofovir izinyanga okungenani eziyisithupha,*
- *unemiyaka 20 – 40 ubudala*
- *kudingeka imiphumela yamagazi ekhombisa ukusebenza kwezinso zakho ngaphambi kokuba uqaliswe lomushanguzo (Serum creatinine test).*

- ***<u>Uyokhishwa</u>*** *uma kutholakala lokhu okulandelayo:*

- ukhulelwe
- unesifo soshukela *(Diabetes)*
- ufutho *kanyenesifo senhliziyo (iHigh-High)*
- *umkhomba ngokusebenza kwezinso esingaphansi 60 ml / min / 1.73m ^{2} (Lokhu ikuyobalwa umphenyi noma udokotela) ngesikhathi uqalwa ukwelashwa ngeTDF*
- *Uma ugula ngengqondo ungakwazi ukuzithathela izinqumo noma ungakwazi ukukhetha okufanele wena noma ungakwazi ukukhuluma ngokwakho.*

Kudingeka inani leziguli ayi72 abadala (abesilisa nabesifazane) kulolu cwaningo.

Yikuphi okuzofanele ukwenze?

- *kufanele ukuba usayine leliFomu elizobe lingigunyaza ukuba ngisebenzise ifayela lakho,nolwazi oluqukethwe yilo, njengokuthi waqala ninimishanguzo okufaka neTDF,isisindo sakho,ubude bakho,imiphumela yegazi esitshengisa ukusebenza kwezinso.konke lokhu kukhombisa ukuvuma ukuba sisebenzise ulwazi lakho kulolucwaningo.emvakokuqala uTDF ezinyangeni ezi12.*

- *Sizosebenzisa ulwazi esizolithola kufayela lakho,njengo bude bakho,isisindo,amasotsha omzimba,izinga lengciwane egazini lakho kanye nokusebenza kwezinso zakho.*
- *ngeke sikubize sithi wozodonsa amagazi aphathelene nalolucwaningo.Amagazi uzowadonsa njengenjwayelo noma kuthiwa awuzukuba ingxenye yalolucwaningo emtholampilo yakho*
- *lonke ulwazi esizolithola kwifayela lakho ngeke lwaziwe muntu9akukhishwa amagama,nezibongo)okuzokwenza singakwazi ukukulandela,yonke imininingwane ezotholakala la iyoba imfihlo sizokunika inambolo okuzoba ngeyakho ukuze uvikeleke.*

Ngabe ikhona inzuzo ozoyithola ngalolucwaningo?

- *Inzuzo ebhekene nawe ngqo ukuthi uzoba nolwazi olunzulu lokuqonda ukuthi lomushanguzo isebenza kanjani ezinsweni zakho . Ucwaningo lizosinika umphumela yokuthi isebenza kanjani iTDF ezinyangeni12 nomuthelela ezinsweni zakho* .uma kukhona okungahambi kahle ifayela lakho liyobonwa udokotela wakho omuhambayo
- *Inzuzo engaqondene nawe ukuthi isizukulwane esilandelayo iyokwazi ukuthola ulwazi ngokusebenza kweTDF nokuthi kunakekelwa kanjani ngayo nokulawula ukusebenza kwayo.*

Ingabe zikhona izingozi ezingakwehlela ngokubamba iqhaza kulolucwaningo?

- *Ukudonswa kwegazi ngokujwayelekile kwakho emtholampiloudonswa udokotela noma unesi wasemtholampilo uma kudonswa igazi uzozwa ubuhlungu okuntshuntshudayo engalweni.* Ngendlela evumelekile engunyazwe *nguMnyango Kazwelonke waseNingizimu Africa.*
- *Okunye ukuyincuphe okungenzeka ukuthi imininingwane yakho ingagcina yaziwa abasebenzi baseMtholampilo wakho ababandekelayo kulolucwaningo*
- *Noma kukhona ubungozi kodwa ukuba yinxenye yalolucwaningo kuzosisiza kakhulu sonke ngokufunda okuningi*

Yini ongayenza uma kunesimo esingenza ukuba ungaphatheki kahle okubangelwa ukuba yingxenye yalolucwaningo?

- *Uma kwenzeka ubaneminye imibuza mayelana nemiphumela yegazi lakho ovumelekile ukuba ubuze umhlengi ongu Sr Sabelo Nkosi okufundela ukubhekwa kwamagazi uzokwazi nokuphendula imibuzo yakho.*
- *Sr Sabelo Nkosi kuyodingekauzokwazi ububhekana gqo neziguli ezibambe iqhaza kulolucwaningo.*
- U*mphenyi ophethe lolucwani uzokwazi ukuxhumana naye ngasosonke isikhathi kulenombolo enikeziwe ngaphezulu(bheka imininingwane yokuxhumana ekhasini 4 of 6).*

Ngubani onemvume oykufinyelela kulolulwazi ?

