ASSESSING RENAL FUNCTION AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS

by

CHOSHI JOEL MABAKANE

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SUPERVISOR: Dr. S Hanser

CO-SUPERVISOR: Dr. B Flepisi (University of Pretoria)

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DECLARATION

I declare that ASSESSING RENAL FUNCTION AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any institution.

Choshi Joel Mabakane	25 October 2021
NAME	**DATE**

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ABSTRACT

The purpose of this study was to investigate the effect of cART on renal function and assess the association between renal function and cardiovascular risk factors in a black rural HIV-positive population in Limpopo Province, Mankweng district. We have conducted a cross-sectional study which included both male and female cART-treated patients (n=84), cART-naïve patients (n=27) and HIV-negative controls (n=44). We have measured biomarkers of renal function (plasma cystatin C, clusterin, retinol binding protein 4 [RBP4]) and determined the estimated glomerular filtration rate (eGFR) using the chronic kidney disease-epidemiology collaboration formula (CKD-EPI). We have also measured blood pressure (BP), body mass index (BMI) and fasting blood glucose (FBG). The prevalence of renal dysfunction was similar among the study groups. A significant difference in RBP4 was found among the groups after controlling for covariates (age, gender, alcohol consumption, BMI, systolic blood pressure and FBG) ($F(2, 146) = [4.479]$, $p=0.010$). The significant difference in RBP4 was specifically observed between the cART-treated and cART-naïve groups ($p=0.008$). Cystatin C, clusterin and eGFR were not significantly different among the study groups after controlling for the covariates. The cardiovascular risk factors age ($\beta=0.207$; $p=0.039$), $CD4^+$ T-cell count ($\beta=-0.236$; $p=0.040$), and duration of cART ($\beta=0.232$; $p=0.043$) were independently associated with cystatin C. The use of cART independently associated with RBP4 ($\beta=0.282$; $p=0.004$). Age ($\beta=-0.363$; $p=0.001$), $CD4^+$ T-cell count ($\beta=0.222$; $p=0.034$) and duration of cART ($\beta=-0.230$; $p=0.034$) independently associated eGFR. Renal dysfunction is common in this HIV-positive population, with similar rates as the HIV-negative population. Plasma cystatin C as a promising alternative renal biomarker need to be re-evaluated in this HIV-positive population. RBP4 may be a more promising renal function biomarker in the HIV-positive population. Cardiovascular risk factors are associated with renal dysfunction in this rural HIV-positive population and $CD4^+$ T-cell count may be an independent predictor for renal function.

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LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
ADA	American diabetes society
ADH	Antidiuretic hormone
ADP	Adenosine diphosphate
AIDS	Acquired immunodeficiency syndrome
AKI	Acute kidney injury
ALLINIs	Allosteric HIV-1 integrase inhibitors
ANOVA	Analysis of variance
ARV	Antiretroviral drug
ATP	Adenosine triphosphate
ATV	Atazanavir
ATV/r	Ritonavir boosted atazanavir
AZT/ZDV	Zidovudine
BMI	Body mass index
BMT	Basement membrane thickness
BP	Blood pressure
cART	Combination antiretroviral therapy
CCR5	cc chemokine receptor type 5
CD4	Cluster of differentiation 4
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
CVD	Cardiovascular disease
CXCR5	cxc chemokine receptor type 4
D:A:D	Data Collection on Adverse Events of anti-HIV Drugs
D4T	Stavudine
DBP	Diastolic blood pressure
DN	Diabetic nephropathy

DNA	Deoxyribonucleic acid
DOR	Doravirine
DRV	Darunavir
DRV/r	Ritonavir boosted darunavir
DTG	Dolutegravir
EC	Eastern cape
ECF	Extracellular fluid
EFV	Efavirenz
eGFR	estimated glomerular filtration rate
ESRD	End stage renal disease
ETR	Etravirine
FDA	Food and drug Administration
FIs	Fusion inhibitors
FS	Free State
FSGS	Focal segmental glomerulosclerosis
FTC	Emtricitabine
G6P	Glucose-6-phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
GFR	Glomerular filtration rate
GLUT4	Glucose transporter 4
HIV	Human immunodeficiency virus
HIV/AIDS	Human immunodeficiency virus and acquired immunodeficiency syndrome
HIVAN	HIV-associated nephropathy
HIVICK	HIV immunocomplex kidney disease
HK	Hexokinase
HOPS	HIV outpatient study
HT	Hypertension
IDV	Indinavir
IFG	Impaired fasting glucose

IGT	Impaired glucose tolerance
INIs	Integrase inhibitors
InSTIs	Integrase strand transfer inhibitors
IQR	Interquartile range
IR	Insulin resistance
IRS	Insulin receptor substrate
ISAK	International society for advancement of Kinanthropometry
KDOQI	Kidney disease outcomes quality initiative
KZN	KwaZulu-Natal
LMICs	Low middle-income countries
LP	Limpopo
LPV	Lopinavir
LPV/r	Ritonavir boosted lopinavir
LSD	Fischer's least significance difference (LSD)
MATE-1	Multidrug extrusion protein 1
MATE-2K	Multidrug extrusion protein 2k
MDRD	Modification of diet in renal disease
MFI	Mean fluorescence intensity
MP	Mpumalanga
MRP-2	Multidrug resistance-associated protein 2
MRP-4	Multidrug resistance-associated protein 4
MTCT	Mother-to-child-transmission
mtDNA	Mitochondrial DNA
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NDoH	National department of health
NICE	National Institute for Health and Clinical Excellence
NKI	National kidney foundation
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIS	Nucleoside reverse transcriptase inhibitors

NtRTIs	Nucleotide reverse transcriptase inhibitors
NVP	Nevirapine
NW	North west
OAT-1	Organic anion transporter 1
OAT-3	Organic anion transporter 3
OCT-2	Organic cation transporter 2
P-gp	P-glycoprotein
PI/r	Ritonavir boosted ritonavir
PIs	Protease inhibitors
RAL	Raltegravir
RAS	Renin angiotensin system
RBP4	Retinol-binding protein 4
RCTs	Randomised controlled trials
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPM	Revolutions per minute
RTEs	Renal tubular epithelial cells
RTIs	Reverse transcriptase inhibitors
RTV	Ritonavir
RVP	Rilpivirine
SA	South Africa
SANA	South African national AIDS council
SBP	Systolic blood pressure
SD	Standard deviation
SMART	Strategies for Management of Antiretroviral Therapy
SPSS	Statistical package for the social sciences
SQV	Saquinavir
SSA	Sub-Saharan Africa
StatsSA	Statistics south Africa
T-20	Enfuvirtide

TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TNF	Tenofovir
TNF-α	Tumour necrosis factor alpha
TPV	Tipranavir
TREC	Turfloop research ethics committee
UL	University of Limpopo
UNAIDS	Joint united nations programme on HIV/AIDS
UP	University of Pretoria
US	United states
UTI	Urinary tract infection
VDR	Vitamin D Receptor
VL	Viral load
WC	Waist circumference
WC	Western cape
WHO	World Health Organisation
WHR	Waist-hip-ratio

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CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

More than 35 years have passed since the first case of human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) was reported in humans, and many countries continue to battle this disease that is now a major global health burden (World Health Organisation [WHO], 2020). The human immunodeficiency virus (HIV) adversely affects the immune system by infecting and destroying immune cells, rendering the host immunocompromised and thus unable to fight off other infections (AIDSinfo, 2016; Statista, 2020). Although there is currently no cure for HIV/AIDS, combination antiretroviral therapy (cART) has proven to effectively control the virus and prevent disease progression to AIDS (Carrion *et al*., 2018).

In the cART era, HIV infection has become a manageable chronic disease and those infected with HIV are living longer because of reductions in AIDS-related illnesses (Adih *et al*., 2011; Deeks *et al*., 2013; Eyawo *et al*., 2017). The Joint United Nations Programme on HIV/AIDS (UNAIDS) had estimated that 38 million people globally are living with HIV where 25.4 million people are on cART (UNAIDS, 2020). In 2019, globally, 1.7 million people were diagnosed with HIV and 690 000 people died of AIDS-related illnesses (UNAIDS, 2020). More than two-thirds of the global HIV infections are in Sub-Saharan Africa (SSA) with the latest statistics indicating that approximately 26 million people are living with HIV in this region (WHO, 2020).

South Africa (SA) has one of the highest prevalence rates of HIV infections in the world, with an estimated prevalence of 19% in adults aged 15–49 years and ranking

4th after Eswatini (27%), Botswana (36%), and Lesotho (23%) (Statista, 2020). The national prevalence is currently estimated to be at 13.0%, which translates to 7.9 million South Africans currently living with HIV (Johnson *et al*., 2017; Statistics South Africa [StatsSA], 2020). Furthermore, over 50% of the HIV-population in SA receives cART (UNAIDS, 2020). Thirty-five years after the discovery of HIV, the HIV epidemic is still on the rise in SA, which is a cause for concern, as more people are initiated onto the nephrotoxic cART (Yedla *et al*., 2015; Simbayi *et al*., 2019).

The burden of renal (kidney) dysfunction in the ageing HIV-positive population is escalating, where HIV, cART, and co-morbidities such as hypertension and diabetes are implicated (Naicker *et al*., 2015; Fiseha and Gebreweld, 2021). The kidney performs several functions including the metabolism and excretion of drugs (da Rocha *et al*., 2015). The kidneys are constantly exposed to ARV drugs among HIV populations. Studying the toxic effects of cART is important as it aids in the scrutiny of the safety and efficacy of ARV drugs to make more informed decisions (Carr, 2002). Regular renal assessment is particularly important for HIV-positive patients starting cART to help prevent renal dysfunction and its complications (Yombi *et al*., 2015).

A plethora of research studies were conducted to understand the effects of cART on renal function (Mouton *et al*., 2016; Diana and Naicker, 2016; Baynes *et al*., 2017; Fulgence *et al*., 2021). Generally, cART is beneficial for renal function, however, certain ARV drugs are associated with renal dysfunction (Naicker *et al*., 2015; Mocroft *et al*., 2016; Ojeh *et al*., 2018; Debeb *et al*., 2021). Initial clinical trials and observational studies have demonstrated an improvement in renal function following cART use (Winston *et al*., 2001; Cosgrove *et al*., 2002; Atta *et al*., 2006; Lucas *et al*., 2004; Mpondo *et al*., 2004; Peters *et al*., 2008; Reid *et al*., 2009).

In observational studies, cART was beneficial among patients with pre-existing HIV-associated nephropathy (HIVAN) and advanced HIV stage (Kalayjian *et al*., 2008; Cooper and Tonelli, 2011). Other studies have reported an improvement in renal function in cART-treated patients in the absence of baseline renal impairment and advanced HIV stage (Leport *et al*., 2009; Kwantwi *et al*., 2017).

Conversely, other observational studies reported increased incidence and prevalence of renal dysfunction following cART use (Mocroft *et al*., 2010; Flanre *et al*., 2011; Scherzer *et al*., 2012; Ryom *et al*., 2013; Bonjoch *et al*., 2014; Ojeh *et al*., 2018; Debeb *et al*., 2021). Specific ARV drugs such as tenofovir disoproxil fumarate (TDF), ritonavir-boosted ritonavir (LPV/r), atazanavir (ATV) and zidovudine (AZT) were implicated. Furthermore, several cardiovascular risk factors such as older age, hypertension, diabetes, and obesity were linked to renal dysfunction (Overton *et al*., 2009; Msango *et al*., 2011; Morlat *et al*., 2013; Okpa *et al*., 2019).

Cardiovascular disease (CVD) is one of the major causes of death among HIV-positive populations (Martin-Iguacel *et al*., 2015). Compared with the HIV-negative individuals, HIV-positive patients have a higher risk of CVD due to a combination of traditional and HIV-related cardiovascular risk factors (Blanco *et al*., 2010; Bocarra *et al*., 2013; Martin-Iguacel *et al*., 2015; Touloumi *et al*., 2020). These includes smoking, hypertension, HIV-related inflammation, immune activation and treatment-related cardiometabolic abnormalities such as diabetes and dyslipidaemia (Anne-Lise *et al*., 2015; Blanco *et al*., 2010; Bocarra *et al*., 2013). It has been reported that these cardiovascular risk factors are also associated with increased risk of renal dysfunction among HIV-positive populations (Ryom *et al*., 2013; Ryom *et al*., 2014; Lee e*t al*., 2017; Hou and Nast, 2018; Pallela e*t al*., 2018).

About two-thirds of global HIV infections occur in SSA which suggest that most HIV populations in this region (SA in particular) are predisposed to HIV-related

cardiovascular risk factors and higher risk of renal dysfunction. Therefore, it is imperative to address cardiovascular risk factors in the cART-treated HIV-positive population when evaluating renal function. A thorough assessment of renal function in the HIV population must take into consideration the cardiovascular risk factors to better manage renal dysfunction (Luyckx *et al*., 2017). The inconsistency on the impact of cART on renal function underpins the need to assess renal function and its association with cardiovascular risk factors in HIV-positive populations, particularly in the Sub-Saharan African region and hence the current study. Routine renal monitoring is a challenge in SSA (Salome *et al*., 2016).

1.2. PROBLEM STATEMENT

The kidneys in the HIV-positive population are continuously exposed to cART and this may cause renal dysfunction. The cardiovascular risk factors in the HIV-positive populations occur frequently and may further increase the risk of renal dysfunction (Nsagha *et al*., 2015; Pallela e*t al*., 2018). In addition, the drugs used for cardiometabolic disease may also contribute to renal dysfunction. The high prevalence of renal dysfunction in the HIV-positive population has serious clinical and economic implications (Ekrikpo *et al*., 2018). The clinically recommended serum creatinine-based method of evaluating renal function has limitations as it is influenced by factors such as age, gender, muscle mass, HIV infection and medications (citimedine, antibiotics, ARV drugs). Further, it has been less sensitive and specific, and these limitations can lead to misdiagnosis of renal dysfunction (Botev *et al*., 2011; Gagneux-Brunon *et al*., 2012; Thomas *et al*., 2017). There is a lack of data on the impact of cART on renal function in the rural HIV-positive population of Mankweng District in Limpopo Province. Therefore, it is important to investigate the impact of cART on renal function in this HIV-positive population of Mankweng District using alternative biomarkers.

1.3. AIM AND OBJECTIVES

1.3.1. Aim

To investigate the effect of cART on renal function and its association with cardiovascular risk factors among in a rural black HIV-positive population living in the Mankweng District of Limpopo Province, South Africa.

1.3.2. Objectives

i. To measure cardiovascular risk factors: cluster of differentiation 4 ($CD4^+$) T-cell count, body mass index, waist circumference, fasting blood glucose and blood pressure.
ii. To measure the plasma levels of cystatin C, retinol-binding protein 4 and clusterin, to determine renal function.
iii. To estimate glomerular filtration rate (GFR) using plasma cystatin C to determine renal function.
iv. To determine the association between cardiovascular risk factors and renal function.

1.4. OUTLINE OF SUBSEQUENT CHAPTERS

Chapter 2 Literature Review: Includes detailed background information of current literature.

Chapter 3 Methodology: Includes discussions on methods and materials employed to collect and analyse data.

Chapter 4 Results: Present and describe the findings of the study.

Chapter 5 Discussion: Interprets and explains the main findings and compares with similar studies. This chapter also highlights the limitations of the study.

Chapter 6 Summary, Recommendations, and Conclusion: Includes a summary of the main findings and highlights their significance with recommendations for future research.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

The term AIDS describes the advanced stage of HIV where the immune cells are severely depleted, and the immune function is severely impaired thus giving rise to opportunistic infections (Center for Disease Control and Prevention [CDC], 2021). HIV is a type of lentivirus that causes slowly progressing disease (Acheson, 2011; Whiteside, 2016). Lentiviruses are a subfamily within the retroviruses family that has the distinctive characteristic of reverse transcription by which the retrovirus ribonucleic acid (RNA) is converted into double-stranded deoxyribonucleic acid (DNA) (Acheson, 2011).

This fully formed double-stranded DNA is ultimately replicated within the infected cell by the host cellular enzymatic machinery (Acheson, 2011). HIV infects humans through sexual contact or blood exchange with an infected person (Harden and Fauci, 2012; Dobra *et al*., 2017). Upon infection, the virus targets and destroys immune cells called $CD4^+$ T-lymphocytes that play a key role in adaptive immunity resulting in impaired immune function and increased susceptibility to opportunistic infections (WHO, 2021).

There are two known strains of HIV in humans called HIV type 1 (HIV-1) and HIV type 2 (HIV-2) (Faria *et al*., 2014; Visseaux *et al*., 2016). HIV type 1 affects most of the world's population and it is responsible for causing the global AIDS pandemic (Sharp *et al*., 2011; Freed and Martin, 2013; Faria *et al*., 2014). In contrast, HIV-2 is a rare strain that is mainly predominant in West Africa (Visseaux *et al*., 2016). Unlike many retroviruses, HIV has a unique ability to rapidly mutate and establish latent

reservoirs that can persist and remain undetectable in humans (Nomaguchi *et al*., 2018). Thus, making it impossible to eliminate the virus with the current ARV treatment (Churchill *et al*., 2016). New infections continue to rise in both developed and developing countries despite the advancement in ARV therapy (Gökengin *et al*., 2016; Girum *et al*., 2018).

2.2. HUMAN IMMUNODEFICIENCY VIRUS AND CURRENT ANTIRETROVIRAL THERAPY PRACTICE IN SOUTH AFRICA

2.2.1. Human immunodeficiency virus epidemic

South Africa (SA) has the worst HIV epidemic with an estimated prevalence of 13% (StatsSA, 2020). The number of HIV-positive people in the country has reached a staggering 7.9 million, representing nearly 20% of global HIV infections (UNAIDS special analysis, 2020; StatsSA, 2020). The HIV epidemic in SA is described as nonhomogeneous whereby there are variations in the prevalence and incidence at provincial, district, and local levels (Simbayi *et al*., 2019; Johnson and Dorrington, 2019). The HIV prevalence and incidence also vary by age, sex, race, and socioeconomic status (Shisana *et al*., 2014; Simbayi *et al*., 2019).

The HIV prevalence in persons aged 15–49 years ranged from 10.3–27.4% across the provinces in 2018, with Western Cape (WC) recording the lowest prevalence and Kwazulu-Natal (KZN) recording the highest (Johnson and Dorrington, 2019). The Limpopo Province displayed an HIV prevalence of 14.5% in persons aged 15–49 years (Johnson and Dorrington, 2019). There has been a steady rise in HIV prevalence observed in all nine provinces albeit provinces such as KZN, Mpumalanga (MP), Free State (FS), and North West (NW) have reached the highest prevalence (Johnson and Dorrington, 2019). The HIV prevalence in persons between the ages of 15–49 years seem to be growing rapidly in LP, WC, and Eastern

Cape (EC) provinces in comparison with other provinces which is a grave concern (Johnson and Dorrington, 2019). A high HIV burden has serious clinical and economic implications.

With regards to racial differences, black South Africans are disproportionately affected by HIV compared to the coloured, white, and Indian racial groups (Simbayi *et al*., 2019). The HIV prevalence in black Africans, coloureds, whites, and Indians was found to be 16,6%, 5.3%, 1.1%, and 0.8%, respectively (Simbayi *et al*., 2019). On the other hand, there was a HIV incidence 0.59% in Black Africans and 0.10% in other races combined, which translates to approximately 220 500 new infections in Black Africans and 10 600 new infections in other races combined (Simbayi *et al*., 2019). One previous study attributed the differences in HIV prevalence and transmission between the racial groups to differences in sexual behaviours, prevalence of concurrency and number of sexual partners (Kenyon *et al*., 2013). Compared to other racial groups, black South Africans have a higher prevalence of concurrency and multiple sexual partners with whom they engage in risky sexual behaviours (Kenyon *et al*., 2013).

In terms of geographical variation, populations living in urban areas have a higher HIV incidence (0.58%) compared to those living in rural areas (0.23%) (Simbayi *et al*., 2019). With regards to sex, new infections were higher in females than in males, particularly in adolescents and young women (Naicker *et al*., 2015; Topazian *et al*., 2020). In SA, the HIV epidemic disproportionately affects women more than men, where it was reported that the incidence rates aged 15–49 years for females and males were 0.93% and 0.69%, respectively (Simbayi *et al*., 2019). Young black females between the ages 15–24 years particularly have a higher incidence rate (1.51%) compared to males of the same age group with an incidence rate (0.49%) (Simbayi *et al*., 2019). The high HIV burden in females may be attributed to behavioural, biological, social, cultural, and economic factors (Naicker *et al*., 2015; Sia *et al*., 2016; UNAIDS, 2018). For example, females are more likely to be

unemployed and poor which promote risky behaviours such as engaging in transactional sex with casual partners and without protection (Bandali, 2011; Mojola, 2011).

The overall incidence of HIV among South Africans has declined in the last decade (Simbayi *et al*., 2019). Furthermore, trends have showed a steady decline in the HIV incidence among people aged 15–49 years in most provinces since the late 1990s (Johnson and Dorrington, 2019). The success of SA in reducing new HIV infections is attributed to several interventions put in place by the public health sector (Simbayi *et al*., 2019). The interventions include amongst others educational awareness programs, scaling-up of HIV testing, condom provision, preventative treatment of high-risk groups, and expansion of the cART programme (Republic of South Africa National Department of Health [NDoH], 2015; Simbayi *et al*., 2019). The number of people receiving cART rapidly increased since the country rolled out one of the largest HIV treatment programmes (Mukumbang *et al.,* 2016).

2.2.2. Combination antiretroviral therapy coverage

The provision of cART in SA has greatly improved in the past decade (Osler *et al*., 2018; Burger *et al*., 2019). All nine SA provinces continue to ensure that maximum cART access is attained for HIV-positive people in their various communities. Almost every public health facility has an HIV testing centre and offers cART services for individuals diagnosed with HIV. In LP province, over 500 public health facilities offer cART services, and this has enabled many communities to have access to HIV treatment closer to their homes (Limpopo Provincial AIDS council, 2017).

The national cART coverage was 62% in 2019 and it is one of the largest cART coverage in the world (WHO, 2020). However, this is not surprising considering the number of HIV infections currently in the country. Approximately 5.3 million South

Africans living with HIV receive cART which is more than half of the country's HIV-positive population (UNAIDS, 2020). The cART coverage at the provincial level ranged from 51–75%, the lowest being NW and the highest being NC (Johnson and Dorrington, 2019). In 2016, the UNAIDS committed to ending the HIV epidemic by establishing what is called the 90-90-90 treatment target strategy (UNAIDS, 2017). The strategy called for 90% of HIV-positive people to be diagnosed by 2020, 90% of whom will be on cART and 90% of whom will have viral suppression. SA appear to have made considerable progress especially at achieving the first indicator where 93% of HIV-positive people now know their status but it is still behind with second indicator with ART coverage that is 20% point below the target (Marinda *et al*., 2020; Nyasulu *et al*., 2021). Although 87% of those on cART are virally suppressed, the second indicator may reflect problems with adherence to treatment (Marinda *et al*., 2020). Although guidelines on the use of cART were developed, adherence to treatment remains key to managing HIV (WHO, 2021).

2.2.3. Guidelines and recommendations for using combination antiretroviral therapy

In 2013, WHO recommended that all people diagnosed with HIV should be initiated onto cART regardless of the $CD4^+$ T-cell count (WHO, 2013). The goal of cART is to attain and maintain full viral suppression, which will restore and preserve immune function as well as reduce HIV-related morbidity, thus prolonging life expectancy, and improving quality of life (Raper and Cobbs, 2016; Meintjes *et al*., 2017). The therapy generally consists of several classes of ARV drugs that suppress viral replication by inhibiting one of the key stages of the HIV life cycle, namely, reverse transcription, integration, and maturation (Figure 2.1, Table 2.1) (Laskey and Siliciano, 2014; AIDSinfo, 2016; Raper and Dobbs, 2016). These stages are

accomplished by three HIV enzymes called reverse transcriptase, protease, and integrase (Laskey and Siliciano, 2014; Meintjes *et al*., 2017).

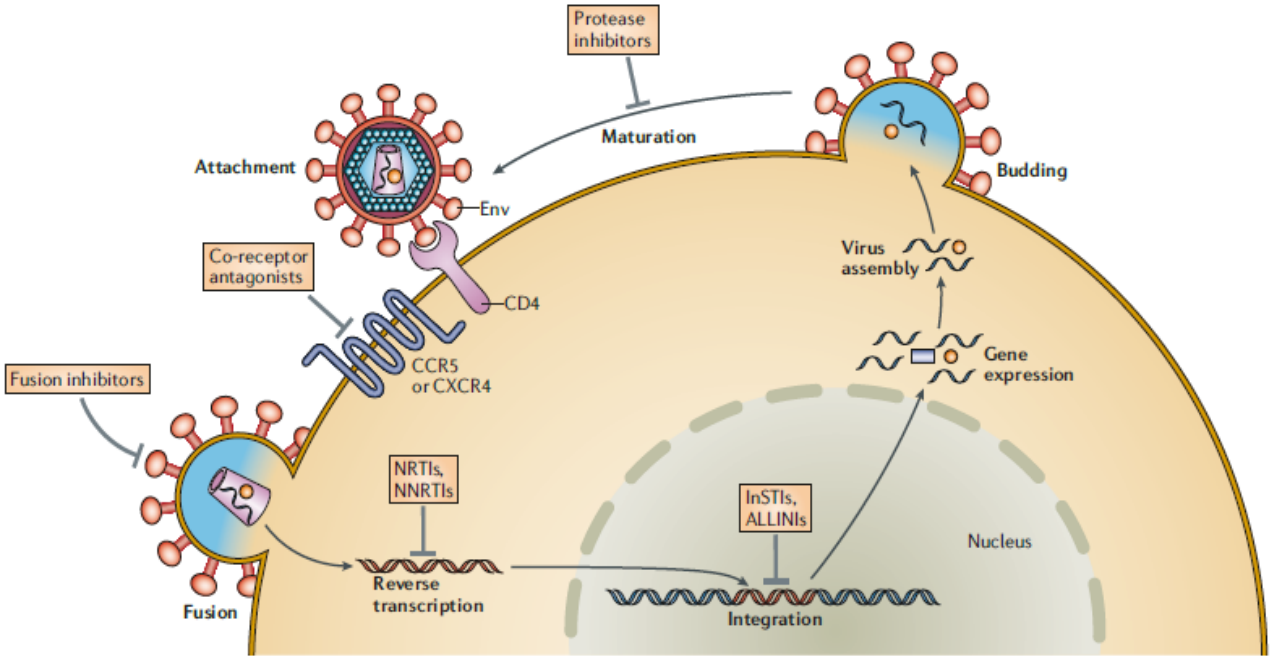
Reverse transcriptase catalyses the process of reverse transcription, which makes a copy of viral DNA from single-stranded viral RNA (Figure 2.1) (Karch, 2017). Integrase and protease are responsible for the insertion or integration of proviral DNA copy into the host genome and the growth or maturation of viral particles (Figure 2.1) (Karch, 2017). A typical cART consists of ARV drugs from at least two drug classes which include nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs) (Table 2.1) (AIDSinfo, 2016; Raper and Dobbs, 2016).

Reverse transcriptase inhibitors (RTIs) are inhibitors of reverse transcriptase (Raper and Dobbs, 2016; Karch, 2017). In contrast, PIs inhibit the activity of the protease enzyme, resulting in immature viral particles which become destroyed by enzymes (Raper and Dobbs, 2016; Karch, 2017). Other ARV classes are integrase inhibitors (INIs) that inhibit the integration of HIV into host cell chromosomes, and fusion inhibitors (FIs) that inhibit the fusing of viral and host cell membranes (Table 2.1) (Laskey and Siliciano, 2014; Raper and Dobbs, 2016; Karch, 2017).

Table 2.1: Classes of antiretroviral drugs

NRTIs	NNRTIs	PIs	FIs	INIs
Lamivudine (3TC) Zidovudine, (AZT/ ZDV) Emtricitabine (FTC) Abacavir (ABC) Tenofovir disoproxil fumarate (TDF) Stavudine (d4T)	Efavirenz (EFV) Rilpivirine (RVP) Nevirapine (NVP) Etravirine (ETR) Doravirine (DOR)	Indinavir (IDV) Lopinavir (LPV) Ritonavir (RTV) Atazanavir (ATV) Tipranavir (TPV) Saquinavir (SQV) Darunavir (DRV)	Enfuvirtide (T-20)	Raltegravir (RAL) Dolutegravir (DTG)

United States Food and drug administration [FDA], 2020



Laskey and Siliciano, 2014

Figure 2.1: Schematic representation of stages of HIV life cycle targeted by antiretroviral drugs. HIV: human immunodeficiency virus; NRTIs: nucleoside reverse transcriptase inhibitors; NNTRIs: nonnucleoside reverse transcriptase inhibitors; InSTIs: integrase strand transfer inhibitors; ALLINIs: Allosteric HIV-1 integrase inhibitors; CD4: cluster of differentiation 4; CCR5: CC chemokine receptor type 5; CXCR4: CXC chemokine receptor type 4; Env: envelope glycoprotein.

In line with WHO guidelines, the NDoH recommends TDF+3TC+DTG (in fixed-dose combination) as the preferred first-line regimen for all patients starting cART in SA (NDoH, 2019). Dolutegravir is an INI recently incorporated as a first-line agent

(WHO, 2019). Before DTG, EFV was part of the preferred first-line regimen and now forms part of an alternative first-line regimen with RTIs (Meintjes *et al*., 2017; WHO, 2019). The change from EFV to DTG was due to the DTG-based regimen offers higher viral suppression than the EFV-based regimen, has a lower risk for drug-drug interactions and development of viral drug resistance (WHO, 2019). Another alternative first-line regimen is TDF+ FTC/3TC+RPV which is used if the viral load (VL) is <100 000 copies/millilitres (ml) (Meintjes *et al*., 2017).

The United States Department of Health and Human Services (HHS) defines viral load (VL) as a measure of the viral content in a blood sample (HHS, 2018). A patient's VL indicates viral suppression and patient adherence to treatment and thus reflects the success of cART (WHO, 2021). A VL of <50 copies/ml is considered undetectable (virally suppressed) and indicates good adherence whereas a VL of >50 copies/ml for at least 6 months is a sign of poor adherence and indicates a need for intervention through the change of regimen type to improve adherence (Meintjes *et al*., 2017; WHO, 2021). Thus, virological monitoring is an integral part of evaluating the success of cART and poor adherence to cART leads to virological failure (WHO, 2013). If a patient experiences virological failure with a first-line regimen, second-line therapy is initiated.

A typical second-line regimen consists of two NRTIs and a ritonavir-boosted PI (PI/r) such as ritonavir-boosted atazanavir (ATV/r), ritonavir-boosted lopinavir (LPV/r), or ritonavir-boosted darunavir (DRV/r) (WHO, 2020). A second-line PI therapy with ATV/r was suggested as the preferred option when switching from first to second-line regimen due to better tolerability, favourable lipid profile, and once-daily dose compared with LPV/r-based therapy (Meintjes *et al*., 2017).

A third-line regimen is initiated if a virological failure occurs with the second-line regimen, however, this step requires careful consideration (WHO, 2013; Meintjes *et*

al., 2017). The patient must have experienced virological failure with a PI-based second-line regimen (and be over one year on therapy) with identified PI resistance (NDoH, 2015). The choice of regimen is guided by a medical expert based on evidence provided of PI resistance, cART history, and adherence to treatment (Meintjes *et al*., 2017). Despite the success of cART in controlling HIV, various side effects of the treatment have been reported (Penkalski, 2017).

2.2.4. Side effects of combination antiretroviral therapy

While the treatment prolongs the lifespan of people living with HIV, substantial evidence has also linked it to various side effects which includes skin rashes, hypersensitivity reactions, liver disease, dyslipidaemia, diabetes, renal dysfunction and CVD (Braithwaite *et al*., 2005; Moreno-Cuerda *et al*., 2006; Peck *et al*., 2014; Nsagha *et al*., 2015; Penkalski, 2017). Renal dysfunction is of particular concern as the kidney are constantly exposed to the highly active nephrotoxic ARV drugs. Moreover, HIV itself also plays a key role in the pathogenesis of renal impairment among HIV-positive patients (Seegmiller *et al*., 2018).

2.3. OTHER RISK FACTORS FOR RENAL DYSFUNCTION

cART is not the only cause of renal dysfunction in the HIV-positive population. There is a complex interplay between multiple factors such as ageing, HIV, immune function, hypertension, obesity and metabolic abnormalities such as hyperglycaemia (diabetes) and dyslipidaemia (Ejerblad *et al*., 2006; Kazancioğlu, 2013; Swanepoel *et al*., 2018; Calza *et al*., 2019). The metabolic abnormalities can be induced by both HIV and cART (Bune *et al*., 2020). HIV-associated nephropathy (HIVAN), hypertension, and diabetes are the major underlying cause of impairment and these conditions tend to co-exist in the adult HIV-positive population (Winston, 2010; US Renal Data System, 2013; Swanepoel *et al*., 2018). Studies have pointed out that

older age is a single major risk factor for renal dysfunction (Calza *et al*., 2019; Sun *et al*., 2019). There is an age-related decline in renal function in both the general and HIV-positive populations which is caused by increased levels of oxidative stress resulting in structural and functional changes (Liu *et al*., 2012; Glassock and Rule, 2016; Gekle, 2017).

2.3.1. Human immunodeficiency virus-induced hyperglycaemia

Compared with the general population, the prevalence of hyperglycaemia, particularly diabetes is higher among HIV-positive patients and it is associated with both HIV and cART toxicity (Noubissi *et al*., 2018). A previous study conducted on animal model has shown direct involvement of HIV in causing glucose metabolism derangement (Non *et al*., 2017). The HIV protein called viral protein R (vpr) protein inhibits the intracellular proliferator-activated receptors-gamma (PPAR-Ɣ) which act as a transcriptional factor during adipocyte differentiation resulting in fatty acid accumulation. The accumulation of fatty acids causes lipotoxicity which results in insulin resistance (IR), hypertriglyceridaemia and fatty acid liver disease (Gavrilova *et al*., 2003; Shrivastav *et al*., 2008; Non *et al*., 2017). The IR leads to abnormally high blood glucose concentrations referred to as hyperglycaemia (Gutierrez and Balasubramanian, 2012). The IR is a state in which insulin (a hormone secreted by pancreas) is unable to stimulate the uptake of glucose into insulin-sensitive tissues such as the adipose tissue, skeletal muscle, and liver tissue tissues cells resulting in abnormally elevated insulin and glucose in the blood (Freeman and Pennings, 2021).

2.3.2. Antiretroviral drug-induced hyperglycaemia

Protease inhibitors are known to cause IR (Ergin *et al*., 2020). There is substantial evidence showing the link between PIs and the development of IR, however, the mechanism of action is not completely understood (Noor *et al*., 2001; Hruz *et al*., 2002; Monier and Wilcox, 2004; Ergin *et al*., 2020). It was suggested that PIs disrupt

the insulin signaling pathway by specifically targeting GLUT4, the transporter that facilitates insulin-stimulated glucose uptake (Murata *et al*., 2000). Indinavir (IDV) is the first PI to be implicated in IR and glucose intolerance (Hruz *et al*., 2002). Indinavir inhibits glucose uptake by adipocytes without affecting GLUT4 translocation (Murata *et al*., 2000).

Nelfinavir was found to have a profound impact than IDV by disrupting key steps in the insulin signaling pathway in addition to inhibiting glucose uptake into adipocytes (Rudich *et al*., 2001). The mechanisms of action of nelfinavir involve the inhibition of PKB/Akt activation in the insulin-signaling pathway which initiates a cascade of events leading to GLUT4 translocation and impaired glucose transport (Rudich *et al*., 2001). Consistent with this phenomenon, other studies demonstrated the inhibition of PI3-k activation by nelfinavir which led to impaired GLUT4 migration, glucose entry into adipocytes. This results in hyperglycaemia (Ben-Romano *et al*., 2004; Rudich *et al*., 2005). High glucose levels in the kidneys can induce structural and functional changes leading to renal impairment (Fakhruddin *et al*., 2017).

2.3.3. Hyperglycemic-induced renal dysfunction

Diabetic nephropathy (DN) is a major complication of type 2 diabetes and hyperglycaemia was implicated in its pathogenesis and progression (Schrijvers *et al*., 2004; Elmarakby and Sullivan, 2012). Diabetic nephropathy is characterized by histopathological and functional abnormalities in the kidney which include glomerular hypertrophy and hyperfiltration, increased basement membrane thickness (BMT), tubular dilatation, glomerulosclerosis, proteinuria, and renal function decline (Schrijvers *et al*., 2004).

Elmarakby and Sullivan explained that hyperglycaemia induces oxidative stress in renal cells thus causing glomerular and tubular tissue damage (Elmarakby and Sullivan, 2012). Since the uptake of glucose is high in the renal tissue in a diabetic

kidney, it exacerbates damage compared to the other tissues in the body (Coward *et al*., 2005; Forbes *et al*., 2008).

A study that investigated the effect of transient hyperglycaemia on renal cell injury using both HIV-infected human podocytes and experimental mouse models with HIVAN showed that hyperglycaemia worsened renal cell injury by downregulating vitamin D receptors (VDR) expression (Rai *et al*., 2015). This resulted in the activation of the renin-angiotensin system (RAS) and the generation of reactive oxygen species (ROS), thereby causing oxidative-induced DNA damage (Rai *et al*., 2015).

It is well established that HIV activates RAS and triggers the formation of ROS in the renal cells which may lead to the development of DN and HIVAN (Durvasula and Shankland, 2008; Salhan *et al*., 2012; Chandel *et al*., 2013). Glucose accelerates the damage by suppressing VDR expression and enhancing the activation of RAS which causes accelerated DNA damage and renal injury which results in renal dysfunction (Rai *et al*., 2015).

2.3.4. Obesity

Obesity is a serious pandemic affecting virtually all age groups and it increases the risk of diabetes and CVD (WHO, 2020). The WHO statistics indicated that more than 650 million adults worldwide are obese and over 1.9 billion are overweight (WHO, 2020). Developed countries have the highest overall prevalence of overweight and obesity than developing countries (WHO, 2020). The overall prevalence of overweight and obesity were 62.5% and 28.6% respectively among the adult western populations while in the adult African population the prevalence of overweight and obesity were 31.1% and 10.6% respectively (WHO, 2020).