- *Akukho amagama noma izibongo ezizokhishwa noma zaziwe abanye abantu kuphela umphenyi waloluchwaningo nomhlengikazi asebenzisana naloluhlelo locwaningo.abase North west University abagunyeziwe ukuba bazokwazi ukufinyelela kulolulwazi kodwa ngeke bebone ukuthi ubani okuthathwe kuye lolulwazi njengoba kuzobe kusetshenziswa izinombolo.*

- *Umphenyi uzoqinisekisa ukuthi alukho ulwazi oluphathelene nawe oluphumayo kuzosetshenziswa izinombolo ozonikwa yona ezosetshenziswa abaphenyi nabafunda ngaloluchwaningo.*
- *Idatha kuzoqikelelwa ukuba lonke ulwazi olutholakala kulolucwaningo lubekwa endaweni ephephile afihlwe,akhiyelwe emakhabetheni nalolu oluyobe lwikwiKhomputha kuyodingeka ingama eliyimfihlo ukuze ukwazi ukungena.*
- *Lonke ulwazi oluzotholakala kulolu cwaningo luzogcinwa iminyaka emihlanu emva kololucwaningo luphothuliwe.*

Kuzokwenzekani ngolwazi oluzotholakala lapha / amasampula?

- *Kuzo qoqwa kube kanye yonke imiphumela.lonke ulwazi luzobekwa kude likhiyelwe lisetshenziswe umphenyi noma kunikezwa abase North West University ukuze bekugcine luphephile emva kwalolucwaningo Umphathi womphenyi walolucwaningo uzoqisekisa ukuthi umphenyi akasali namaphepha azobe ephethe lolucwaningo nangayiphi indlela.*
- *Akukho amagazi uyothathwa futhi agcinwe emva kwalolucwaningo ukuze ayobhekwa ehlaziywe.*
- *Abahlanganyeli banelungelo lokuhoxa noma yingasiphi isikhathi nangaphambi kokuqalwa kocwaningo ngaphandle kwesizathu noma ukubandlululwa nokugxekwa futhi lololwazi akauvumelekile ukuba luphume.*

Ingabe uzokhokhelwa na ngokuba yinxenye yalolucwaningo futhi ngabe zikhona yini izindleko kulolucwaningo?

Yebo. Bonke abazobamba iqhaza kulolucwaningo kuzohlelwa indlela yokuxhumana nabo,ngenkathi kuqhubeka loluchwaningo bayocelwa uma beze emtholampilo ngokujwayelekile ukuba banaloyini uthando lokubamba iqhaza kulolucwaningo,abaphoqelekile ukuba bevume ngalesosikhathi

Bazonikwa isikhathi sokuyocabanga ukuba bayathanda yini ukuba yingxenya yalolu cwaningo uma bevuma bayonikwa iphepha okuyomele begcwalise kulo ukuba bayavuma ukuba yingxenye yalolucwaningouma kwenzeka uza emtholampilo kungasilo usuku lakho lokuza uyokukhokhelwa imali engangoR30 yokugibela,yalolosuku kuphela.

Zonke iziguli ezivuma ukubamba iqhaza kulolucwaningo kumele zibuyise amaphepha esivumelwano lingakapheli iviki ziwanike uSr Sabelo Nkosi okunguyena oyowafaka ekharibhothini elivaliwe eliyolandwa umphenyi wocwaningo maviki wonke.

Uma kukhona okunye ongathanda ukukwazi noma ukwenze?

- *Ungaxhumana nomphenyi kulenombolo 072 9668457 uma unemibuzo eminye noma unezinkinga.*
- *Ungaxhumana Health Research Ethics Committee nge uNkk Carolien van Zyl ngesikhathi 018 299 2089; carolien.vanzyl@nwu.ac.za uma unazo ukukhathazeka noma izikhalazo ukuthi abakaze ngokwanele iphendulwa umcwaningi.*
- *Uzothola ikhophi yalolulwazi kanyene fomu lwesivumelwano sakho wedwa.*

Uzokwazi kanjani ngokutholakala kulolucwaningo?

- *Yonke imiphumela yamagazi iyofakwa emqulweni yokugcina ulwazi ngempilo yakho osemtholampilo wakho kufakwe abasebenzi bezempilo ukuze masikhona isidingo sokushintsha imishanguzo yakho bekwazise ukuze wenze izinqumo ezithile mayelana nokwelashwa kwakho njengoba lokhu kuyingxenye standard ukwelashwa kanye nokunakekelwa nemihlahlandlela.*

- U*mbiko wokugcina ngalolu cwaningo uzokwethulwa emtholampilo uma ucwaningo seluphothuliwe.*
- *Ukhululekile ukubaxhumane nomphenyi uma udinga eziminye izimpendulo mayelana nomphumela walolucwaningo ngqo.*

Isifungo sokuqinisekisa:

Ngokusayina ngezansi, mina(igama) .... ngiyavuma ukuthatha isikhundla sallolu ucwaningo lokubhekwa ukusebenza kwezinso zami: *ukuphepha kwetenofovir njengoba lisetshenziswe inhlanganisela ukwelashwa ngezidambisi gciwane.*

Ngiyavuma ukuthi:

- Ngifunde lolu lwazi kanye nefomulwemvume futhi kubhalwe ngolimi engilaziyo ukulikhuluma nokubhala.
- Ulwazi luchaziwe kimi ngolimi engilaziyo nengilizwayo engikhululekile ukulikhuluma.
- Nginikeziwe ithuba lokubuza imibuzo kubobobabili abantu ,obekade esinikeza amaphepha emvume kanye nomphenyi futhi bangiphendule ngokugculisayo
- Ngiyaqonda ukuthi ukubamba iqhaza kulolu cwaningo **kungokuzithandela** futhi angizange ngiphoqwenoma kubenencindezelo ukuba ngithathe ingxenye.
- Ngiyaqonda ukuthi Imiphumela ethile yami yokulashwa izosetshenziswa beyithatha kwifayela lami emtholampilo.
- Ngingakhetha ukushiya kulolucwaningo nangoba isiphi isikhathi futhi kungabi nokuhlukumezeka ngalesosinqumo sami.
- Kungase umcwaningi angicele ukuba ngishiye phakathi uma kunezinkinga ezikhona noma uma ngingayilandeli imithetho yalolucwaningo ngaphambi kokuphela kocwaningo.