Overweight and obesity are also major concerns in SA, where it was reported that 53.8% of the adult population is overweight (WHO, 2020).

The prevalence of overweight or obesity among people living with HIV has risen over the years and concerns are risen that it is increasing the incidence of diabetes (Hanttu *et al*., 2021). Although comparative studies on the prevalence of overweight or obesity among HIV-positive population with general population are scarce (Hanttu *et al*., 2021). Overweight and obesity are collectively defined as excessive body fat accumulation associated with health risks. BMI which is defined as body mass in kilograms (kg) divided by square meter of height and is a common tool used to determine both overweight and obesity (WHO, 2020). Although BMI is a commonly used tool, it does not exclusively measure body fatness due to muscle weight contributing to total body mass (Dagan *et al*., 2013).

Waist circumference (WC) is an alternative tool that provides a simple measure of abdominal obesity (visceral obesity) and it is strongly associated with the risk of diabetes and CVD (Vasiljevic *et al*., 2017; Ross *et al*., 2020). Abdominal obesity in men is defined by a WC of more than 102 cm whereas in women it is confirmed when the WC is greater than 88 cm (Hall *et al*., 2014). Increased adiposity has been associated with renal impairment (Hall *et al*., 2019).

2.3.6. Obesity-induced renal dysfunction

Hall *et al*. (2014) explained that the causal link between obesity and renal dysfunction is mediated by obesity-induced hypertension. Hypertension is defined as persistently elevated blood pressure of above 140/90-millimeter mercury (mmHg) (Kaplan, 2010). The upper number is systolic blood pressure which is the maximum pressure in the arteries when the heart contracts. The lower number is diastolic blood pressure which is the minimum pressure in the arteries when the heart relaxes (Sherwood, 2015). Both the prevalence of obesity and hypertension among HIV-positive population are high which raises concern about renal function.

According to Hall *et al*. (2014), obesity increases tubular reabsorption of sodium and impairs the mechanism of sodium excretion. In response to increased sodium reabsorption, renal vasodilation and glomerular hyperfiltration occur to compensate for increased sodium reabsorption leading to maintenance of sodium balance. Excessive fat accumulation over time leads to failure of the compensatory mechanisms to counteract increased sodium reabsorption which results in hypertension. Hypertension causes glomerular damage and renal dysfunction (Hall *et al*., 2014).

Obesity can also impair renal function by inducing inflammation and oxidative stress. Although obesity-induced high blood pressure (hypertension) and diabetes are major driving mechanistic links for the development of renal dysfunction (Sherwood, 2015; Hall *et al*., 2014). The kidneys perform important functions in the human body including the metabolism and elimination of drugs.

2.4. RENAL FUNCTION

2.4.1. Function of the kidney

The kidneys are a pair of bean-shaped organs that lie between the upper rear part of the abdominal cavity and back muscles (Gueutin *et al*., 2012). Both kidneys maintain homeostasis by regulating acid-base balance, electrolyte concentrations, and extracellular fluid (ECF) volume (Gueutin *et al*., 2012). By adjusting the electrolyte content and ECF compartment, the kidney contributes to the long-term regulation of blood pressure (BP) in the human body (Gueutin *et al*., 2012; Raghavendra and Vidya, 2013). The kidney also performs other functions such as urine formation, metabolic wastes excretion (urea, uric acid, ammonia, creatinine), and hormone production such as erythropoietin, aldosterone, renin, anti-diuretic hormone (ADH) amongst others (Gueutin *et al*., 2012; Raghavendra and Vidya, 2013).

Each kidney has a functional unit called the nephron that carries out most of the kidney's functions through three major processes called glomerular filtration, tubular reabsorption, and secretion (Inui *et al*., 2000; Sherwood, 2015; Raghavendra and Vidya, 2013). With its high-capacity transport systems, the kidney can prevent loss of essential substances such as nutrients and electrolytes that were filtered by the glomerulus while simultaneously facilitating secretion of wastes and xenobiotics (Inui *et al*., 2000).

Glomerular filtration is defined as the passive transfer of blood components including water, proteins, ions, and nutrients from the blood into the renal tubule. Reabsorption and secretion involve the transfer of substances either by active or passive transport between the proximal tubular lumen and peritubular capillary blood (Gueutin *et al*., 2012; Raghavendra and Vidya, 2013). These processes accomplish the kidney's main function which is to maintain homeostasis (Ferguson and Waikar, 2012). A variety of methods such as biopsy, urinalysis and blood tests are used to evaluate renal function (Filler *et al*., 2014).

2.4.2. Markers of renal function

2.4.2.1. Glomerular filtration rate

Glomerular filtration rate (GFR) describes the amount of filtrate passing through the kidney glomeruli per unit time (Kaufmann *et al*., 2019). The GFR is regarded as the best measure of overall renal function globally (Estrella and Fine, 2010; Schaeffner, 2017). Direct measurement of GFR, referred to as the "gold standard", is accomplished by measuring the urinary clearance of an exogenous substance such as inulin or iothalamate (Berg *et al*., 2014; Delanaye *et al*., 2016). Although it is a gold standard, measuring GFR directly is expensive, time-consuming, and

cumbersome (Hsu and Bansal, 2011). As a result, the technique has limited use in clinical practice in resource limited settings (Delanaye *et al*., 2016).

Alternatively, renal function can be estimated by using an endogenous substance measurable in a biological specimen such as serum or plasma (Estrella and Fine, 2010; van Veldhuisen *et al*., 2016). The endogenous substance is termed a biomarker and it is mainly used to diagnose disease progression or therapeutic drug monitoring (Lemley, 2007; Khan and Pandey, 2014). In the context of renal function, both serum and urinary biomarkers are useful in the evaluation of renal function although urinary biomarkers are ideal for evaluating drug-induced damage (Fernando *et al*., 2019). Creatinine is a commonly used serum renal biomarker for estimating GFR (Delanaye *et al*., 2017).

2.4.2.2. Creatinine

Serum creatinine is a widely used endogenous biomarker for estimating GFR and it is recommended by the National Kidney Foundation (NKF) for clinical renal function assessment (NKF, 2020). Creatinine is the end-product of muscle catabolism generated from conversion of creatine and phosphocreatine (Gagneux-Brunon *et al*., 2012; Thomas *et al*., 2017). Several equations were developed to estimate GFR and the two most used are CKD-EPI and Modification of diet in renal disease (MDRD) (Levey and Inker, 2017). Other studies use the Cockcroft-Gault formula to determine creatinine clearance; however, this method requires a 24-hour timed collection of urine which may be inconvenient (Levey *et al*., 2003; Stevens *et al*., 2006; Estrella and Fine, 2010).

Serum creatinine is used to estimate GFR to evaluate renal function in daily clinical practice and research (NKF, 2020). However, reports have showed that creatinine has several limitations (Gagneux-Brunon *et al*., 2012; Delanaye *et al*., 2017; Thomas

et al., 2017). Serum creatinine is impacted by other factors such as dietary protein, muscle mass, gender, and age (Thomas *et al*., 2017; NKF, 2020).

Moreover, certain medications such as cimetidine, cisplatin, antibiotics, and ARV drugs have been shown to decrease creatinine excretion by inhibiting tubular secretion (Delanaye *et al*., 2017; Thomas *et al*., 2017). This results in elevated levels of blood creatinine which will lead to false estimation of GFR (Stevens and Levey, 2007). Thus, the use of serum creatinine to evaluate renal function especially in clinical practice raise concerns about its reliability. Other studies conducted in HIV-positive populations showed that serum creatinine is also affected by the infection itself (Maia *et al*., 2005; Gagneux-Brunon *et al*., 2012). For example, the wasting syndrome associated with advanced HIV stage and cART-related mitochondrial toxicity reduces the lean mass and production of creatinine (Gagneux-Brunon *et al*., 2012).

A study also reported that serum creatinine is a non-specific and insensitive to renal damage which only detects when a significant loss of nephrons has occurred (De Oliveira *et al*., 2019). Although the equation used to estimate GFR take in consideration age, gender, race, and body surface area, it is uncertain whether serum creatinine can be a reliable tool to determine renal function in the HIV-positive population. Therefore, these limitations prompt for alternative biomarkers for renal function evaluation that will be of clinical value. Cystatin C seems to be a better alternative to creatinine and there has been a growing interest in the use of plasma cystatin C (Gagneux-Brunon *et al*., 2012; Gupta *et al*., 2014).

2.4.2.3. Cystatin C

Cystatin C is a small-sized protein (13 kilodaltons) produced at a constant rate by nuclear cells (Vaidya *et al*., 2008). Cystatin C is freely filtered and completely

metabolised by the kidneys following glomerular filtration and it is not subjected to extra-renal excretion or tubular secretion as in the case of creatinine (Inker *et al*., 2018). Plasma cystatin C is also not affected by muscle mass, and it is very sensitive in detecting early renal impairment (Murty *et al*., 2013; Ebert and Shlipak, 2020; John *et al*., 2020). Thus, plasma cystatin C is considered superior to creatinine as a marker of GFR and it has been recommended for use in clinical practice (Grubb, 2017; Ebert and Shlipak, 2020).

However, certain factors such as current smoking, inflammation and viral loads have an impact on plasma cystatin C (Bagshaw and Bellomo, 2010; Overton *et al*., 2012; Longenecker *et al*., 2015). In terms of age and gender, previous studies have reported little or no effect on serum cystatin C (Finney *et al*., 2000; Weinert *et al*., 2010; Murty *et al*., 2013; Ebert and Shlipak, 2020). Cystatin C may therefore be a useful alternative biomarker. There are other available useful biomarkers which include retinol-binding protein 4 (RBP4) and clusterin (Murata *et al*., 2009; Henze *et al*., 2010; Anderson *et al*., 2014).

2.4.2.4. Retinol-binding protein 4

Retinol-binding protein 4 is a carrier protein that originates from the liver and its function is to transport vitamin A from the liver to the peripheral tissues (Fiseha and Gebreweld, 2016). The RBP4 is filtered by the glomerulus and reabsorbed by the proximal tubular epithelial cells which in turn becomes catabolized (Vaidya *et al*., 2008). Serum RBP4 is sensitive to change in GFR (Ni *et al*., 2018). Compared to serum RBP4, urinary RBP4 is sensitive to drug-induced tubular toxicity (Mahfouz *et al*., 2016). Increased serum RBP4 levels were correlated with renal function decline (eGFR) among diabetic patients with renal dysfunction (Murata *et al*., 2009; Mahfouz *et al*., 2016; Domingos *et al*., 2017). Patients with renal dysfunction were reported to have elevated levels of serum RBP4 (Frey *et al*., 2008; Henze *et al*., 2010; Fernando

et al., 2019). Although increased serum RBP4 indicates renal dysfunction, data also showed that elevated serum RBP4 may be associated with obesity (Liu *et al*., 2016).

2.4.2.5. Clusterin

Clusterin is a glycoprotein that plays an important role in the reproductive system. Clusterin derived its name from its ability to cluster Sertoli cells (Adiyanti and Loho, 2012). It also plays a role in immune function, and it is important for cell regression and cell aggregation (Redondo *et al*., 2008). It was reported that renal disease caused by toxicant-induced renal injury results in the expression of clusterin in the kidney and urine (Davis II *et al*., 2004; Kharasch *et al*., 2006).

The expression of clusterin on tubular cells was also seen in polycystic kidney disease and renal cell carcinoma (Zellweger *et al*., 2001). When a renal injury occurs, clusterin mRNA becomes translated into clusterin in the renal tubular epithelium (Khan and Pandey, 2014). A post-translation process converts clusterin into α and β subunits and the α-subunit is excreted while β-subunit accumulates in the tubular cell cytoplasm (Saunders *et al*., 1994). The upregulation of both clusterin mRNA and protein levels is a potential indicator of nephrotoxicity (Girton *et al*., 2002).

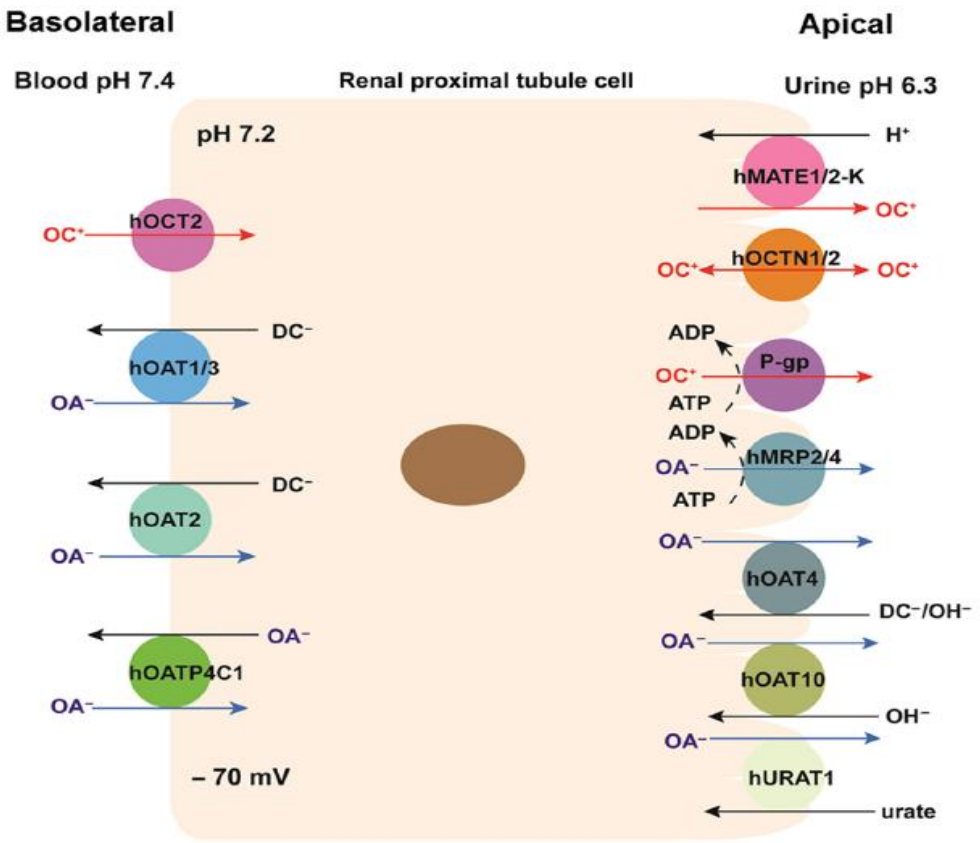
Studies demonstrating a change in blood clusterin with renal function are currently lacking. In one study nine renal biomarkers in HIV-uninfected AKI and CKD patients were measured (Anderson *et al*., 2014). Serum clusterin and cystatin C were significantly elevated in the CKD group as compared to the control group (Anderson *et al*., 2014). The study also observed serum cystatin C and other biomarkers significantly differed between AKI and CKD groups (Anderson *et al*., 2014). There is no literature demonstrating how blood clusterin level changes with renal function in

the HIV population. Various transporters on the renal tubular cells play an important role in the reabsorption and secretion of drugs (Wang, 2016).

2.4.3. Renal transport of drugs

Renal drug transporters are predominantly expressed on the basolateral and apical membranes of proximal tubules and are selective based on the charge of the drug (cationic or anionic) (International transporter consortium *et al*., 2010; Morrissey *et al*., 2013). Studies have shown that the secretion of anionic drugs in humans is mediated by organic anion transporters 1 and 3 (OAT-1 and OAT-3) situated on the basolateral membrane and multidrug resistance-associated proteins 2 and 4 (MRP2 and MRP4) situated on the apical membrane (Figure 2.2) (Masereeuw and Russel, 2001; Morrissey *et al*., 2013).

On the other hand, cationic drugs are secreted by organic cation transporter 2 (OCT-2) on the basolateral membrane and multidrug and toxin extrusion proteins 1 and 2-K (MATE1 and MATE2-K) on the apical membrane (Figure 2.2) (Masereeuw and Russel, 2001; Morrissey *et al*., 2013). The secretion of larger and more hydrophobic cations is mediated by P-glycoprotein (P-gp) on the apical membrane (Figure 2.2) (Yin and Wang, 2016). Several other transporters on the tubular epithelium have also been found to play a role in renal handling of drugs and metabolic wastes (Yin and Wang, 2016).



Yin and Wang, 2016

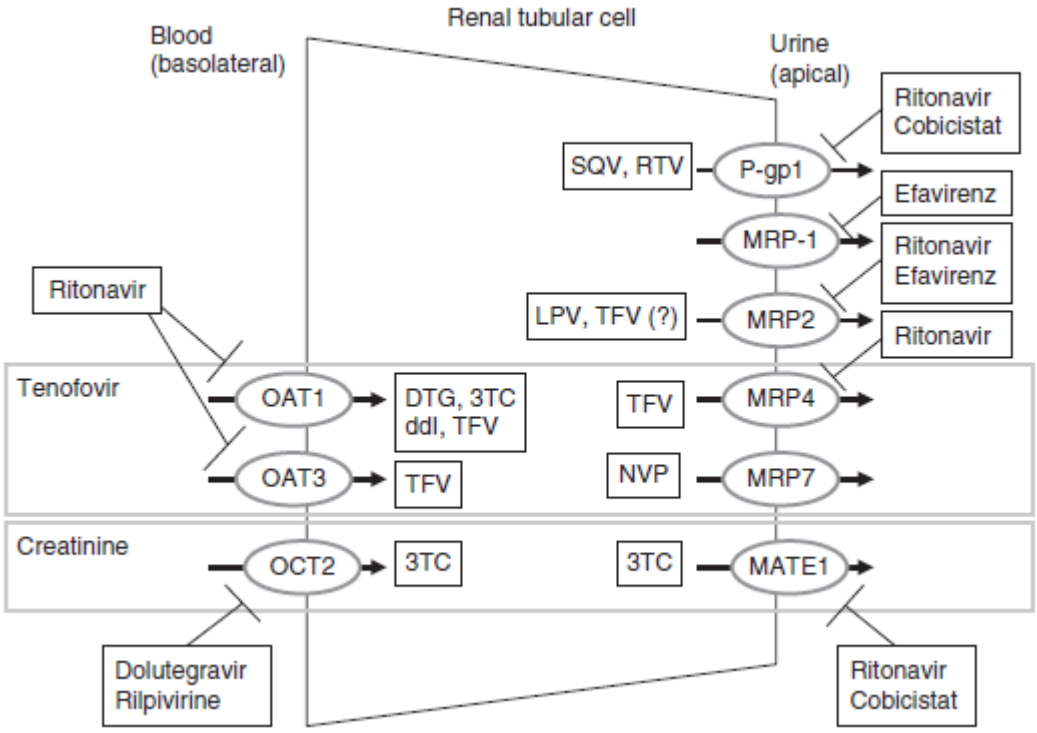
Figure 2.2: Major drug transporters involved in renal handling of drugs in human renal proximal tubule cells. ADP: adenosine diphosphate; ATP: adenosine triphosphate; DC: dicarboxylate; OH: hydroxide; OA: organic anion; and OC: organic cation; hOCT: human organic cation transporter; hOAT: human organic anion transporters; hMATE: human multidrug and toxin extrusion proteins; P-gp: P-glycoprotein; hMRP: human multidrug resistance-associated proteins. pH: potential of hydrogen; and H: hydrogen.

2.4.4. The effects of antiretroviral drugs on renal drug transporters

Several ARV drugs and pharmacoenhancers (ritonavir or cobicistat) have inhibitory effects on the basolateral and apical membrane transporters of proximal tubule cells

(Figure 2.3). These inhibitory effects tend to affect the secretion of other drugs thereby increasing their plasma concentrations or intracellular concentrations leading to tubular toxicity (Yombi *et al*., 2014). In addition, tubular secretion of creatinine is also affected by some of the ARV drugs and pharmacoenhancers which reduces renal clearance (Jotwani *et al*., 2017).

The secretion of creatinine is mediated by OCT2 and OCT3, and possibly OAT2 and OAT-3 on the basolateral membrane and by MATE-1 on the apical membrane (Jotwani *et al*., 2017). Dolutegravir (DTG) and RPV inhibit OCT2 and thus, creatinine transport into tubule cells causing high plasma concentrations (Yombi *et al*., 2014; Jotwani *et al*., 2017). On the other hand, ritonavir and cobicistat inhibit MATE-1 and consequently creatinine secretion on the apical side (Jotwani *et al*., 2017). Renal drug toxicity was also linked with inhibitory effects of ritonavir on MRP-2 and MRP-4, and polymorphisms in these apical membrane transporters (Yombi *et al*., 2014).



Yombi *et al*., 2014

Figure 2.3: Schematic representation of inhibitory effects of antiretroviral drugs and pharmacoenhancers on renal membrane transporters. 3TC: lamivudine; ddI: didanosine; DTG: dolutegravir; LPV: lopinavir; MATE: multidrug and toxin extrusion protein; MRP: multidrug resistance protein; NVP: nevirapine; OAT: organic anion transporter; OCT: organic cation transporter; P-gp: P-glycoprotein; SQV: saquinavir; TFV: tenofovir.

2.5. THE IMPACT OF HUMAN IMMUNODEFICIENCY VIRUS ON RENAL FUNCTION

2.5.1. Human immunodeficiency virus-associated renal diseases

Renal dysfunction can manifest as HIVAN, HIV immune complex kidney disease (HIVICK), acute kidney injury (AKI), chronic kidney disease (CKD), or treatment-related nephrotoxicity (Wyatt, 2017). In the context of this study, renal dysfunction is

defined as an estimated glomerular filtration rate (GFR) of <60 mL/min/1.73m^2 (also referred to as CKD) (Kidney Disease: Improving Global Outcomes [KDIGO] CKD Work Group, 2013). The HIVAN develops during advanced HIV stages, and it is characterised by focal segmental glomerulosclerosis (FSGS) with tubular microcysts formations, interstitial fibrosis, and inflammation (Atta *et al*., 2006; Wyatt *et al*., 2008; Hughes *et al*., 2021). Moreover, HIVAN is associated with proteinuria and a reduction in GFR that progresses to end-stage renal disease (ESRD) within weeks to months (Wyatt *et al*., 2008; Swanepoel *et al*., 2018).

2.5.2. Prevalence and incidence of renal diseases in human immunodeficiency virus populations

The epidemiology of renal diseases among the HIV population around the world and in Africa is not well studied. The true incidence and prevalence of HIVAN are still unknown. Data has shown that the prevalence of HIVAN ranged from 38–48.5% globally (Naicker *et al*., 2015; Diana and Naicker, 2016). Evidence shows that HIVAN is becoming a less frequent entity among the HIV-positive population in the era of cART (Wyatt, 2017).

With the scale-up of cART, the rates of HIVAN have seen a significant drop (Swanepoel *et al*., 2018). In one observational study, cART reduced the risk of HIVAN by 60% in HIV-positive African Americans and none of the patients who started cART at CD4$^+$ T-cell counts >200 cells/mm^3 developed HIVAN (Lucas *et al*., 2004). Thus, demonstrating the beneficial role of cART in reducing HIVAN (Lucas *et al*., 2004). This suggests that HIV is less likely to contribute to renal dysfunction in the cART-treated HIV-positive patients compared to untreated HIV-positive patients. Thus, untreated HIV-positive populations are particularly at a higher risk of developing HIV-related renal disease.

Despite the decline in HIVAN incidence rates due to cART, the HIV-positive black population still has a 6-fold higher risk of ESRD than their white counterparts partly due to genetic susceptibility (Abraham *et al*., 2015). It was suggested that the racial disparity in HIVAN and associated ESRD is driven by susceptibility risk variants called Apolipoprotein L1 variants which are found mainly in people of African descent (Kopp *et al*., 2011; Fine *et al*., 2012; Parsa *et al*., 2013).

This was supported by a study from SA that found the HIV-positive black cART-naïve patients with two Apolipoprotein L1 variants have an 89-fold higher risk of developing HIVAN compared with the HIV-positive controls (Kasembeli *et al*., 2015). The evidence from human and animal studies showed that the risk variants, which are highly expressed in glomeruli podocytes, interacted with HIV to induce pathological changes characteristic of HIVAN (Chan *et al*., 2009; Tzur *et al*., 2010; Ma *et al*., 2015).

2.5.3. Pathogenesis of Human immunodeficiency virus-associated renal disease

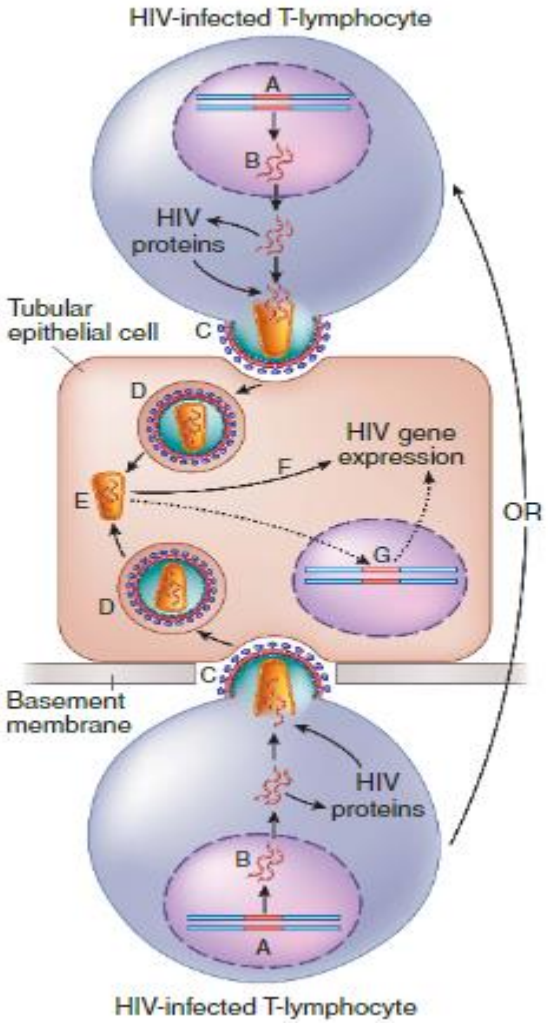
The pathogenesis of HIVAN begins with the infection of renal glomeruli and tubular epithelial cells (Rosenberg *et al*., 2015; Li *et al*., 2017). It is not completely understood how HIV enters the renal epithelial cells as they do not express viral CD4 receptors or CC chemokine receptor type 5 (CCR5)/CXC chemokine receptor type 4 (CXCR4) co-receptors, but a few mechanisms were proposed (Husain *et al*., 2018). Ray and colleagues explained the infection of human renal tubular epithelial cells (RTEs) by HIV isolate via a CD4 independent pathway, and cell-cell transfer of HIV between infected mononuclear cells and RTEs (Ray *et al*., 1998).

Other studies have proposed what is generally accepted to be a more efficient way of virus transfer which involves T-lymphocytes to RTEs with direct cell-cell contact

and no involvement of CD4 receptors (Figure 2.4) (Hubner *et al*., 2009; Chen *et al*., 2011). The virological synapses between T-lymphocytes and RTEs allow HIV uptake and subsequently gene expression in the RTEs (Chen *et al*., 2011).

The new mechanism involving infection of glomeruli epithelial cells (podocytes) was recently described by Li and colleagues (Li *et al*., 2017). According to this novel mechanism, a transmembrane tumour necrosis factor-alpha (TNF-α) promotes HIV entry into podocytes and subsequent integration into host DNA (Li *et al*., 2017). When HIV enters the renal epithelial cell, gene expression occurs leading to cell cycle dysregulation, cytoskeletal dysregulation, inflammation, and cell death. Renal epithelium injury and death result in collapsing glomerulosclerosis, tubular microcysts, and interstitial fibrosis, characteristic of HIVAN (Rednor and Ross, 2018).

Polymorphisms in the apolipoprotein gene explain the excess risk of HIVAN in people of African ancestry although the role of apolipoprotein L1 risk variants in promoting renal injury in the context of HIV infection is not well studied (Rednor and Ross, 2018). Studies are needed to elucidate the mechanisms by which the apolipoprotein risk alleles in the presence of HIV induce renal injury particularly in people of African ancestry.



Ross, 2014

Figure 2.4: Schematic representation of the proposed mechanism of HIV transfer from T-lymphocytes to renal tubular epithelial cells. HIV: human immunodeficiency virus.

The HIVICK has not received much attention as HIVAN and the causal relationship between HIV infection and HIVICK is not well studied. The role of cART on reducing HIVICK risk and other forms of renal disease is also less clear than on HIVAN (Wyatt, 2017). Although cART decreases HIVAN risk in the HIV-positive population, certain ARV drugs have been associated with renal dysfunction and risk of progression to ESRD (Mocroft *et al*., 2010; Wyatt, 2017; McMahon *et al*., 2018).

2.6. THE IMPACT OF COMBINATION ANTIRETROVIRAL THERAPY ON RENAL FUNCTION

2.6.1. The beneficial role of combination antiretroviral therapy on renal function

The impact of cART on renal function was studied in diverse settings. The cART is generally considered beneficial on renal function, however, certain ARV drugs such as TDF, ATV, ATV/r, or LPV/r are associated with renal dysfunction (Mocroft *et al*., 2010; Flanre *et al*., 2011; Scherzer *et al*., 2012; Ryom *et al*., 2013; Bonjoch *et al*., 2014; Ojeh *et al*., 2018; Debeb *et al*., 2021). The beneficial effect of cART on renal function was demonstrated in initial randomised clinical trials (RCTs), observational studies, and case reports (Betjes and Verhagen, 2002; Chemlal *et al.,* 2000; Kirchner, 2004; Scheurer, 2004; SMART Study Group, 2006; Kalayjian *et al*., 2008). Recent findings also show that cART improves renal function in HIV-positive populations (Kwantwi *et al*., 2017; Adedeji *et al*., 2019; Bani *et al*., 2020).

In many observational studies, cART improved renal function among HIV populations with HIVAN, impaired baseline renal function, advanced HIV stage, and low $CD4^+$ T-cell counts (Winston *et al*., 2001; Cosgroove *et al*., 2002; Atta *et al*., 2006; Mpondo *et al*., 2014). However, other findings showed cART improved renal function in HIV-positive patients with normal baseline renal function (and $CD4^+$ T-cell count > 200 cells/mm^3) and that renal function remained stable after 16 months for up to 7 years (Leport *et al*., 2009; Kwantwi *et al*., 2017).

It was suggested that cART improves renal function by reducing HIV viremia and increasing $CD4^+$ T-cell count among patients with HIVAN (Choi *et al*., 2009; Kalayjian *et al*., 2012). Combination antiretroviral therapy may slow down the progression of HIVAN by suppressing HIV replication in the kidneys thereby reducing

the pathological effects and leading to renal function recovery (Barisoni *et al*., 2001; Winston *et al*., 2001; Atta *et al*., 2006; Choi *et al*., 2009). On the contrary, cART was associated with renal dysfunction in other HIV-positive populations (Baynes *et al*., 2017; Calza *et al*., 2019).

2.6.2. Evidence of combination antiretroviral therapy-associated renal dysfunction

Substantial evidence has emerged showing that ARV drugs such TDF, ATV, ATV/r, LPV/r, and IDV are associated with renal dysfunction. This includes AKI, renal function decline, CKD, renal tubular dysfunction, nephrolithiasis, and in severe cases, Falconi syndrome (Schmid *et al*., 2007; Mocroft *et al*., 2010; Rockwood *et al*., 2011; Hamada *et al*., 2012; Scherzer *et al*., 2012; Campo *et al*., 2013; Mwafongo *et al*., 2013; Zolopa *et al*., 2013; Ryom *et al*., 2013; Jose *et al*., 2014; Ojeh *et al*., 2018; Debeb *et al*., 2021).

cART-induced renal dysfunction may be caused by direct tubular toxicity, crystal-induced obstruction, interstitial nephritis, or indirectly by metabolic disorders such as hyperglycaemia diabetes or dyslipidaemia (Karras *et al*., 2003; Brewster and Perazella, 2004; Anderson *et al*., 2007; Izzedine *et al*., 2007; Crane *et al*., 2007; Overton *et al*., 2009; Herlitz *et al*., 2010; Ryom *et al*., 2014). Although these mechanisms are not yet completely understood. Substantial evidence shows that TDF is mostly implicated (Ryom *et al*., 2013; Ryom *et al*., 2014; Scherzer *et al*., 2012). Although there is a new prodrug called tenofovir alafenamide fumarate (TAF) that provides better renal safety and switching from TDF to TAF results in improved renal function (Mills *et al*., 2016; Gupta *et al*., 2019). However, the long-term renal safety of TAF still needs to be elucidated (Mills *et al*., 2016).

Tenofovir disoproxil fumarate is a highly effective NRTI that is commonly used as a backbone agent in several first-line regimens (Wearne *et al*., 2020). Although initial randomised control trials (RCTs) have demonstrated the efficacy and renal safety of TDF reporting no or minimal nephrotoxicity for periods up to 34 months, substantial evidence of TDF-associated renal dysfunction emerged from observational studies and case reports (Fux *et al*., 2007; GoicoecheaM *et al*., 2008; Flanre *et al*., 2011; Bonjoch *et al*., 2014; Kumarasamy *et al*., 2018; Ojeh *et al*., 2018; Debeb *et al*., 2021).

This is a call for concern especially in SSA and many other countries where TDF is a part of the preferred first-line regimen. In resource-limiting settings such as SSA routine renal screening is not possible which may therefore lead to the advancement of renal complications among the HIV population. The limitations presented in the commonly used serum creatinine-based eGFR method in determining renal function may further increase renal complication. It is therefore imperative to conduct studies to assess renal function in cART-exposed populations in SA, particularly in the Limpopo province where data is scant.

The concurrent use of TDF and PI/r such as ATV/r or LPV/r leads to greater renal dysfunction compared to TDF with NNRTIs (Buchacz *et al*., 2006; Goicoechea *et al*., 2008; Kiser *et al*., 2008; Calza *et al*., 2011; Albini *et al*., 2012; Young *et al*., 2012; Baxi *et al*., 2014). Several risk factors such as older age, diabetes, hypertension, high BMI, low $CD4^+$ T-cell counts or high VLs, prolonged treatment exposure, and concomitant use of PI were previously implicated in renal dysfunction (Calza *et al*., 2011; Msango *et al*., 2011; Kazancioğlu, 2013; Morlat *et al*., 2013; Ryom *et al*., 2013; Yimbi *et al*., 2014; Kalayjian *et al*., 2012; Calza *et al*., 2019; Okpa *et al*., 2019).

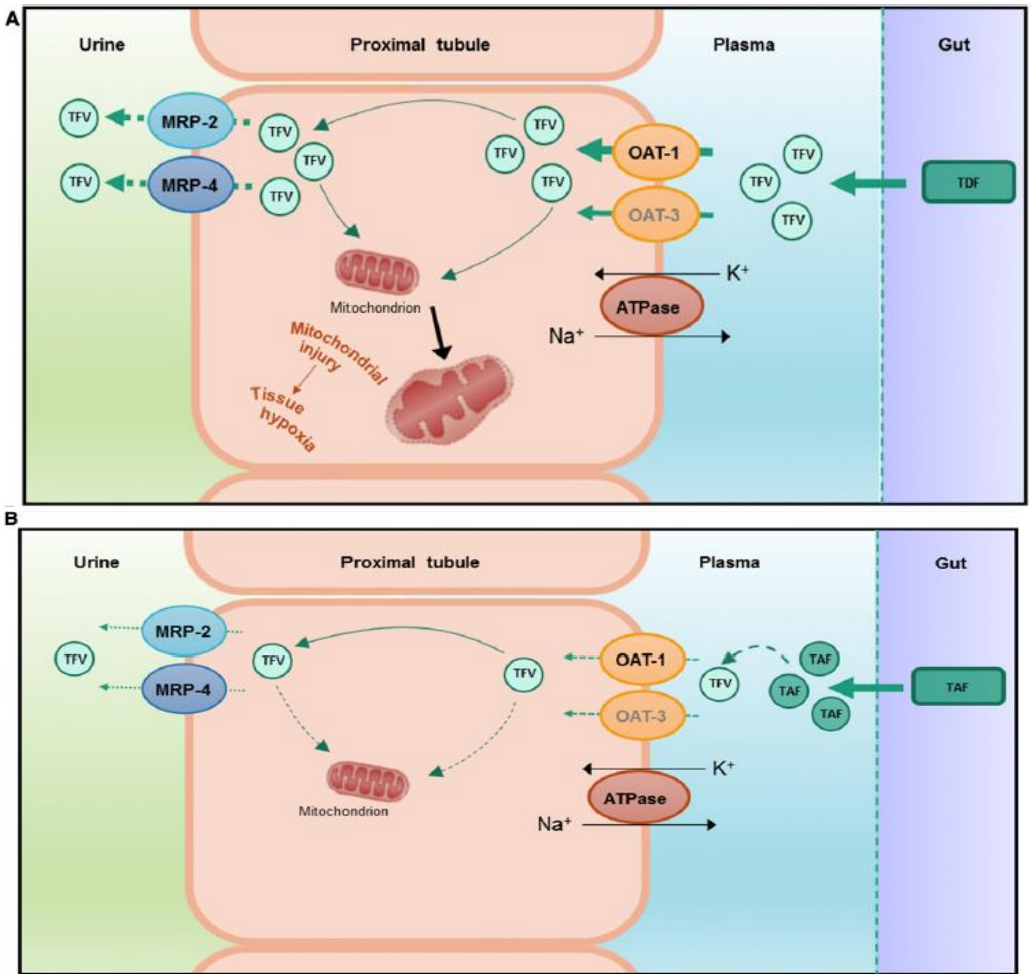
Despite the extensive evidence, the extent to which cART causes renal dysfunction is regarded as mild-to-moderate, and severe renal dysfunction is not common (Reid *et al*., 2009; Ryom *et al*., 2013; Laprise *et al*., 2013; Mulenga *et al*., 2014). While

some evidence pointed out that the loss of renal function due to cART occurs mostly during the first 12 months, others have reported a continuous renal function loss for up to 5–6 years, thus implying that the effects of TDF are cumulative (Gallant and Moore, 2009; Nishijima *et al*., 2014; Mocroft *et al*., 2016). It seems that prolonged exposure to TDF poses a risk to renal failure. Evidence shows that TDF causes renal dysfunction by inducing mitochondrial toxicity (Perazella, 2010).