Isayinwe e *(indawo)* .... ngomhlaka *(usuku)* .... 20 ....

…….. ……..

Isiginesha yomhlanganyeli **Isiginesha kafakazi**

Isifungo sokuqinisekisa ukuthola kwemvume:

I *(igama)* .... amemezele ukuthi:

- ulwazi olukulencwadi luchaziwe ukuthi ....
- umhlanganyeli ukhuthaziwe ukuba abuze imibuzo futhi mina ngithathe isikhathi ukuyiphendula.
- Nganelisekile wukuthi yena / watshelwa ngokwanele, uqonda zonke izici zocwaningo, njengoba kuxoxwa ngenhla.
- Ngisebenzise / angiazange ngisebenzise unotolika.

Isayinwe e *(indawo)* .... ngomhlaka *(usuku)* .... .20 ....

…… ……

Isiginesha umuntu ukuthola kwemvume **Isiginesha yofakazi**

Isifungo sokuqinisekisa komcwaningi:

I *(igama)* .... amemezele ukuthi:

- ulwazi oluku lencwadi luchazwe ku ... .
- ngibakhuthazile ukuba babuze imibuzo futhi ngathatha isikhathi esanele ukuyiphendula.
- Nganelisiwe wukuthi yena / watshelwa ngokwanele, ukuqonda zonke izici zocwaningo, njengoba kuxoxwa ngenhla.
- Ngikwenzile / angikwezanga ukusebenzisa unotolika.

Isayinwe e *(indawo)* .... ngomhlaka *(usuku)* .... 20 ....

…….. …….

Isiginesha of umcwaningi **Isiginesha lobufakazi**

ANNEXURE 3: PATIENT INFORMATION RECORD FROM HEALTH FILES

1. Patient Details (Study number:)

NAME	
SURNAME	
DOB	
Gender	M / F
Account Number	
Phone number	

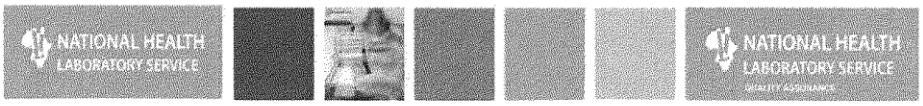
2. Long –Term Record

mm/yy HIV diagnosed		Tenofovir start date (At this clinic)	/ /
Previous ARV treatment		Current ARV treatment	
Duration on previous treatment (PMTCT/HAART/PEP)		Duration on current treatment	
Other chronic medical condition		Chronic medication (other than ARVs)	

3. Clinical Information

	Baseline	12 months
Height (m)		
Viral load (copies/ml)		
CD4 cell count (cells/µl)		
Serum creatinine (µmol/l)		
Weight (kg)		
CrCl according to CG method		

ANNEXURE 4: NHLS STANDARD OPERATING PRECEDURES TO DETERMINE SERUM CREATININE VALUES



STANDARD OPERATING PROCEDURE

Title: Creatinine

Document number: GPL3164

Version number: 2
(Changes from previous version highlighted)

Written by: T Naidoo

Checked by: N/A

Approved by: R Bridgemohan

Active date: 07-05-2012

Date of next review	*Date reviewed*	*Reviewed by*	*Action*
07-05-2013			

Date withdrawn: .....

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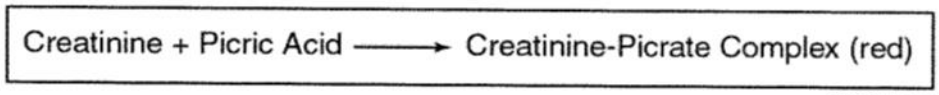
From: To:0086622437 14/10/2014 08:40 #025 P.001/004

1. OBJECTIVE

The creatinine test is intended for the quantitative determination of creatinine in serum and urine. Creatinine is an end-product of creatine metabolism. It is found in the blood and in muscle, and is excreted by the kidneys. The level of creatinine is an indicator of kidney function, and measurements thereof are useful in the diagnosis and treatment of renal disease. Decreased values are not clinically significant. Increased values may be found in all instances of renal impairment.

2. PRINCIPLE

Creatinine concentration is measured by means of the Jaffe rate method. A precise volume of sample is injected in a reaction cup containing an alkaline picrate solution. Creatinine from the sample combines with the reagent to produce a red color complex. Absorbance readings are taken at both 520 nm and 560 nm at 25.6 seconds after sample introduction. The differential absorbance has been shown to be a direct measure of the concentration of creatinine in the sample.