2.6.3. Tenofovir disoproxil fumarate induced nephrotoxicity and renal dysfunction

The mechanism of TDF-induced nephrotoxicity is not completely understood. Animal model data showed that tenofovir (TFV), an active absorbable metabolite, causes mitochondrial DNA (mtDNA) depletion and mitochondrial dysfunction in the renal proximal tubule cells (Figure 2.5) (Perazella, 2010). The TDF is eliminated almost exclusively by the kidneys via a combination of glomerular filtration (70–80%) and tubular secretion (20–30%) (Ryom *et al*., 2014). After oral intake, TDF is converted to TFV in the gut (Jackson *et al*., 2013).

In the proximal tubule cell, TFV uptake occurs via OAT-1 and OAT-3 whereas extrusion or efflux occurs via MRP4 and MRP-2 (Figure 2.5) (Ray *et al*., 2006; Imaoka *et al*., 2007). Disturbances in the transport pathway of TFV due to increased OAT-1 activity or reduced MRP efflux activity can result in TFV accumulation in the tubule cells which then cause mtDNA depletion resulting in mitochondrial dysfunction (Figure 2.5). Mitochondrial dysfunction results in the deficit of energy molecule adenosine triphosphate (ATP) in the proximal tubule cell leading to impaired cell function and cell injury or death. This ultimately results in proximal tubulopathy and dysfunction (Perazella, 2010). In one study, proximal tubular dysfunction was associated with GFR decline, of which the mechanism of action has not been elucidated (Swanepoel *et al*., 2018).



Jotwani *et al*., 2017

Figure 2.5: Renal handling of tenofovir, an active metabolite of both tenofovir disoproxil fumarate and tenofovir alafenamide (TAF), in the proximal tubule cells. (A) TDF is converted to TNF within the plasma. TNF is transported into tubule cells via OAT-1 and OAT-3 on the basolateral membrane. The TNF efflux into urine space occurs via MRP-2 and MRP-4 on the apical membrane. Intracellular accumulation of TNF causes mitochondrial dysfunction and tissue hypoxia. (B) TAF is stable within plasma and conversion of TAF to TNF largely occurs within tubular cells, resulting in lower plasma concentrations and risk of tubular damage.

Polymorphisms on MRP-4 and MRP-2 and the inhibitory effects of ritonavir on MRP-2 and MRP-4 were associated with high TFV toxicity (Yombi *et al*., 2014). Some PI/r have shown out to slow down the renal clearance of tenofovir leading to increased plasma exposure by 20–30% which could explain the greater risk of nephrotoxicity associated with TDF and PI/r combination (Kearney *et al*., 2006; Gilead Sciences, 2007).

2.7. SUMMARY

Since the kidneys are constantly exposed to cART in the HIV-positive patients and the commonly used serum creatinine has significant limitations, it would be imperative to investigate the effect of cART on renal function in this specific population using alternative markers. There is substantial evidence of cardiovascular risk in the HIV-positive population and therefore, it is equally important to assess its association with renal function.

CHAPTER 3: METHODOLOGY

3.1. INTRODUCTION

This chapter describes the methods and materials employed to obtain and analyse data. The ethical considerations are also discussed towards the end of the chapter.

3.2. STUDY DESIGN

This was a cross-sectional study enrolling adult HIV-positive and HIV-negative subjects. The patients were recruited from February–May 2018.

3.3. STUDY POPULATION AND SETTING

A total of 155 subjects including 84 HIV-positive patients on cART (cART-treated group), 27 HIV-positive cART-naïve patients (cART-naïve group), and 44 HIV-negative subjects (healthy control) were recruited from Mankweng Hospital and referral clinics, situated in the Polokwane Municipality under Capricorn district in LP province, SA. The referral clinics included: Makanye, Nobody, Evelyn Lekganyane, Molepo, Mothiba, and Sebayeng. Males and females were 52 and 108, respectively. HIV-positive patients received cART for at least 2 months (2–151 months). All the subjects were black Africans.

3.4. ELIGIBILITY CRITERIA

The eligible groups consisted of adult subjects who were HIV-positive on cART, HIV-positive cART-naïve, and HIV-negative (HIV-free). All the subjects should not have

been diagnosed with any of the conditions before the initiation of the study: renal dysfunction, history of kidney transplant or dialysis, Tuberculosis (TB) or urinary tract infections (UTIs), history of diabetes, CVD such as coronary artery disease or acute myocardial ischemia and hypertensive taking long-term medications other than cART. The subject must have fasted on the previous night before visiting the clinic and not be pregnant or breastfeeding if female.

3.5. SAMPLING STRATEGY AND SAMPLE SIZE CALCULATION

3.5.1. Sampling strategy

A consecutive sampling method was employed whereby patients who met the eligibility criteria and signed informed consent forms criteria were selected to participate in the study. These patients were categorised into strata based on their HIV and cART status.

3.5.2. Sample size calculation

The sample size was determined on a formula considering the prevalence of HIV among the people of Limpopo province (Day and Gray, 2012), confidence interval (CI) of 95%, and margin error of 5%. The formula has been described below. This was also verified by the statistician.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

n = calculated sample size
t = confidence level at 95% (standard value of 1.96)
p = estimated prevalence of HIV of 11.4% (0.114)
m = margin of error at 5% (standard value of 0.05)

$$n = \frac{(1.96)^2 \times 0.114(1 - 0.114)}{(0.05)^2}$$

$$= 155.21 \sim 155$$

3.6. ORGANISATIONAL PROCEDURE

The researchers visited the clinics and informed relevant stakeholders including clinic managers and matrons about the study. Potential participants were identified by the nurse/counsellor who arranged the dates with the researcher for collection of data. The clinic nurses and HIV counsellors assisted with the recruitment of patients. On the day of data collection, the participants were informed about the study verbally and through consent letters written in Sepedi and English (Appendix A). An informed consent form that was also written in Sepedi and English was given to participants to sign (Appendix B). The interviews were conducted using the questionnaires to confirm eligibility and capture data (Appendix C). The patient's medical files were screened to collect relevant information, blood was collected by the registered nurse (Appendix D), and anthropometric measurements were taken. The blood samples were analysed at the University of Limpopo (UL) Medical laboratory (Appendix E) and the University of Pretoria (UP) Department of Immunology (Appendix F).

3.7. DATA COLLECTION

3.7.1. Questionnaire and medical files

A structured questionnaire was customised according to the study objectives to collect data which included sociodemographic characteristics, medical information and history, and anthropometric data. The questionnaire was written in English. Medical information retrieved from the medical files included HIV status, year of HIV diagnosis, treatment status, specific regimen, and duration of cART. To confirm the reliability and validity of the information, it was collected from the participants for first

time at the start of data collection and second time at a later stage. The medicals files were checked for corresponding information obtained during the interview.

3.7.2. Blood pressure

The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using the digital automatic Omron M2 blood pressure monitor (Omron Healthcare, Japan) following the manufacturer's instructions. Measurements were taken in a quiet room when the participant was in a relaxed, upright seated position. Thick or tight clothing was removed from the patient's right upper arm where the cuff was accurately wrapped and then the arm placed on the table to ensure that the same level is maintained between the cuff and participant's heart. Upon applying the cuff and ensuring that the air plug and main unit were connected, the reading was taken. The BP was measured twice on the right arm at a 5-minute interval and the average was calculated. Both the SBP and DBP were classified as normal, optimal, high normal, and hypertensive according to the South African HT practice guidelines (Seedat *et al*., 2014) (Appendix G).

3.7.3. Blood collection and preparation

Approximately 3–5ml of venous blood was withdrawn from the median cubital vein and was transferred into EDTA and BD vacutainer tubes (Beckton Dickinson, New Jersey, United States). These vacutainer tubes containing blood were placed on ice to avoid clotting. The samples were taken to the laboratory where it was centrifuged into serum and plasma samples using the Allegra X-30 benchtop centrifuge (Beckman Coulter, Indianapolis, United States) at 3000 revolutions per minute (RPM) and 18˚C for 20 minutes. Following centrifugation, the plasma and serum samples were aliquoted into labelled Eppendorf tubes and subsequently stored in the bio-freezer at -80°C until further analysis.

3.7.4. Biochemical analyses

3.7.4.1. Human immunodeficiency virus testing

The HIV was tested using Alere Determine™ HIV-1/2 antigen/antibody combo (Orgenics, Israel). This is an immunochromatographic test for simultaneous and separate detection of free HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2. The device is a laminated strip that consists of a sample pad that contains monoclonal biotinylated anti-HIV-1 p24 antibody and conjugate pad that contains monoclonal anti-HIV-1 p24 antibody-colloidal selenium with HIV-1 and HIV-2 recombinant antigen-colloidal selenium, and a nitrocellulose membrane containing an immobilised mixture of recombinant and synthetic peptide HIV-1 and HIV-2 antigens in the lower test area, immobilised streptavidin in the upper test area, and an immobilised mixture of anti-HIV-1 antibodies, HIV-1/2 antigens, and HIV-1 p24 recombinant antigen and anti-HIV-1 monoclonal antibody in the control area (Orgenics, 2014).

A 50µL of serum was applied to the sample pad which moved through the conjugate pad and nitrocellulose membrane. HIV in the sample binds with the monoclonal biotinylated anti-HIV-1 p24 antibody from the sample pad and then with monoclonal anti-HIV-1 p24 antibody-colloidal selenium from the conjugate pad to form a complex. This complex moves through the solid phase until it reaches the upper test area where it is captured by immobilised streptavidin labelled antigen thus forming a single pink/red antigen line. The formation of the pink/red antigen line confirms the presence of HIV. In the absence of HIV-1 antigens, the pink/red antigen line does not form. When HIV-1/2 antibodies are present in the sample, they bind with recombinant gp41 (HIV-1) and gp36 (HIV-2) antigen-colloidal selenium conjugate pad. This complex moves through the solid phase until it reaches the lower test area labelled antibody where it is captured by immobilised HIV-1 and HIV-2 synthetic peptide antigens and recombinant gp41 antigen forming a single pink/red antibody

line. The pink/red antibody line is not formed in the absence of HIV antibodies. A procedural control line containing a mixture of HIV-1/2 antigens, anti-HIV-1 antibody, and HIV-1 p24 recombinant antigen and anti-HIV-1 p24 monoclonal antibody is incorporated in the nitrocellulose membrane to ensure assay validity (Orgenics, 2014).

3.7.4.2. Cluster of differentiation 4 count determination

The $CD4^+$ T-count was determined using the factory-calibrated Alere PIMA™ analyser (Alere Technologies GmbH, Germany). The PIMA test consisted of a disposable test cartridge that contains dried reagents coded with $CD3^+$ and $CD4^+$ detection antibodies, and a PIMA analyser that analyse blood samples. The test specifically determines the absolute count of $CD4^+$ T-cells in whole blood. At the start, a test with PIMA bead standard was run to confirm device functionality. A Pima test cartridge containing 25µL of whole blood sample was then inserted in the analyser which processed the sample. Upon completion, the cartridge was removed from the analyser and the test results were retrieved and captured. The $CD4^+$ count was classified into various categories according to WHO immunological classification (WHO, 2006) (Appendix G).

3.7.4.3. Fasting blood glucose determination

Serum glucose was analysed using the Cobas Integra® 400 plus analyser (Roche Holding AG, Basel, Switzerland) based on the hexokinase (HK) colorimetric method. In the hexokinase colorimetric method, glucose is phosphorylated by HK with phosphate from ATP in the presence of magnesium ions forming glucose-6-phosphate (G-6-P) and ADP. Glucose-6-phosphate is subsequently oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase (G6P-DH) with the simultaneous conversion of nicotinamide adenine dinucleotide phosphate ($NADP^+$)

to reduced nicotinamide adenine dinucleotide phosphate (NADPH). The amount of NADPH formed at an increase in absorbance at 340 nanometres (nm) is in direct proportion to the glucose concentration of the sample. Glucose was classified as normal, prediabetes or diabetes using the American Diabetes Association (ADA) reference ranges (ADA, 2019) (Appendix G).

3.7.4.4. Luminex immunoassay

Luminex 200^{TM} instrument system with xPonent 4.2 software (Merck KGaA, Germany) was used to analyse plasma concentrations of cystatin C in ng/mL, clusterin in ng/mL, and RBP4 in (ng/mL). The Milliplex® map human kidney injury magnetic bead panel 6 assay was used (Merck Group, 2021).

A. Principle of Luminex

The Luminex sorts pre-coated analyte-specific beads (same size) which are ligated with specific mean fluorescence intensities (different concentrations of the red dye). Luminex 200 has two lasers to examine the pre-coated analyte-specific beads. The lasers examine the spectral property (the concentration of the red dye content) of the beads and the reaction around the analyte (antibody) of the specific bead.

B. Assay procedure

Three antibody-bead vials (for each of the three selected biomarkers) were sonicated and vortexed. Approximately 150µL of each antibody-bead vial was then pipetted into mixing bottle and mixed with 450µL of bead diluent to make the final volume of 3mL. Two quality controls (labelled 1 & 2) were reconstituted with 250µL of deionised water then vortexed and left to sit for 10 minutes. A wash buffer was subsequently prepared by mixing 540mL of deionised water with 60mL wash buffer

in a graduated cylinder. Kidney Injury panel standard 6 was prepared by reconstituting with 250mL deionised water in a vial labelled standard 6. Five working standards (labelled 1–5) were also prepared in polypropylene microfuge tubes by serial dilutions by adding 50µL reconstituted standard 6 to standard tube 5–1.

Approximately 200µL of prepared assay buffer was added into 96-well sample plate which was then sealed with foil and placed on the plate shaker for 10 minutes. After 10 minutes, the plate was removed from the shaker and the assay buffer was decanted. The remnants of assay buffer were further removed by tapping the plate against an absorbent towel. Then 25µL of each prepared standard and control was added in appropriate plate wells followed by 25µL of assay buffer. 25µL of diluted plasma samples (dilution factor of 1:3000) was added in appropriate wells in duplicates. The mixing bottle containing antibody-immobilised beads was vortexed followed by inoculation of 25µL of the mixed beads in appropriate wells. The sample plate was then sealed with foil and incubated overnight at 4°C. After incubating, the plate was washed on an automatic magnetic plate washer. 25µL of biotinylated detection antibodies was also added in every well. The sample plate was sealed and incubated for 1 hour at room temperature. After incubating, 25µL of the streptavidin-Phycoerythrin conjugate was added in wells containing detection antibodies. The sample plate was sealed with foil and incubated for 30 minutes. After 30 minutes of incubating, the contents were removed off and the plate was washed on the magnetic plate washer. 25µL of sheath fluid was then added in all wells and the beads were resuspended on a plate shaker for 5 minutes. Subsequently, the plate was analysed on Luminex 200 analyser.

On running the samples, the analyser was pre-warmed for 30 minutes prior to the analysis. The probe height was adjusted to a 96-well plate. The start-up routine was performed with maintenance using a reagent-filled automatic maintenance plate (distilled water and 70% ethanol). A test protocol was created with standard curves on the xPonent software. After creating the protocol, the samples were analysed,

and results were captured. After completing the analysis, the last automatic maintenance was performed.

3.7.4.5. Determination of estimated glomerular filtration rate

The eGFR was determined with an online GFR calculator, which excludes the race factor (NKF, 2020). CKD-EPI formula was used to estimate GFR with plasma cystatin C. This formula considers age, sex, race and is adjusted for body surface area (Levey *et al*., 2009). The CKD-EPI formula was chosen for this study as it is more accurate than the MDRD equation in estimating GFR and it is currently recommended by the NKF (Stevens and Levey, 2010). The eGFR was classified into various stages according to NKF Kidney Disease Outcomes Quality Initiative (NKF KDOQI) criteria (NKF, 2020) (Appendix G). Renal dysfunction was defined as eGFR <60mL/min/1.73m^2 (also called CKD).

3.8. CARDIOVASCULAR RISK FACTORS

3.8.1. Body mass index

The BMI was calculated using weight and height with the formula below. The BMI was classified as underweight, normal weight, overweight or obese using the WHO and National Institute for Health and Clinical Excellence (NICE) cut-off points (WHO, 1995; NICE, 2006) (Appendix G).

$$BMI = \frac{Weight\ (kg)}{Height\ (m^2)}$$

3.8.1.1. Weight

The weight was measured using the electronic body weight scale (Pee Electricals; Delhi, India) according to the International Standards for Anthropometric Assessment of the International Society for Advancement of Kinanthropometry (ISAK) (ISAK, 2001). The patient was wearing minimal clothing and barefooted when the measurement was taken with the weight scale placed on the flat floor. The measurement was recorded in kilograms (kg).

3.8.1.2. Height

The height was measured using the stadiometer (Seca gmbh & co. kg, Germany) according to ISAK guidelines (ISAK, 2001). The patient was in an upright position without shoes on the stadiometer which was placed on the flat floor in a vertical position. The head was positioned in Frankfort horizontal plane as the patient stood upright with feet and heels parallel together on the stadiometer base as well as buttocks and upper part of the back including the head touching the stadiometer. The measurement was recorded in metres (m).

3.8.2. Waist circumference

The WC was measured with the circumference tape measurer (Seca gmbh & co. kg, Germany) following the WHO measurement protocol (WHO, 2008). The tape measurer was placed around the participant's waist between the top of the iliac crest and the lowermost rip bone at the level of umbilicus in the midaxillary line. The measurement was recorded in cm. The WC was classified as normal and abnormal according to the WHO cut-off points (WHO, 2008) (Appendix G).

3.9. ETHICAL CONSIDERATIONS

The present study was approved by the research committees from the School of Molecular and Life Science (reference number SMLS/SRC/2019/03; Appendix H) and Faculty of Science and Agriculture (proposal number 92 of 2019; Appendix I). The ethical clearance was granted by the Turfloop Research Ethics Committee (TREC) of the UL (project number TREC/315/2019: PG; Appendix J). The permission to do the research in the hospital and clinics were requested and granted by the Limpopo Department of Health, Primary Health Care, and Social Development (Appendix K&L).

The study was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from study participants before taking part in the study. Participation was completely voluntary. The potential risks and benefits of the study were explained to participants before the start of data collection. To protect participants from harm, a registered nurse collected blood samples. Standardised procedures were adhered to throughout data collection. Questionnaires were treated with strict confidentiality. Unique codes were assigned to each patient to maintain strict confidentiality and anonymity. One patient was interviewed at a time to adhere to privacy. Any participant who experienced emotional discomfort during data collection was referred to a registered counsellor in the clinic. All patients were given feedback on the results.

3.10. STATISTICAL ANALYSIS

Statistical analysis was conducted with the use of the statistical package for social science (SPSS) software version 26. The data was cleaned prior to analysing. Codes were assigned to individual study participants. A normality test was conducted to ensure all variables were normally distributed. Normality was confirmed with Shapiro Wilk test and gaussian distribution curves. Descriptive statistics were performed to

characterise the study population. The results were presented as mean ($\bar{x}$) and standard deviation (SD) for continuous variables, and as frequencies (n) and percentages (%) for categorical variables.

Comparisons between the study groups for continuous variables were done using One-Way Analysis of Variance (ANOVA) (with Bonferroni post-hoc test]. The independent sample T-test was used to compare significant differences between the cART-treated and cART-naïve groups. The Chi-square test was for comparisons of categorical data between the study groups. Comparisons of renal biomarker levels were also done with Analysis of covariance (ANCOVA) by adjusting for age, gender, alcohol consumption, BMI, SBP and FBG, $CD4^+$ T-cell count. Graphs and pie-charts were constructed in excel SPSS and GraphPad prism.

Pearson correlation and multiple linear regression analysis were performed to determine associations between cardiovascular risk factors and renal function for the cART-treated group, HIV-positive group, and control groups. For the cART-naïve group, no Pearson correlation and multiple regression were not performed because of the small sample size (n=27). The independent variables for multiple stepwise regression analysis were selected by statistical methods. The renal function markers and CVD risk factors were converted into standardised scores before running the multiple regression. For the cART-treated group, the independent variables included age, gender, alcohol consumption, duration of cART, BMI, SBP and FBG and $CD4^+$ T-cell count. For the HIV-positive group, the independent variables included age, gender, alcohol, BMI, SBP, FBG, $CD4^+$ T-cell count and cART use. For the control group, the independent variables included age, BMI, SBP and FBG. The level of significance was set at a probability value (p-value) of <0.05.

CHAPTER 4: RESULTS

4.1. SOCIODEMOGRAPHIC CHARACTERISTICS

The study population consisted of cART-treated patients (n = 84), cART-naïve patients (n=27) and HIV-negative controls (n = 44). In terms of age, the mean ± standard deviation ($\bar{x}$ ± SD) of the total population was 40.50 ± 12.62 years (Table 4.1). There was a significant difference in age across the three study groups (p=0.027, Table 4.1), however, further analysis showed that the age of the cART-treated group (42.96 ± 10.51) was significantly higher compared to the control group (37.07 ± 16.40) (p=0.035, Table 4.1). A significant difference in participant age groups was further found among the three study groups (p<0.01, Table 4.1). In the total study population, most participants were in the age group of 30–39 years (32.2%) (Table 4.1). The age group 40-49 years had most HIV-positive participants on cART (34.5%) as compared to other age groups while the age group 30-39 years had the most HIV-positive participants that were cART-naive (33.3%) as compared to other age groups (Table 4.1). The control group consisted mostly of young adults aged 18–29 years (40.9%) (Table 4.1). The total population consisted predominantly of females (66.5%) as compared to males (33.5%) (Table 4.1). Females had the highest percentage of participants across the HIV-positive groups (Table 4.1). There was no significant difference in terms of education level, however, secondary education had the highest percentage of participants across the study population (Table 4.1).

Table 4.1: Sociodemographic characteristics. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation.

	TOTAL N=155	cART-TREATED N=84	cART-NAÏVE N=27	CONTROL N=44	*P-VALUE*
Age (years), $\bar{x}$ ± SD	40.50 ± 12.62	42.96 ± 10.51	38.44 ± 10.09	37.07 ± 16.40	**0.027***
AGE GROUP, n (%)					
18-29	32 (20.6)	7 (8.3)	7 (26.0)	18 (40.9)	**0.001****
30-39	50 (32.3)	27 (32.2)	9 (33.3)	14 (31.8)	
40-49	40 (25.8)	29 (34.5)	8 (29.6)	3 (6.8)	
50-59	20 (12.9)	14 (16.7)	2 (7.4)	4 (9.1)	
≥ 60	13 (8.4)	7 (8.3)	1 (3.7)	5 (11.4)	
GENDER, n (%)					
Female	103 (66.5)	58 (69.0)	16 (59.3)	29 (65.9)	0.642
Male	52 (33.5)	26 (31.0)	11 (40.7)	15 (34.1)	
EDUCATION LEVEL, n (%)					
No formal education	8 (5.2)	6 (7.1)	1 (3.7)	1 (2.3)	0.719
Primary	21 (13.5)	13 (15.5)	2 (7.4)	6 (13.6)	
Secondary	109 (70.3)	56 (66.7)	22 (81.5)	31 (70.5)	
Tertiary	17 (11.0)	9 (10.7)	2 (7.4)	6 (13.6)	

Bonferroni post hoc: Age significant with p=0.035 between cART-treated and control groups. ***** *Significant at p<0.05.* ****** *Significant at p<0.01.*

4.2. CLINICAL CHARACTERISTICS

There was no significant difference in terms of weight among the three study groups, however, the highest weight was observed in the control group (71.25 ± 15.91) as compared to the cART-treated group (67.92 ± 15.05) and cART-naïve group (64.46 ± 14.88) (Table 4.2). There was no significant difference in terms of height among the three study groups, however, height was higher in the cART-naïve group (1.64 ± 0.07) as compared to the cART-treated group (1.61 ± 0.11) and control group (1.61 ± 0.09) (Table 4.2).

There was a significant difference in $CD4^+$ T-cell count between the cART-treated and the cART-naïve groups and it was significantly higher in the cART-treated group (440.25 ± 221.60) as compared to cART-naïve group (319.22 ± 284.72; p=0.034; Table 4.2).

Overall, a significant difference was observed in the WHO clinical stages between the cART-treated group and the cART-naïve groups ($p<0.01$, Table 4.2). The cART-treated group had majority of participants in WHO clinical stage I (35.1%) whereas the cART-naïve group had most participants in WHO clinical stage IV (47.8%) (Table 4.2). The percentage of HIV-positive participants with WHO clinical stage iv (known as AIDS) was higher in the cART-naïve group (47.8%) as compared to the cART-treated group (14.3%) (Table 4.2).

The cART-treated group was approximately 57.09 ± 41.35 months on cART. Furthermore, most patients in the cART-treated group were on the first-line regimen (81%) which comprised mainly of TDF+FTC+EFV (95%), followed by AZT+3TC+NVP (3%) and ABC+3TC+EFV (2%) (Figure 4.1A and 4.1B). The second-line regimen (which accounted for 19% in the line of regimen) comprised mainly of AZT+3TC+LPV/r users (94%), and TDF+FTC+LPV/r (6%) (Figure 4.1A and 4.1C). Most cART-treated patients were using a TDF-based regimen (78%).

Table 4.2: Clinical characteristics. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; $CD4^+$: Cluster of differentiation 4; WHO: world health organisation.

	TOTAL N=155	cART-TREATED N=84	cART-NAÏVE N=27	CONTROL N=44	*P-VALUE*
Weight (kg), $\bar{x}$ ± SD	68.26 ± 15.34	67.92 ± 15.05	64.46 ± 14.88	71.25 ± 15.91	0.186
Height (m), $\bar{x}$ ± SD	1.62 ± 0.10	1.61 ± 0.11	1.64 ± 0.07	1.61 ± 0.09	0.569
$CD4^+$ T-cell count (cells/µl), $\bar{x}$ ± SD	412.41 ± 241.52	440.25 ± 221.60	319.22 ± 284.72	**-**	**0.034***
Duration on cART (months), $\bar{x}$ ± SD	57.09 ± 41.35	57.09 ± 41.35	-	-	
WHO CLINICAL STAGE, n (%)					
I (≥ 500 cells/µl)	32 (32.0)	27 (35.1)	5 (21.8)	**-**	**0.008****
II (350-499 cells/µl)	25 (25.0)	22 (28.5)	3 (13.0)	-	
III (200-349 cells/µl)	21 (21.0)	17 (22.1)	4 (17.4)	-	
IV (<200 cells/µl)	22 (22.0)	11 (14.3)	11 (47.8)	-	

***** *Significant at p<0.05.* ****** *Significant at p<0.01.*

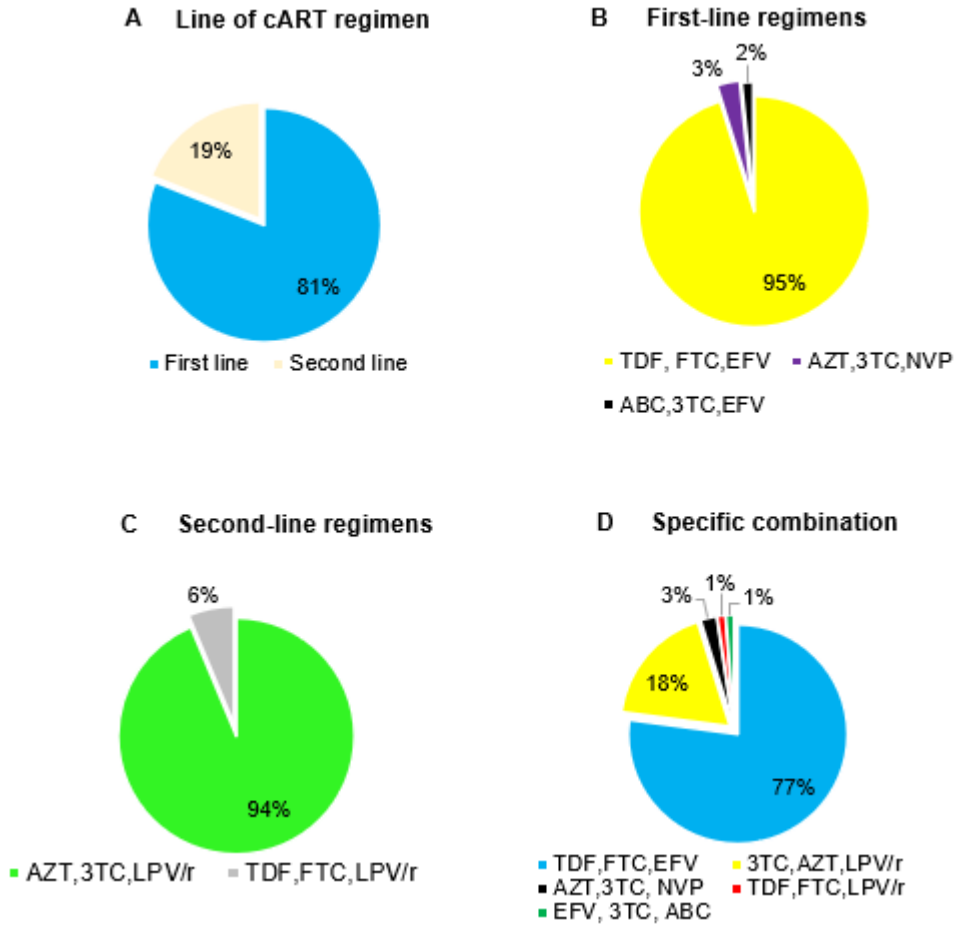


Figure 4.1: The distribution of the different lines of regimen and specific combinations. (A) First- and second-line regimen (B) Specific combinations of the first-line regimen. (C) Specific combinations of second-line regimen. (D) Specific combinations general combination antiretroviral therapy. TDF: Tenofovir disoproxil fumarate; FTC: Emtricitabine; EFV: Efavirenz; 3TC: Lamivudine; AZT: Zidovudine; LPV/r: Lopinavir/ritonavir; NVP: Nevirapine; ABC: Abacavir.

4.3. CARDIOVASCULAR RISK FACTORS

There was no significant difference in BMI among the three study groups, however, BMI was higher in the control group (27.51 ± 6.07) as compared to the cART-

treated group (26.27 ± 6.12) and cART-naïve group (24.22 ± 5.77) (Table 4.3). In terms of the BMI categories, no significant difference was observed among the three study groups. However, the prevalence of obesity was higher in the control group (38.6%) as compared to the cART-treated group (26.2%) and the cART-naïve group (7.4%) (Table 4.3).

Waist circumference showed a marginally significant difference among the three study groups (p=0.052, Table 4.3). The WC was higher in the control group (88.13 ± 13.22) as compared to the cART-treated group (84.46 ± 11.47) and cART-naïve group (81.35 ± 9.66) (p=0.052, Table 4.3) There was no significant difference in the WC categories among the three groups, however, the prevalence of abnormal WC (abdominal obesity) was higher in the control group (40.9%) as compared to the cART-treated group (28.6%) and cART-naïve group (14.8%) (Table 4.3).

No significant difference was found for FBG levels among the three study groups, however, FBG level was higher in the cART-treated group (5.49 ± 1.80) as compared to the cART-naïve group (5.10 ± 1.43) and control group (4.99 ± 0.97) (Table 4.3). There was also no significant difference in the FBG categories among the three study groups. However, the prevalence of prediabetes was higher in the cART-treated group (31.0%) as compared to the control group (22.7%) and the cART-naïve group (7.4%) (Table 4.3). Furthermore, the prevalence of diabetes was higher in the cART-naive group (11%) as compared to the cART-treated group (4.8%) and the control group (2.3%).

Systolic blood pressure (SBP) was higher in the cART-treated group (120.87 ± 20.02) as compared to the cART-naïve group (118.07 ± 14.25) and control group (117.95 ± 14.13) (Table 4.3) (non-significant). In terms of diastolic blood pressure (DBP), no significant difference was found among the three study groups. However, DBP was higher in the cART-treated group (75.18 ± 10.36) as compared to the cART-naïve group (73.19 ± 9.63) and control group (74.18 ± 8.75) (Table 4.3). No significant difference was observed in terms of the blood pressure categories among the three study groups. The prevalence of hypertension was

higher in the cART-treated group (17.9%) as compared to the cART-naïve group (11.1%) and control group (9.1%) (Table 4.3).

Table 4.3: The cardiovascular risk factors of the study population. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure.

	TOTAL N=155	**cART-TREATED N=84**	**cART-NAÏVE N=27**	**CONTROL N=44**	***P-VALUE***
BMI (kg/m^2), $\bar{x}$ ± SD	26.27 ± 6.11	26.27 ± 6.12	24.22 ± 5.77	27.51 ± 6.07	0.088
WC (cm), $\bar{x}$ ± SD	84.97 ± 11.87	84.46 ± 11.47	81.35 ± 9.66	88.13 ± 13.22	0.052
FBG (mmol/l), $\bar{x}$ ± SD	5.28 ± 1.55	5.49 ± 1.80	5.10 ± 1.43	4.99 ± 0.97	0.187
SBP (mmHg), $\bar{x}$ ± SD	119.55 ± 17.55	120.87 ± 20.02	118.07 ± 14.25	117.95 ± 14.13	0.601
DBP (mmHg), $\bar{x}$ ± SD	74.55 ± 9.77	75.18 ± 10.36	73.19 ± 9.63	74.18 ± 8.75	0.629
BMI CATEGORY, n (%)					
Underweight	9 (5.8)	6 (7.1)	2 (7.4)	1 (2.3)	0.127
Normal weight	62 (40.0)	31(36.9)	14 (51.9)	17 (38.6)	
Overweight	43 (27.7)	25 (29.8)	9 (33.3)	9 (20.5)	
Obesity	41 (26.5)	22 (26.2)	2 (7.4)	17 (38.6)	
WC CATEGORY, n (%)					
Normal	109 (70.3)	60 (71.4)	23 (85.2)	26 (59.1)	0.062
Abnormal	46 (29.7)	24 (28.6)	4 (14.8)	18 (40.9)	
FBG CATEGORY, n (%)					
Normal	109 (70.3)	54 (64.2)	22 (81.5)	33 (75.0)	0.080
Prediabetes	38 (24.5)	26 (31.0)	2 (7.4)	10 (22.7)	
Diabetes	8 (5.2)	4 (4.8)	3 (11.1)	1 (2.3)	
BLOOD PRESSURE CATEGORY, n (%)					
Normotension	81 (52.3)	44 (52.3)	14 (51.9)	23 (52.3)	0.833
Optimal	29 (18.7)	14 (16.7)	5 (18.5)	10 (22.7)	
High normal	23 (14.8)	11 (13.1)	5 (18.5)	7(15.9)	
Hypertension	22 (14.2)	15 (17.9)	3 (11.1)	4 (9.1)	

4.4. THE EFFECT OF COMBINATION ANTIRETROVIRAL THERAPY ON RENAL FUNCTION

4.4.1. The estimated glomerular filtration rate of the study population

There was no significant difference in the level of eGFR≥ 60 mL/min/1.73m^2 among the three study groups (Figure 4.2). However, the level of eGFR≥ 60 mL/min/1.73m^2 was higher in the control group (97.7%) as compared to the cART-naïve group (96.3%) and cART-treated group (88.1%) (Figure 4.2). There was also no significant difference in the level of eGFR<60 mL/min/1.73m^2. However, the level of eGFR<60 mL/min/1.73m^2 was higher in the cART-treated group (11.9%) as compared to the cART-naïve group (3.7%) and control group (2.3%) (Figure 4.2).

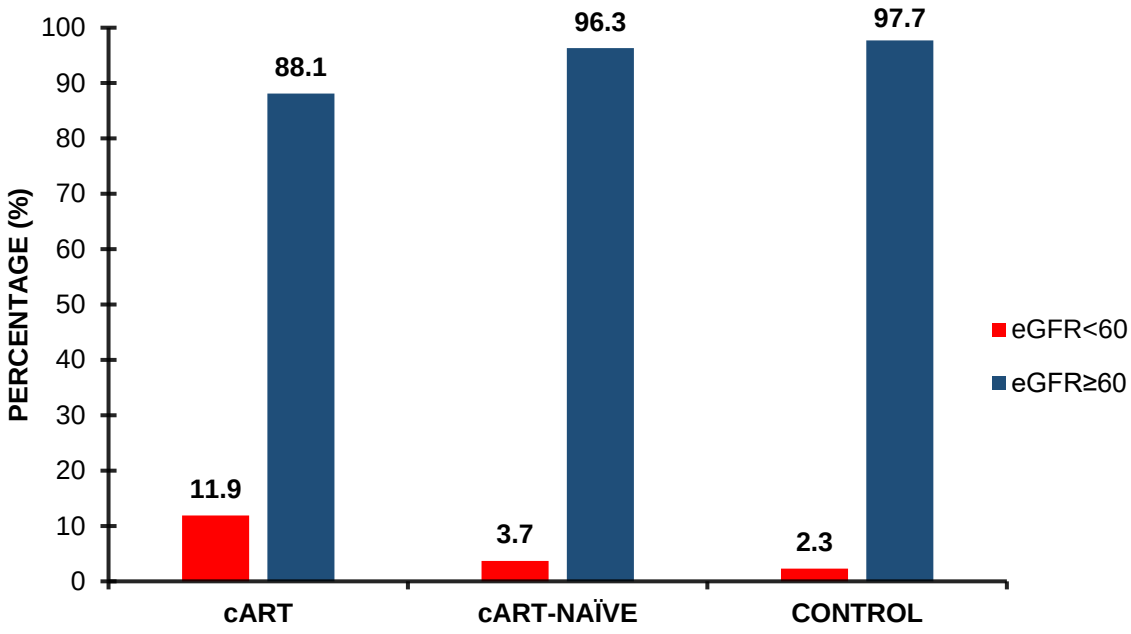


Figure 4.2: Estimated glomerular filtration rate levels

4.4.2. Biomarkers of renal function

There was no significant difference observed in cystatin C levels among the three study groups, however, cystatin C levels were higher in the cART-treated group (0.89 ± 0.60) as compared to the cART-naïve group (0.77 ± 0.61) and control group (0.71 ± 0.22) (Table 4.4, Figure 4.3A). A significant difference in RBP4 levels was observed among the three study groups ($F(2,152) = [5.340]$, $p<0.01$, Table 4.4). Further analysis showed that RBP4 levels were significantly higher in the cART-treated group (20218.59 ± 13949.49) as compared to the cART-naïve group (11559.94 ± 6935.99) ($p<0.01$;Table 4.4, Figure 4.3B). No significant difference in terms of clusterin levels was found among the three study groups, however, clusterin level was higher in the cART-treated group (3682947.30 ± 13399114.26) as compared to the cART-naïve (1018749.38 ± 473416.39) and control group (1031887.89 ± 316234.36) (Table 4.4, Figure 4.3C). The eGFR levels initially showed a significant difference among the three study groups ($F(2, 152) = [3.242]$, $p=0.042$, Table 4.4). The eGFR was lower in the cART-treated group (104.18 ± 37.09) as compared to the cART-naïve group (119.81 ± 38.62) and control group (117.64 ± 27.69). Further analysis showed that the eGFR level was the lower in the cART-treated group (104.18 ± 37.09) as compared to the cART-naïve group (119.81 ± 38.62) and control group (117.64 ± 27.69), however, non-significant (Table 4.4, Figure 4.3D).