E015216L.EPS

3. RESPONSIBILITY

A qualified medical technologist or medical technician, or a registered student medical technologist or student medical technician will process the test, and the result will be verified by a qualified medical technologist/technician.

4. FREQUENCY OF TESTING

Daily

5. SPECIMEN COLLECTION AND HANDLING

Upon receipt, the patient identification details on the specimen are checked to ensure that they correlate with the information on the request form. If not, the specimen will be rejected. The specimen is logged in on the computer and labeled.

6. SAMPLE TYPE and SAMPLE STORAGE

Venous blood in an SST tube with gel. The only additive is a clot activator.
Tubes of blood are to be kept closed at all times and in a vertical position. Ideally, the sample should be centrifuged within 2 hours from the time of collection. Serum should be tested within 8 hours if the sample is kept at room temperature, or within 48 hours if the sample is kept between 2°C and 8°C. If testing cannot be done within 48 hours, the sample should be frozen between -15°C and -20°C. Frozen samples should be thawed only once
Urine – 24 hour urine sample collected in a bottle containing 5ml of 10% thymol in iso-propanol. Storage as for serum.

7. EQUIPMENT AND MATERIALS

Equipment: SYNCHRON CX7 analyser or DXC800 analyser
Reagents: CX7 – Creatinine reagent product no 403340
DXC800 - Creatinine reagent product no 472525

8. CALIBRATION

CX7: Calibration is done daily using Synchron CX calibrators 1, 2 and 3
DXC800: Calibration is done daily using Synchron Systems Aqua Cal 1, 2 and 3

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From: To:0086622243 79 14/10/2014 08:43 #025 P.002/004

9. QUALITY CONTROL
Internal quality control: Refer to CHE0990
External quality control: Refer to CHE0989

10. SAFETY PRECAUTIONS
All controls and specimens should be treated as potentially infectious.
Protective gear comprising laboratory coats and disposable gloves should be worn when doing this test.
All waste matter should be discarded into a bucket containing a solution of Biocide.

11. PREPARATION OF REAGENTS, CONTROLS, CALIBRATORS AND SAMPLES
Reagents: CX3 – Carefully pour the Picric Acid Solution into the Buffer bottle. Replace cap and mix at least 10 times by gentle inversion. Allow the reagent to equilibrate to room temperature for a minimum of 12 hours before loading onto the instrument. Once reconstituted and loaded, reagent is stable for 30 days or until the expiry date on the bottle. Prior to use, reagent is stored at room temperature.
DXC800: Carefully pour the Picric Acid Solution into the Buffer bottle. Replace cap and mix at least 10 times by gentle inversion. Allow the reagent to equilibrate to room temperature for a minimum of 12 hours before loading onto the instrument. Once reconstituted and loaded, reagent is stable for 30 days or until the expiry date on the bottle. Prior to use, reagent is stored at room temperature.
Calibrators: CX3 - Synchron CX calibrators 1, 2 and 3 which are stable until the expiration date printed on the bottles when stored at 2°C - 8°C. No preparation necessary.
DXC 800: Synchron Systems Aqua Cal 1, 2 & 3 which are stable until the expiration date printed on thebottles when stored at 2°C - 8°C. No preparation necessary.
Controls: Synchron controls do not require preparation, are stored at between -15°C and -20°C and are stable until the expiry date on the box.
Samples: Serum - Samples should be centrifuged at 4200 r.p.m. for 5 minutes
Urine: Measure the urine volume (for 24 hour collections), mix the specimen then aliquot a few mls into a labeled bar-coded tube for processing. If urine is turbid, it should be centrifuged before analysis.

12. METHOD
1. At the start of the day check the reagent levels, and if less than 50% full, load more reagent, recording the lot number and expiry date on the Reagent Replacement Chart.
2. Go to the calibration screen, and select the chemistry for calibration, and proceed with calibration.
3. Do the control run using Synchron controls in barcoded tubes.
4. When control results have printed, and no flags appear, thus indicating that controls are within the specified limits, process samples in barcoded tubes.

13. REFERENCE RANGES (source: LabTrak)

Age	Male	Female	Unit
0d - 30d	44 - 106	44 - 80	umol/l
1m - 12m	35 - 62	35 - 53	umol/l
1y - 3y	35 - 62	35 - 62	umol/l
4y - 6y	44 - 71	44 - 71	umol/l
7y - 9y	53 - 80	44 - 80	umol/l
10y - 12y	53 - 88	53 - 88	umol/l
13y - 15y	53 - 106	62 - 97	umol/l
16y - 17y	71 - 123	71 - 106	umol/l
18 y →	80 - 115	53 - 97	umol/l

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From: To:0086622437 9 14/10/2014 08:44 #025 P.003/004

Urine: Males 7.1 – 17.7 mmol/24 hours
Females 5.3 – 15.9 mmol/24hours

14. ALERT / CRITICAL VALUES

Critical levels: Serum: <20 and >2200 umol/l
Urine: n/a

15. REPORTING OF RESULTS

Results are verified electronically and are reported in umol/l for serum and mmol/l or mmol/24hrs for urine.

16. INTERFERENCES & POTENTIAL SOURCES OF VARIABILITY

Gross haemolysis may interfere with this methodology therefore grossly haemolysed samples should not be processed.
Lipaemia may interfere with this methodology therefore lipaemic samples should not be processed.

17. PROCEDURE FOR ABNORMAL RESULTS

Serum results of less than 20 and greater than 2200 must be checked.
Specimens giving results of "OIR" high must be diluted 1 in 2 with saline and re analysed.
A urine giving a result of "OIR low" must be reprogrammed as a serum and rerun.

18. PERFORMANCE SPECIFICATIONS

The performance specifications for the DXC800 including linearity, precision, uncertainty of measurement, detection limit, measuring interval, accuracy and sensitivity & specificity, are incorporated in the manufacturer's instructions contained in the SYNCHRON CLINICAL SYSTEMS CHEMISTRY INFORMATION MANUAL Version November 2006 The performance specifications for the CX7 including linearity, precision, uncertainty of measurement, detection limit, measuring interval, accuracy and sensitivity & specificity, are incorporated in the manufacturer's instructions contained in the SYNCHRON CX® SYSTEMS CHEMISTRY INFORMATION MANUAL Version May2000. Both manuals are available in electronic format on CD.

19. REFERENCES

i) Clinical Chemistry – Principles, Procedures, Correlations 2nd edition – Bishop, Duben-Engelkirk& Fody.
ii) Interpretative Clinical Chemistry – Bernard E Statland (published by Beckman Coulter).
iii) SYNCHRON CX® SYSTEMS CHEMISTRY INFORMATION MANUAL Version: May 2000.
iv) SYNCHRON CLINICAL SYSTEMS CHEMISTRY INFORMATION MANUAL Version: November 2006.

In the event of a dispute concerning this document, the electronic version stored on Q-Pulse will be deemed to be the correct version

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From: To:0086622437 14/10/2014 08:46 #025 P.004/004

ANNEXURE 5: EQUIPMENT INFORMATION SHEET – DETERMINING SERUM CREATININE CONCENTRATION

SYNCHRON® System(s)
Chemistry Information Sheet

CREm
Creatinine
REF 472525

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

CREm reagent, when used in conjunction with SYNCHRON LX® System(s), UniCel® DxC 800 System and SYNCHRON® Systems AQUA CAL 1 and 2, is intended for the quantitative determination of creatinine concentration in human serum, plasma or urine.

CLINICAL SIGNIFICANCE

Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.

METHODOLOGY

The SYNCHRON® System(s) determine creatinine concentration by means of the Jaffe rate method.[1]

A precise volume of sample (16.5 microliters serum or 5.5 microliters urine) is injected in a reaction cup containing an alkaline picrate solution. The ratio used is one part sample to 35 parts reagent for serum and one part sample to 105 parts reagent for urine. Creatinine from the sample combines with the reagent to produce a red color complex. Absorbance readings are taken at 520 nanometers between 19 and 25 seconds after sample injection. The absorbance rate has been shown to be a direct measure of the concentration of creatinine in the sample.[2,3,4]

CHEMICAL REACTION SCHEME

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[5] Freshly drawn serum, plasma or properly collected urine (random/timed) are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.[6]
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.[6]
3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container should be kept in the refrigerator or on ice during the timed period. No preservative is required.[7]

ADDITIONAL SPECIMEN STORAGE AND STABILITY CONDITIONS AS DESIGNATED BY THIS LABORATORY:

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

CRITERIA FOR SAMPLE REJECTION AS DESIGNATED BY THIS LABORATORY:

PATIENT PREPARATION

SPECIAL INSTRUCTIONS FOR PATIENT PREPARATION AS DESIGNATED BY THIS LABORATORY:

SPECIMEN HANDLING

SPECIAL INSTRUCTIONS FOR SPECIMEN HANDLING AS DESIGNATED BY THIS LABORATORY:

REAGENTS

CONTENTS

Each kit contains the following items:

Two Alkaline Buffer Bottles (1600 mL)
Two Picric Acid Solution Bottles (400 mL)

VOLUMES PER TEST

Sample Volume	Serum 16.5 µL
	Urine 5.5 µL
Total Reagent Volume	570 µL

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

ALKALINE BUFFER:	
Sodium Hydroxide	0.188 mol/L
PICRIC ACID SOLUTION:	
Picric Acid	0.05 mol/L

Also non-reactive chemicals necessary for optimal system performance.

EUROPEAN HAZARD CLASSIFICATION

Picric Acid Solution	C;R1-35	Explosive when dry.
		Causes severe burns.
	S28	After contact with skin, wash immediately with plenty of water.
	S35	This material and its container must be disposed of in a safe way.
	S37/39	Wear suitable gloves and eye/face protection.
Creatinine Alkaline Buffer	C;R35	Causes severe burns.
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S37/39	Wear suitable gloves and eye/face protection.
	S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems AQUA CAL 1 and 2
At least two levels of control material
Saline

REAGENT PREPARATION

Carefully pour 400 mL of Picric Acid Solution into the 1600 mL Alkaline Buffer bottle. Replace cap and mix at least 10 times by gentle inversion.

1. Carefully pour 400 mL of Picric Acid Solution into the 1600 mL Alkaline Buffer bottle.
2. Replace cap and mix at least 10 times by gentle inversion.
3. Record preparation date on the end label.
4. If excessive foam is produced when mixing, allow foam to dissipate before loading.