Table 4.4: Biomarkers and the stages of glomerular filtration rate. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate.

	TOTAL N=155	**cART-TREATED N=84**	**cART-NAÏVE N=27**	**CONTROL N=44**	***P-VALUE***
Cystatin C (mg/l), $\bar{x}$ ± SD	0.82 ± 0.52	0.89 ± 0.60	0.77 ± 0.61	0.71 ± 0.22	0.185
RBP4 (ng/ml), $\bar{x}$ ± SD	18006.64 ± 12327.33	20218.59 ± 13949.49	11559.94 ± 6935.99	17739.77 ± 10189.38	**0.006****
Clusterin (ng/ml), $\bar{x}$ ± SD	2466302.41 ± 9929323.01	3682947.303 ± 13399114.26	1018749.38 ± 473416.39	1031887.89 ± 316234.36	0.254
eGFR (ml/min/1.73m^2), $\bar{x}$ ± SD	110.72 ± 35.48	104.18 ± 37.09	119.81 ± 38.62	117.64 ± 27.69	**0.042***
eGFR CATEGORY, n (%)					
Normal or high	119 (76.8)	57 (67.8)	22 (81.5)	40 (90.9)	0.309
Mildly decreased	24 (15.5)	17 (20.2)	4 (14.8)	3 (6.8)	
Mildly to moderately decreased	6 (3.9)	5 (6.0)	0 (0.0)	1 (2.3)	
Moderately to severely decreased	2 (1.3)	2 (2.4)	0 (0.0)	0 (0.0)	
Severely decreased	1 (0.6)	1 (1.2)	0 (0.0)	0 (0.0)	
Renal failure	3 (1.9)	2 (2.4)	1 (3.7)	0 (0.0)	

Bonferroni post hoc: RBP4 significant with p=0.004 between cART-treated and cART-naïve groups.
***** *Significant at p<0.05.* ****** *Significant at p<0.01.*

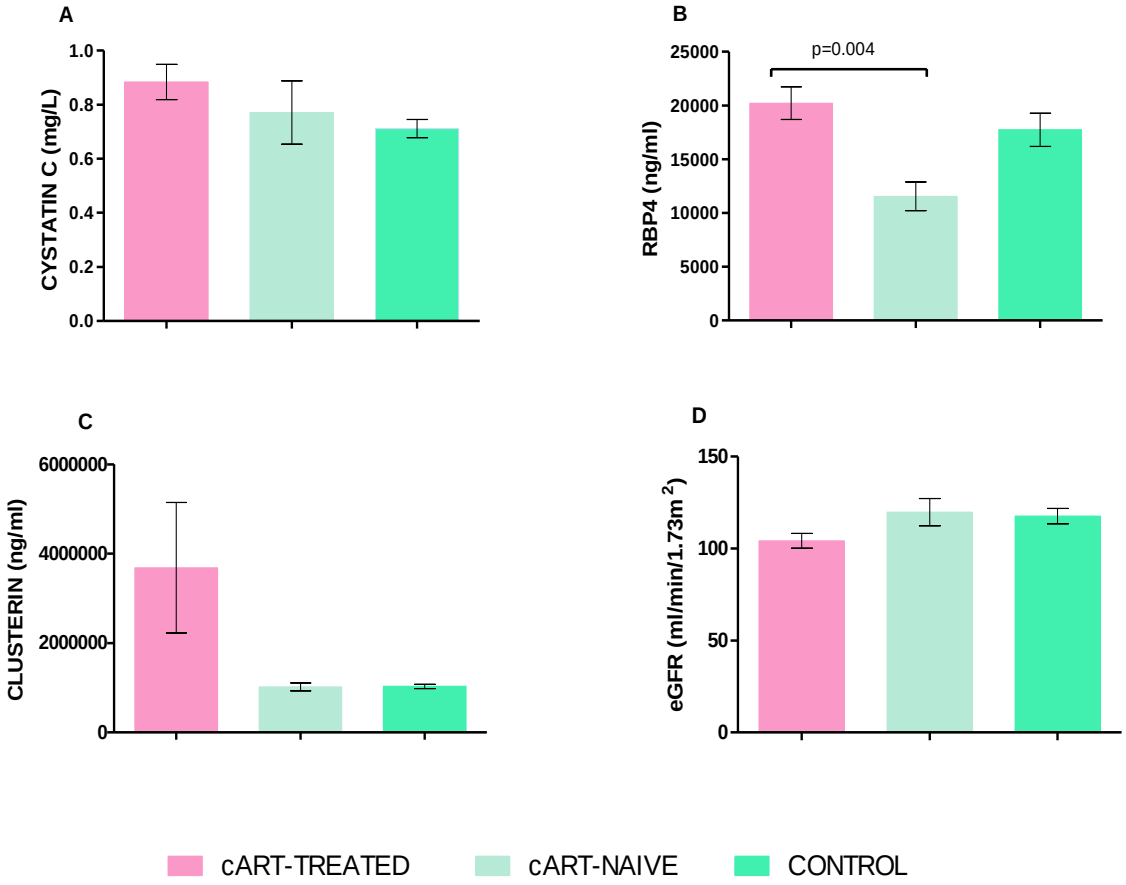


Figure 4.3: The renal function marker levels. RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate.

There was no significant difference in cystatin C levels among the three study groups after adjusting for covariates (age, gender, alcohol consumption, BMI, SBP and FBG) (Table 4.5). However, cystatin C levels remained higher in the cART-treated group (0.88 ± 0.60) as compared to the cART-naïve (0.77 ± 0.61) and control group (0.71 ± 0.22) (Figure 4.4A). A significant difference in RBP4 levels was observed among the three study groups after adjusting for the above-mentioned covariates (F (2, 146) = [4.749], p=0.010, Table 4.5). The RBP4 level was specifically significantly higher in the cART-treated group (20218.59 ± 13949.49) as compared to the cART-naïve group (11559.94 ± 6935.99) after adjusting for covariates (p<0.01, Table 4.5, Figure 4.4B). In terms of clusterin levels, no significant difference was observed among the three groups after adjusting for the above-mentioned covariates. However, clusterin levels remained

higher in the cART-treated group (3682947.303 ± 13399114.26) as compared to the cART-naïve group (1018749.38 ± 473416.39) and control group (1031887.89 ± 316234.36) (Table 4.5, Figure 4.4C). The eGFR levels did not significantly differ among the three study groups after adjusting for the above-mentioned covariates. However, the eGFR level remained lower in the cART-treated group (104.18 ± 37.09) as compared to the cART-naïve group (119.81 ± 38.62) and control group (117.64 ± 27.69) (Table 4.5, Figure 4.4D).

Table 4.5: Renal function markers levels after adjusting for age, gender, alcohol consumption, body mass index, systolic blood pressure, and fasting blood glucose. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate.

	cART-TREATED N=84	**cART-NAÏVE N=27**	**CONTROL N=44**	***P-VALUE***
Cystatin C (mg/l), $\bar{x}$ ± SD	0.88 ± 0.60	0.77 ± 0.61	0.71 ± 0.22	0.223
RBP4 (ng/ml), $\bar{x}$ ± SD	20218.59 ± 13949.49	11559.94 ± 6935.99	17739.77 ± 1018.38	**0.010***
Clusterin (ng/ml), $\bar{x}$ ± SD	3682947.303 ± 13399114.26	1018749.38 ± 473416.39	1031887.89 ± 316234.36	0.235
eGFR (ml/min/1.73m^2), $\bar{x}$ ± SD	104.18 ± 37.09	119.81 ± 38.62	117.64 ± 27.69	0.240

RBP4 significant at p=0.008 between cART-treated and cART-naïve groups. *Significant at p<0.05.

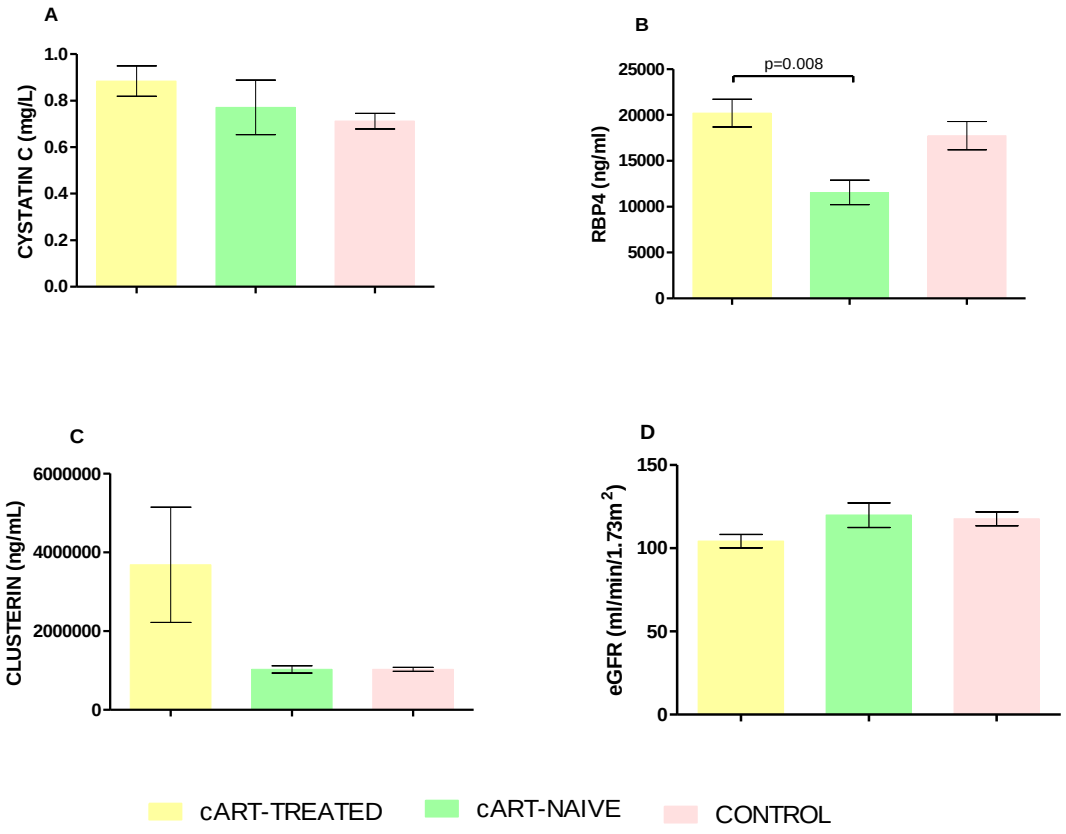


Figure 4.4: Renal function markers levels after adjusting for age, gender, alcohol consumption, body mass index, systolic blood pressure and fasting blood glucose. cART: combination antiretroviral therapy; eGFR: estimated glomerular filtration rate; RBP4: retinol-binding protein 4; p: significance level.

Further analysis showed that there was no significant difference in cystatin C levels between the cART-treated and cART-naïve groups after adjusting for covariates (age, gender, alcohol consumption, BMI, SBP, FBG, and $CD4^+$ T-cell count). However, the cystatin C level was higher in the cART-treated group (0.88 ± 0.60) as compared to the cART-naïve group (0.77 ± 0.61) (Table 4.6, Figure 4.5A). There was a significant difference in RBP4 levels between the cART-treated group and the cART-naïve group after adjusting for age, gender, alcohol consumption, BMI, SBP, FBG, and $CD4^+$ T-cell count (F (1, 91) = [6.665], p=0.011, Table 4.6). The RBP4 level remained significantly higher in the cART-treated group (20218.59 ±

13949.49) as compared to the cART-naïve group (11559.94 ± 6935.99) after adjusting for covariates (Table 4.6, Figure 4.5B).

No significant difference was found for clusterin between the cART-treated group and the cART-naïve group after adjusting for age, gender, alcohol consumption, BMI, SBP, FBG, and $CD4^+$ T-cell count. Clusterin remained higher in the cART-treated group (3682947.303 ± 13399114.26) as compared to the cART-naïve group (1018749.38 ± 473416.39) (non-significant) (Table 4.6, Figure 4.5C). The eGFR showed no significant difference between the cART-treated and cART-naïve groups after adjusting for age, gender, alcohol consumption, BMI, SBP, FBG, and $CD4^+$ T-cell count. However, eGFR remained lower in the cART-treated group (104.18 ± 37.09) as compared to the cART-naïve group (119.81 ± 38.62) (Table 4.6, Figure 4.5D).

Table 4.6: Renal function markers levels between combination antiretroviral therapy-treated and combination antiretroviral therapy-naïve groups after adjusting for age, gender, alcohol consumption, body mass index, systolic blood pressure and fasting blood glucose and $CD4^+$ T-cell count. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate.

	cART-TREATED N=84	**cART-NAÏVE N=27**	***P-VALUE***
Cystatin C (mg/l), $\bar{x}$ ± SD	0.88 ± 0.60	0.77 ± 0.61	0.698
RBP4 (ng/ml), $\bar{x}$ ± SD	20218.59 ± 13949.49	11559.94 ± 6935.99	**0.011***
Clusterin (ng/ml), $\bar{x}$ ± SD	3682947.303 ± 13399114.26	1018749.38 ± 473416.39	0.446
eGFR (ml/min/1.73m^2), $\bar{x}$ ± SD	104.18 ± 37.09	119.81 ± 38.62	0.385

*Significant at p<0.05

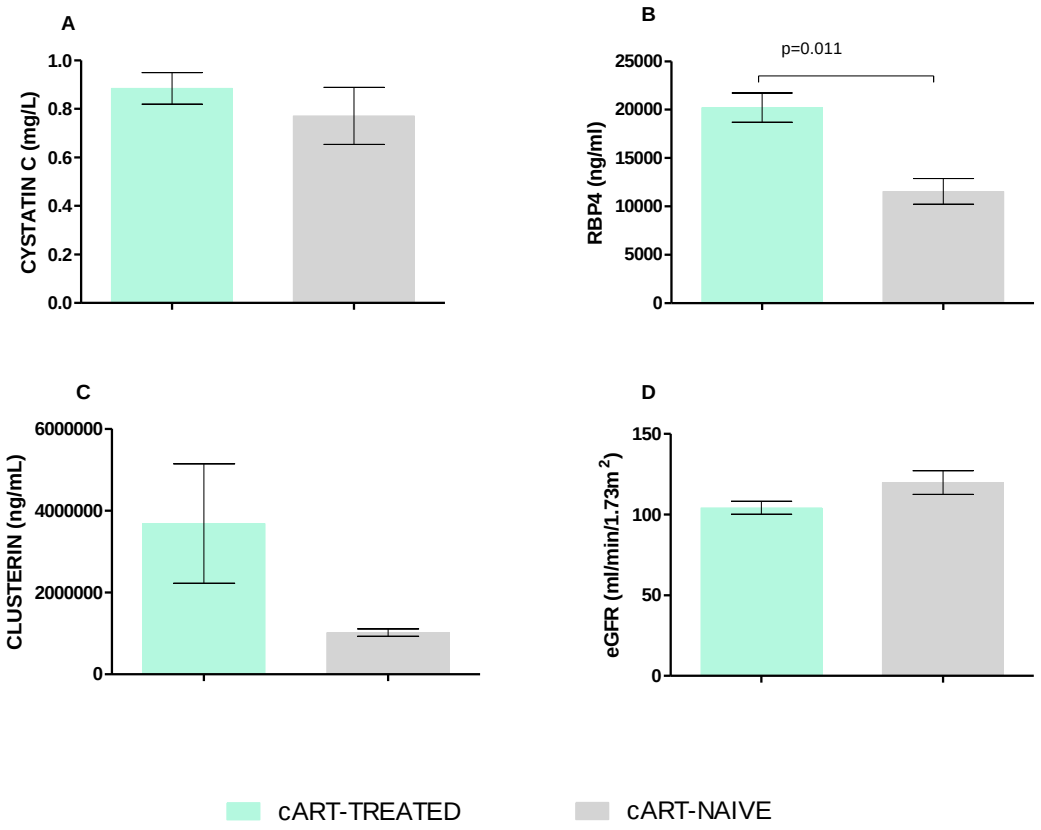


Figure 4.5: Renal function markers levels between combination antiretroviral therapy-treated and combination antiretroviral therapy-naïve groups after adjusting for age, gender, alcohol consumption, body mass index, systolic blood pressure, fasting blood glucose, and $CD4^+$ T-cell count. cART: combination antiretroviral therapy; eGFR: estimated glomerular filtration rate; RBP4: retinol-binding protein 4. p: significance level.

4.4.3. Biomarkers of renal function among first-line regimen, second-line regimen, and combination antiretroviral therapy-naïve groups

The renal marker levels were also compared among the participants on the first-line regimen, second-line regimen, and the cART-naïve group as shown in table 4.7 and figure 4.6. There was no significant difference in cystatin C levels among the participants on the first-line regimen, second-line regimen, and the cART-naïve group. Although no significant difference was observed, cystatin C was higher in

the participants on second-line regimen (0.93 ± 0.38) as compared to the first-line regimen (0.87 ± 0.64) and cART-naïve group (0.77±0.61) (Table 4.7, Figure 4.6A).

The RBP4 was found to be significantly different among the participants on the first-line regimen, second-line regimen, and the cART-naïve group (F (2, 108)= [4.951], p<0.01, Table 4.7). Further analysis showed that RBP4 was significantly higher in the participants on the first-line regimen (19814.55 ± 14394.36) as compared to the cART-naïve group (11559.94 ± 6935.99) (p=0.015, Table 4.7, Figure 4.6B). Furthermore, it was observed that RBP4 was also significantly higher among participants on the second-line regimen (21935.75 ± 12138.95) as compared to the cART-naïve group (11559.94 ± 6935.99) (p=0.032, Table 4.7, Figure 4.6B).

There was no significant difference in clusterin levels among the participants on the first-line regimen, second-line regimen, and the cART-naïve group. However, clusterin was higher among participants on the first-line regimen (4259691.20 ± 14852493.72) as compared to the second-line regimen (1231785.72 ± 431236.80) and cART-naive group (1018949.38 ± 473416.39) (Table 4.7, Figure 4.6C). In terms of eGFR levels, no significant difference was found among participants on the first-line regimen, second-line regimen, and the cART-naïve group. However, the eGFR level was lower in the second-line regimen (93.38 ± 34.11) as compared to the cART-naïve group (119.81 ± 38.62) and first-line regimen (106.72 ± 37.54) (Table 4.7, Figure 4.6D).

Table 4.7: Renal function markers among participants on the first-line regimen, second-line regimen, and combination antiretroviral therapy-naïve group. cART: combination antiretroviral therapy; N: number of participants; P: significance level; $\bar{x}$: mean; SD: standard deviation; RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate.