5. Freshly prepared creatinine reagent may contain micro air bubbles that may result in calibration failure or calibration with low span. To prevent this phenomenon, allow the prepared reagent to sit with the cap loosened for a minimum of 30 minutes (or over night) before loading onto the instrument.

NOTICE
Do not reuse old reagent containers or mix fresh reagent with old reagent.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

Alkaline Buffer and Picric Acid Solution stored unopened and unmixed at room temperature are stable until the expiration dates indicated on each bottle. The combined Creatinine Reagent is stable on-instrument for 30 days from the date of preparation, or by expiration date of either component, if sooner. Do not freeze or refrigerate.

If reagent is frozen in transit, thaw completely, warm to room temperature and mix thoroughly by gently inverting bottle a least 10 times.

NOTICE
At reduced temperature, a precipitate may form in the Alkaline Buffer or combined Creatinine Reagent. Do not filter the precipitate. DO NOT USE combined Creatinine Reagent until all precipitate is completely redissolved. It will redissolve upon warming to +21°C to +25°C without any loss of reactivity. A +25°C water bath may be used to warm reagent. Mix after redissolving precipitate by inverting bottle 10 times.

REAGENT STORAGE LOCATION:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems AQUA CAL 1 and 2

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

1. If unopened, the calibrators should be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable at room temperature for 30 days.
2. Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

CALIBRATOR STORAGE LOCATION:

CALIBRATION INFORMATION

1. The system must have a valid calibration in memory before controls or patient samples can be run.
2. Under typical operating conditions the CREm assay must be calibrated every 72 hours or with each new bottle of reagent and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 Systems *Instructions for Use* (IFU) manual.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.
4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addItion, these controls should be run with each new calibration, with each new bottle of reagent, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of addItional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

TABLE 1 QUALITY CONTROL MATERIAL

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.

4. After loading samples and controls onto the system, follow the protocols for system operations.

For detailed testing procedures, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

The SYNCHRON® System(s) performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.[8]

TABLE 2 REFERENCE INTERVALS

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Literature	Serum or Plasma (Male)	0.9 – 1.3 mg/dL	80 – 115 µmol/L
	Serum or Plasma (Female)	0.6 – 1.1 mg/dL	53 – 97 µmol/L
	Urine (Male)	800 – 2000 mg/24 hrs	7.1 – 17.7 mmol/24 hrs
	Urine (Female)	600 – 1800 mg/24 hrs	5.3 – 15.9 mmol/24 hrs
SYNCHRON	Serum or Plasma (Male)	0.64 —1.27 mg/dL	57 — 113 µmol/L
	Serum or Plasma (Female)	0.44 1.03 mg/dL	39 91 µmol/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (9, 10, 11) for guidelines on establishing laboratory-specific reference intervals.

ADDITIONAL REPORTING INFORMATION AS DESIGNATED BY THIS LABORATORY:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

TABLE 3 COMPATIBLE ANTICOAGULANTS

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	AVERAGE PLASMA-SERUM BIAS (mg/dL)[a]
Ammonium Heparin	14 Units/mL	NSI
Lithium Heparin	14 Units/mL	NSI
Sodium Heparin	14 Units/mL	NSI

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	AVERAGE PLASMA-SERUM BIAS (mg/dL)[a]
Potassium Oxalate/Sodium Fluoride	2.0 / 2.5 mg/mL	NSI

LIMITATIONS

If urine samples are cloudy or turbid, it is recommended that they be centrifuged before transfer to a sample cup.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

TABLE 4 INTERFERENCES

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[b]
Acetoacetic Acid	Acetoacetic Lithium Salt	5 mg/dL	+ 0.04 mg/dL[c]
		50 mg/dL	+ 0.4 mg/dL[c]
		125 mg/dL	+ 0.9 mg/dL
		500 mg/dL	+ 3.5 mg/dL
Bilirubin (unconjugated)	Bovine	20 mg/dL	- 0.2 mg/dL
Cefaclor	NA[d]	100 µg/dL	+ 0.2 mg/dL
Cefoxitin	Cefoxitin sodium salt	50 µg/mL	+ 0.2 mg/dL
Cephalothin	NA	50 µg/mL	+ 0.2 mg/dL
α-D-Glucose	NA	1000 mg/dL	+ 0.2 mg/dL
Fluorescein	Fluorescein Disodium Salt	220 mg/dL	Results suppressed
Glutathione	NA	1.5 mmol/L	+ 0.2 mg/dL
Hemoglobin	RBC hemolysate	500 mg/dL	NSI[e]
L-Dopa	NA	160 mg/dL	- 0.2 mg/dL
Lipemia	Intralipid[f]	500 mg/dL	NSI
	Human	Serum Index 8	NSI
Methyl dopa	NA	10 mg/dL	- 0.2 mg/dL
Pyruvic acid	NA	5 mg/dL	+ 0.2 mg/dL
Sulfasalazine	NA	60 mg/dL	NSI
Sulfobromophthalein	Sulfobromophthalein sodium salt	2.0 mg/dL	NSI

2. Lipemic samples with visual turbidity >3+, or with a Lipemia Serum Index >10, should be ultracentrifuged and the analysis performed on the infranate.
3. Refer to References (12,13,14,15) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical ranges:

TABLE 5 ANALYTICAL RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	0.1 – 25 mg/dL	8.84 – 2210 µmol/L
Urine	10 – 400 mg/dL	0.88 – 35.36 mmol/L

Samples with activities exceeding the high end of the analytical range should be diluted with saline and reanalyzed.

REPORTABLE RANGE (as determined on site):

TABLE 6 REPORTABLE RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for this analyte determination is 0.1 mg/dL (8.84 µmol/L) for serum or plasma and 10 mg/dL (0.88 mmol/L) for urine.

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or Plasma (in the range of 7 to 130 mg/dL):

Y (SYNCHRON LX Systems)	= 0.964X - 0.02
N	= 97
MEAN (SYNCHRON LX Systems)	= 6.78
MEAN (SYNCHRON CX7 DELTA)	= 7.04
CORRELATION COEFFICIENT (r)	= 0.9993

Urine (in the range of 18.2 to 399 mg/dL):

Y (SYNCHRON LX Systems)	= 0.997X - 1.70
N	= 94
MEAN (SYNCHRON LX Systems)	= 135.8
MEAN (SYNCHRON CX7 DELTA)	= 138.0
CORRELATION COEFFICIENT (r)	= 0.9983

Serum or Plasma (in the range of 1.0 to 24.3 mg/dL):

Y (UniCel DxC Systems)	= 1.037X - 0.01
N	= 137
MEAN (UniCel DxC Systems)	= 2.8
MEAN (SYNCHRON LX Systems)	= 2.7
CORRELATION COEFFICIENT (r)	= 0.999

Urine (in the range of 17.9 to 412.7 mg/dL):

Y (UniCel DxC Systems)	= 1.000X + 0.97
N	= 110
MEAN (UniCel DxC Systems)	= 136.1
MEAN (SYNCHRON LX Systems)	= 135.2
CORRELATION COEFFICIENT (r)	= 1.000

Serum (in the range of 4.42 to 22.45 mg/dL):

Y (UniCel DxC Systems)	= 1.01X - 0.03
N	= 39
MEAN (UniCel DxC Systems)	= 4.42
MEAN (Isotope Dilution Mass Spectroscopy reference procedure (16))	= 4.40
CORRELATION COEFFICIENT (r)	= 0.9996

Refer to References (17) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit imprecision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the imprecision of various methods, proficiency test summaries, and literature sources.

TABLE 7 MAXIMUM PERFORMANCE LIMITS

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[g]		% CV
		mg/dL	µmol/L	mg/dL	µmol/L	
Within-run	Serum/Plasma	0.1	9	3.3	300	3.0
Total	Serum/Plasma	0.2	13	3.3	300	4.5
Within-run	Urine	2.0	177	66.7	5900	3.0
Total	Urine	3.0	265	66.7	5900	4.5

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[18] Each laboratory should characterize their own instrument performance for comparison purposes.

TABLE 8 NCCLS EP5-T2 PRECISION ESTIMATE METHOD

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[h]	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	% CV
Within-run	Serum	Control 1	1	80	0.57	0.03	4.8
	Serum	Control 2	1	80	7.86	0.08	1.0
	Urine	Control 1	1	80	90.90	0.71	0.8
	Urine	Control 2	1	80	244.73	1.72	0.7
Total	Serum	Control 1	1	80	0.57	0.05	8.2
	Serum	Control 2	1	80	7.86	0.25	3.1
	Urine	Control 1	1	80	90.90	2.28	2.5
	Urine	Control 2	1	80	244.73	6.94	2.8

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on SYNCHRON LX Systems or UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

Revision History

REVISION AF

Revised Reagent Preparation and the Reagent Storage and Stability section.

REVISION AG

Updated corporate address; updated European Hazard Classification, removed EDTA Acceptable Anticoagulant claim, and removed insert reference from content description.

REVISION AH

Added Revision History.

REVISION AJ

Added new language requirement: Czech, and Korean.

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18. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices* Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

EC REP Beckman Coulter Ireland, Inc., Mervue Business Park, Mervue, Galway, Ireland 353 91 774068

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.

ENDNOTES

a NSI = No Significant Interference (within ± 0.2 mg/dL or 6%).

b Plus (+) or minus (-) signs in this column signify positive or negative interference.

c The observed effect at 5 and 50 mg/dL levels of acetoacetic acid are calculated based on the extrapolation of the interference data collected with 0, 125, 250, 375, and 500 mg/dL of acetoacetic acid.

d NA = Not applicable.

e NSI = No Significant Interference (within ±0.2 mg/dL or 6%).

f Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

g When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

h The point estimate is based on the data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer`s instructions

ANNEXURE 6: LETTER OF ACKNOWLEDGEMENT FROM DISTRICT MANAGER

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE DISTRICT MANAGER
Tel: 034 328 7002
Fax: 03431 32123
E-mail: pravina.naidoo@kznhealth.gov.za
www.kznhealth.gov.za

ENQUIRIES : ABDUS-SAMAD CASSIM

TO	:	ARTHI GAJEE
FROM	:	DISTRICT MANAGER
		MRS. A.M.E.T TSHABALALA
DATE	:	31/10/2014
REF	:	17/1
RE	:	INTENTION TO CONDUCT RESEARCH

I hereby acknowledge your communique dated 10th of October 2014 (intention of conducting research at Newcastle Clinic in Amajuba District).

This letter confirms to the respective that the District Office of Health is aware of your request to conduct research and serves for the purpose of your ethics approval.

This letter is not a confirmation of permission being granted to conduct research at the requested facility.

Thank you

.....

MRS. A.M.E.T TSHABALALA
DISTRICT MANAGER
AMAJUBA DISTRICT

ANNEXURE 7: ETHICS LETTER OF APPROVAL FROM NWU HREC COMMITTEE

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

Private Bag X6001, Potchefstroom
South Africa 2520

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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: THE RENAL SAFETY PROFILE OF TENOFOVIR AS USED IN COMBINATION ANTIRETROVIRAL THERAPY

Project Leader: Dr M Viljoen

Ethics number: N W U - 0 0 0 4 4 - 1 5 - A 1
(Institution | Project Number | Year | Status)

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2015-08-18 **Expiry date**: 2015-12-30 **Category** N/A

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 (principle 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-RERC. Would there be deviated 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: 2015.08.18 19:28:05 +02'00'

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

ANNEXURE 8: ETHICS APPROVAL LETTER FROM THE DOH KWA-ZULU NATAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
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Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM235/15
NHRD Ref.: KZ_2015RP40_426
Enquiries : Ms G Khumalo
Telephone : 033 – 395 3189

Dear Mrs A Gajee

Subject: Approval of a Research Proposal

1. The research proposal titled **'The renal safety profile of tenofovir as used in combination antiretroviral therapy'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

The proposal is hereby **approved** for research to be undertaken at Newcastle Primary Healthcare Clinic.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 07/09/15

ANNEXURE 9: LETTER OF GOODWILL FROM UNIT MANAGER AT PHC CLINIC

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Newcastle Clinic
Corner of Sutherland and Murchison street
Newcastle
2940

TO WHOM IT MAY CONCERN

Dear Sir / Madam

I hereby provide a goodwill concent to Mrs. Artal Gajee to conduct her research for Masters in Pharmacy at Newcastle Primary Health Care Clinic.

We will Provide her with a room to analyse her files.

Kind Regards

Mrs. Gugu Ngema (OMN)

NEWCASTLE PRIMARY
HEALTH CARE CLINIC
C/O Sutherland & Murchison Street
Private X6521
Tel (034) 326 7734
Fax (034) 326 7[illegible]

1/1 Pharmacy\Newcastle\Hospital 0543280000 2015/May/20 11:10:43 AM

ANNEXURE 10: GOOD CLINICAL PRACTICE COURSE CERTIFICATE

BCOMPLIANT cc

CERTIFICATE OF COMPETENCE

THIS SERVES TO CERTIFY THAT

Arthi Anandrai Gajee-Eloff

HPCSA Registration No: *Pharmacy Council 17954*

HAS SUCCESSFULLY PASSED A

GCP INTRODUCTION COURSE

IN PRETORIA

FACILITATOR SIGNATURE: RETHA BRITZ

16 NOVEMBER 2012
DATE:

SUMMATIVE TEST SCORE: **90 %**

CPD Accreditation Number: MDB015/003/01/2012
CPD Points allocated: 4 Ethics & 6 Clinical CPD Points

ANNEXURE 11: HEALTH SA GESONDHEID GUIDELINES

HEALTH SA GESONDHEID

Journal of Interdisciplinary Health Sciences

AUTHOR INFORMATION PACK

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ISSN: 1025-9848

DESCRIPTION

Health SA Gesondheid - Journal of Interdisciplinary Health Sciences is an open access, peer-reviewed
interdisciplinary and interprofessional scholarly journal that aims to promote communication,
collaboration and teamwork between professions and disciplines within the health sciences to address
problems that cross and affect disciplinary boundaries.
Health SA Gesondheid - Journal of Interdisciplinary Health Sciences publishes original articles on issues related to public health, including implications for practical applications and service delivery
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Health SA Gesondheid - Journal of Interdisciplinary Health Sciences explores issues and posits
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GUIDE FOR AUTHORS

INTRODUCTION

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ANNEXURE 12: MICROSOFT EXCEL® RESEARCH TOOL

Long Term Record								
Study number	Date diagnosed	Previous ARV Treatment	TDF Start at this clinic	Current ARV Treatment (PMCT/HAART/PEP)	Duration on previous ARV Treatment (PMCT/HAART/PEP)	Duration on current ARV Treatment (FDC)	Other chronic medical condition	Chronic Medication (other than ARVs)

Clinical information									
Baseline (IST time on TDF)									
Study number	Age	Gender	Constant	Viral Load (cp/ml)	CD 4 count	Serum creatinine (µmol/l)	Weight (Kg)	CrCl (ml/min)	BMI

Clinical information							
12 month follow up							
Study number	Age	Viral load (cp/ml)	CD 4 count	Serum Creatinine (µmol/l)	Weight	CrCl (ml/min)	BMI

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EDITING EXCELLENCE

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editingexcellencepotch@gmail.com

This is to certify that the degree,

Master in Pharmacy

of

Arthi Gajee

has been edited by

Valerie Viljoen, Editing Excellence.

The dissertation has been edited and includes the following:

Pages v - viii

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Date:29 June 2016

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