	FIRST LINE N=68	**SECOND LINE N=16**	**cART-NAÏVE N=27**	***P-VALUE***
Cystatin C (mg/l), $\bar{x}$ ± SD	0.87±0.64	0.93±0.38	0.77±0.61	0.652
RBP4 (ng/ml), $\bar{x}$ ± SD	19814.55±14394.36	21935.75±12138.95	11559.94±6935.99	**0.009****
Clusterin (ng/ml), $\bar{x}$ ± SD	4259691.20±14852493.72	1231785.72±431236.80	1018949.38±473416.39	0.385
eGFR (ml/min/1.73m^2), $\bar{x}$ ± SD	106.72±37.540	93.38±34.11	119.81±38.62	0.078

Bonferroni post hoc: RBP4 significant at p=0.015 between first line and cART-naïve groups.
RBP4 significant at p=0.032 between second line and cART-naïve groups. *Significant at p<0.05.
** Significant at p<0.01.

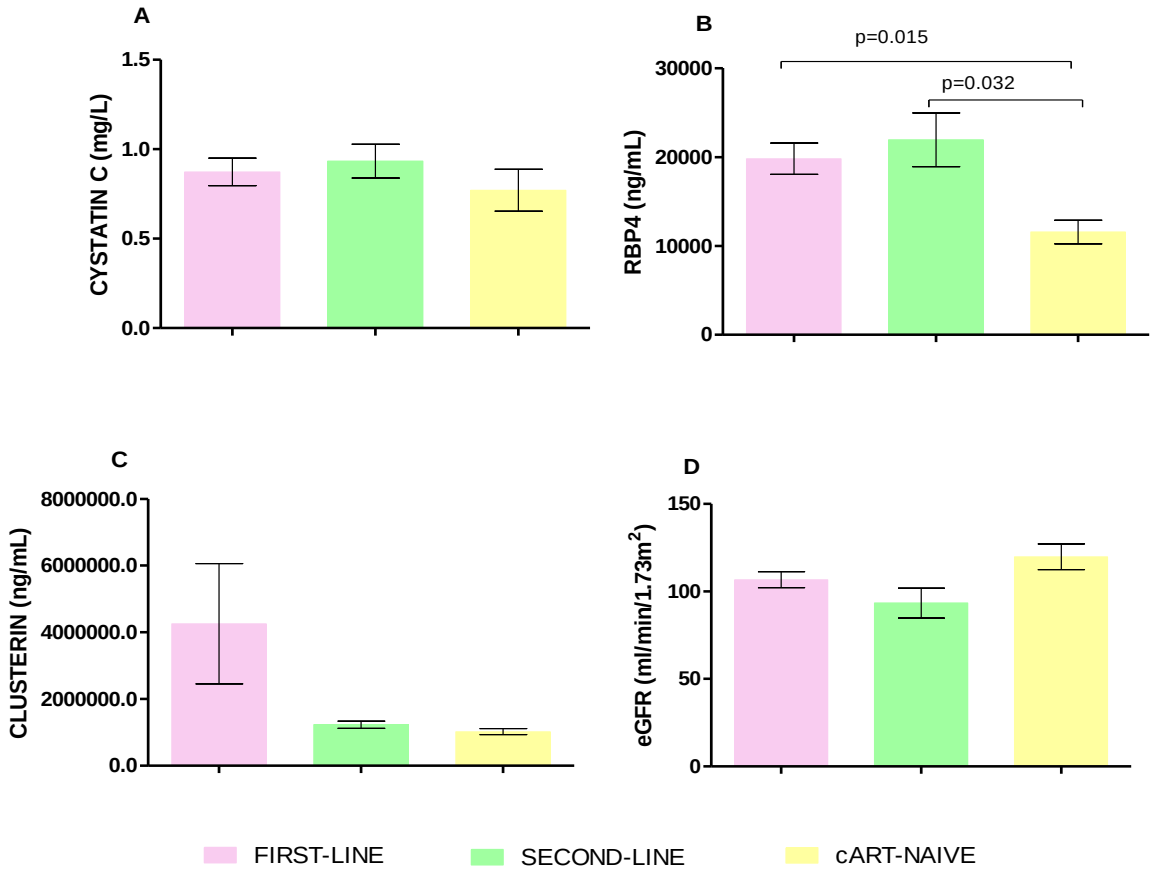


Figure 4.6: Renal function marker levels among participants on the first-line regimen, second-line regimen and combination antiretroviral therapy-naïve group. RBP4: retinol-binding protein 4; eGFR: estimated glomerular filtration rate; RBP4: retinol-binding protein 4; p: significance level.

4.4.4. The association between duration on combination antiretroviral therapy and renal function biomarkers

A significant positive association was observed between the duration of cART and cystatin C (r=0.23, p=0.046, Figure 4.7A). There was a non-significant positive association between the duration of cART and RBP4 (r=0.11, Figure 4.7B). There was a non-significant negative association between the duration of cART and clusterin (r=-0.06, Figure 4.7C). A significant negative association was found between the duration of cART and eGFR (r=-0.32, p=0.004, Figure 4.7D).

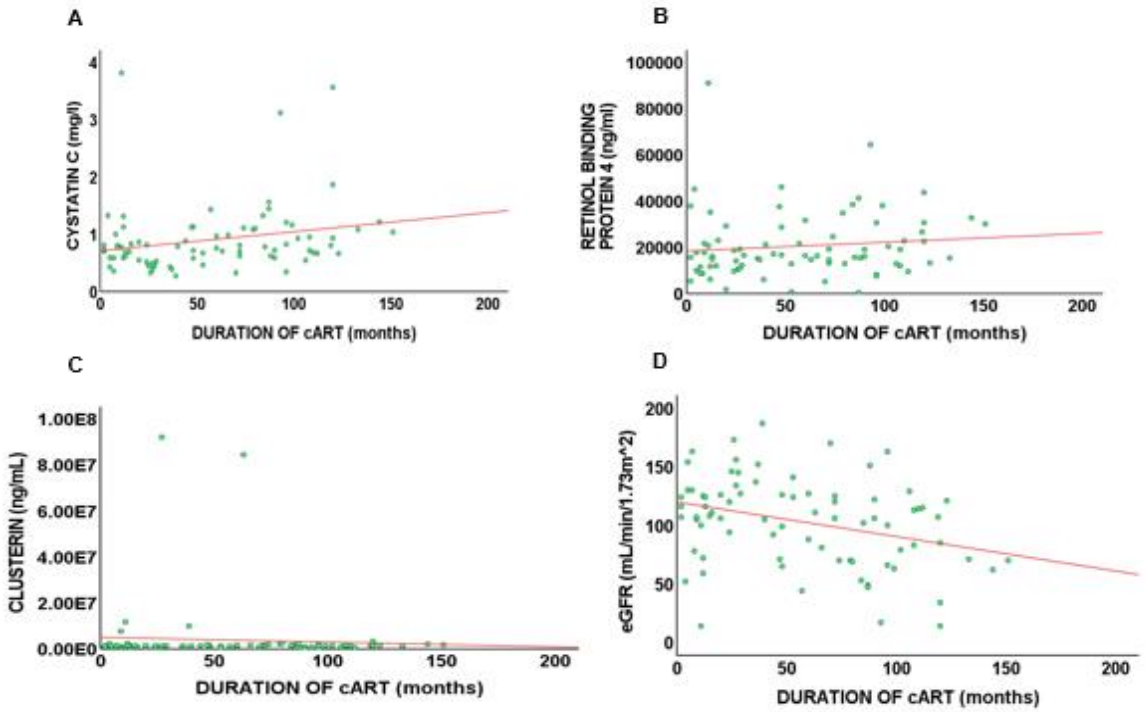


Figure 4.7: Scatter plots for association between duration on cART and renal function. cART: combination antiretroviral therapy; eGFR: estimated glomerular filtration rate.

4.5. ASSOCIATION BETWEEN CARDIOVASCULAR RISK FACTORS AND RENAL FUNCTION

4.5.1. Pearson correlations

The associations between cardiovascular risk factors and renal function were determined using Pearson correlation and multivariate regression analysis. The Pearson correlation analysis for the cART-treated group (n=84) showed a significant negative association between age and eGFR (r=-0.387, p<0.01, Table 4.8). There was a significant positive association between duration on cART and cystatin C (r=0.255, p=0.046, Table 4.8). Furthermore, a significant negative association was found between duration on cART and eGFR (r=-0.323, p<0.01, Table 4.8). A significant negative association was observed between $CD4^+$ T-cell count and cystatin C (r=-0.240, p=0.035, Table 4.8). Furthermore, a significant positive association was observed between $CD4^+$ T-cell count and clusterin (r=0.236, p=0.039, Table 4.8) as well as between $CD4^+$ T-cell count and eGFR (r=0.227, p=0.047, Table 4.8).

Table 4.8: Pearson correlation of cardiovascular risk factors with biomarkers of renal function in the cART-treated group. cART: combination antiretroviral therapy; N: number of participants; $CD4^+$ T-cell count: cluster of differentiation 4 cell count; BMI: body mass index; SBP: systolic blood pressure; FBG: fasting blood glucose; eGFR: estimated glomerular filtration rate; r: correlation coefficient; p: significance level; RBP4: retinol-binding protein 4.

cART-TREATED (N=84)					
		Cystatin C	**RBP4**	**Clusterin**	**eGFR**
Age	*R*	0.207	0.159	-0.072	-0.387
	P	0.067	0.149	0.518	**0.000****
Gender	*R*	0.168	0.152	-0.098	-0.151
	P	0.126	0.168	0.374	0.171
Alcohol consumption	*R*	0.076	0.078	0.079	-0.069
	P	0.493	0.479	0.476	0.535
Duration of cART	*R*	0.225	0.110	-0.062	-0.323
	P	**0.046***	0.335	0.589	**0.004****
$CD4^+$ T-cell count	*R*	-0.240	-0.193	0.236	0.227
	P	**0.035***	0.092	**0.039***	**0.047***
BMI	*R*	0.040	0.053	-0.052	0.004
	P	0.721	0.632	0.639	0.974
SBP	*R*	-0.085	-0.042	-0.031	-0.011
	P	0.441	0.706	0.781	0.919
FBG	*R*	0.049	0.072	-0.050	-0.111
	P	0.658	0.517	0.650	0.315

*Correlation significant at p<0.05. ** Correlation is significant at p<0.01.

Pearson correlation analysis was also performed for the HIV-positive group (n=111) which consisted of a combination of participants from the cART-treated group (n=111) and the cART-naïve group (n=27) (Table 4.9). The HIV-positive group was created due to the small sample size of the cART-naïve group. A significant negative association was found between age and eGFR (r=-0.374, p<0.01, Table 4.9). There was a significant positive association between cART use and RBP4 (r=0.284, p<0.01, Table 4.9). A significant positive association was observed between the duration of cART and cystatin C (r=0.255, p=0.046). Further, a significant negative association was found between duration on cART

and eGFR (r=-0.323, p<0.01, Table 4.9). The $CD4^+$ T-cell count had a significant positive association with clusterin (r=0.210, p=0.036, Table 4.9).

Table 4.9: Pearson correlation of cardiovascular risk factors with biomarkers of renal function in the HIV-positive group. cART: combination antiretroviral therapy; N: number of participants; $CD4^+$ T-cell count: cluster of differentiation 4 cell count; BMI: body mass index; SBP: systolic blood pressure; FBG: fasting blood glucose; eGFR: estimated glomerular filtration rate; r: correlation coefficient; p: significance level. HIV: human immunodeficiency virus. RBP4: retinol-binding protein 4.

HIV-POSITIVE (N=111)					
		Cystatin C	**RBP4**	**Clusterin**	**eGFR**
Age	*r*	0.191	0.188	-0.041	-0.374
	P	0.045	0.048	0.671	**0.000****
cART use	*R*	0.082	0.284	0.098	-0.178
	P	0.393	**0.002****	0.305	0.062
Gender	*R*	0.061	0.046	-0.095	-0.021
	P	0.527	0.633	0.321	0.827
Alcohol consumption	*R*	0.094	0.072	0.068	-0.092
	P	0.328	0.452	0.477	0.336
Duration of cART	*R*	0.225	0.110	-0.062	-0.323
	P	**0.046***	0.335	0.589	**0.004****
$CD4^+$ T-cell count	*R*	-0.109	-0.039	0.210	0.049
	P	0.282	0.700	**0.036***	0.627
BMI	*R*	0.108	0.133	-0.028	-0.114
	P	0.258	0.163	0.772	0.235
SBP	*R*	-0.041	-0.009	-0.021	-0.051
	P	0.667	0.927	0.826	0.596
FBG	*R*	0.052	0.069	-0.034	-0.142
	P	0.587	0.476	0.724	0.137

*Correlation significant at p<0.05. ** Correlation is significant at p<0.01.

For the control group (n=44), the Pearson correlation analysis did not reveal significant associations between the cardiovascular risk factors and renal function as seen in Table 4.10.

Table 4.10: Pearson correlation of cardiovascular risk factors with biomarkers of renal function in the control group. N: number of participants; BMI: body mass index; SBP: systolic blood pressure; FBG: fasting blood glucose; eGFR: estimated glomerular filtration rate; r: correlation coefficient; p: significance level. RBP4: retinol-binding protein 4.

	CONTROL (N=44)				
		Cystatin C	**RBP4**	**Clusterin**	**eGFR**
Age	*R*	0.117	0.151	0.181	0.274
	P	0.449	0.328	0.328	0.072
Gender	*R*	0.098	-0.038	0.095	0.179
	P	0.526	0.809	0.540	0.244
Alcohol consumption	*R*	0.167	0.231	0.192	-0.229
	P	0.278	0.131	0.213	0.134
BMI	*R*	-0.222	-0.234	0.095	0.088
	P	0.147	0.126	0.541	0.580
SBP	*R*	0.194	0.128	-0.042	-0.212
	P	0.207	0.407	0.785	0.167
FBG	*R*	0.203	0.078	0.224	-0.251
	P	0.185	0.616	0.143	0.100

4.5.2. Multiple regression analysis

The multiple-adjusted stepwise analysis for the cART-treated group showed that $CD4^+$ T-cell count independently negatively associated cystatin C (β=-0.236, p=0.040, Table 4.11). Age was independently negatively associated with eGFR (β=-0.363; p<0.01, Table 4.11). The duration on cART was independently positively associated with cystatin C (β=0.232, p=0.043, Table 4.11) and independently negatively associated with eGFR (β=-0.230, p=0.034, Table 4.11). The $CD4^+$ T-cell count independently positively associated with both clusterin (β=0.263, p=0.025, Table 4.11) as well as eGFR (β=0.222, p=0.034, Table 4.11).

Table 4.11: Multiple stepwise analysis of cardiovascular risk factors and renal function biomarkers in the combination antiretroviral therapy-treated group. cART: combination antiretroviral therapy; N: number of participants; **adj.** R^2: adjusted R square; $CD4^+$ T-cell count: cluster of differentiation 4; CI: confidence interval; eGFR: estimated glomerular filtration rate; P: significance level: β: standardised beta weight.

cART-TREATED (N=84)				
	Cystatin C		**Clusterin**	
	β (95% CI)	***P***	**β (95% CI)**	***P***
Model 1 (adj. R^2=0.044; p=0.041)			***Model 1*** (adj. R^2=0.056; p=0.025)	
$CD4^+$ T-cell count	-0.240 (-0.366; -0.007)	**0.041***	0.263 (568847.071; 8029033.546)	**0.025***
Model 2 (adj. R^2=0.086; p=0.016)				
$CD4^+$ T-cell count	-0.236 (-0.329; -0.008)	**0.040***		
Duration on Cart	0.232 (0.005; 0.299)	**0.043***		
	eGFR			
	β (95% CI)	***P***		
Model 1 (adj. R^2=0.162; p=0.000)				
Age	-0.417 (-29.288; -9.355)	**0.000****		
Model 2 (adj. R^2=0.203; p=0.000)				
Age	-0.422 (-29.255; -9.809)	**0.000****		
$CD4^+$ T-cell count	0.227 (0.766; 19.085)	**0.034***		
Model 3 (adj. R^2=0.243; p=0.000)				
Age	-0.363 (-26.616; -7.003)	**0.001****		
$CD4^+$ T-cell count	0.222 (0.767;18.633)	**0.034***		
Duration on Cart	-0.230 (-17.623; -0.715)	**0.034***		

*Correlation significant at p<0.05. ** Correlation is significant at p<0.01.

The stepwise regression analysis for the HIV-positive group showed that age independently positively associated with cystatin C (β=0.207, p=0.039, Table 4.12). The use of cART independently positively associated with RBP4 (β=0.282, p<0.01, Table 4.12). The $CD4^+$ T-cell count independently positively associated with clusterin (β=0.210, p=0.036, Table 4.12) whereas age independently negatively associated with eGFR (β=-0.393, p<0.01, Table 4.12).

Table 4.12: Multiple stepwise regression analysis of cardiovascular risk factors and renal function biomarkers in the HIV-positive group. cART: combination antiretroviral therapy; N: number of participants; adj.R^2: adjusted R square; $CD4^+$ T-cell count: cluster of differentiation 4; CI: confidence interval; eGFR: estimated glomerular filtration rate; P: significance level: β: standardised beta weight. HIV: human immunodeficiency virus; RBP4: retinol-binding protein 4.

HIV-POSITIVE (N=111)						
	Cystatin C		**RBP4**		**Clusterin**	
	β (95% CI)	***P-VALUE***	**β (95% CI)**	***P-VALUE***	**β (95% CI)**	***P-VALUE***
Model 1 (adj. R^2=0.033; p=0.039)			***Model 1*** (adj. R^2=0.070; p=0.004)		***Model 1*** (adj. R^2=0.034; p=0.036)	
Age	0.207 (0.015; 0.565)	**0.039***				
cART use, yes or no	-	-	0.282 (0.231; 1.223)	**0.004****		
$CD4^+$ T-cell count					0.210 (0.018; 0.504)	**0.036***
	eGFR					
	β (95% CI)	***P-VALUE***				
Model 1 (adj. R^2=0.146; p=0.000)						
Age	-0.393 (-0.473; -0.268)	**0.000****				

*Correlation significant at p<0.05. ** Correlation significant at p<0.01.

The multiple stepwise analysis for the control group did not reveal any association found between the cardiovascular risk factors and renal function. However, using the multiple enter regression analysis, the cardiovascular risk factors associated with renal function (non-significant) (Table 4.13).

Table 4.13: Multiple (enter method) regression analysis of cardiovascular risk factors and renal function biomarkers in the control group. cART: combination antiretroviral therapy; N: number of participants; **adj.R^2**: adjusted R square; CI: confidence interval; eGFR: estimated glomerular filtration rate; P: significance level: β: standardised beta weight; RBP4: retinol-binding protein 4; BMI: body mass index; SBP: systolic blood pressure; FBG: fasting blood glucose.

CONTROL (N=44)						
	Cystatin C (adj. R^2=0.029; p=0.281)		**RBP4 (adj. R^2=-0.008; p=0.466)**		**Clusterin (adj. R^2=-0.013; p=0.494)**	
	β (95% CI)	***P-VALUE***	**β (95% CI)**	***P-VALUE***	**β (95% CI)**	***P-VALUE***
Age	0.046 (-0.091; 0.122)	0.774	0.142 (-0.120; 0.301)	0.390	0.148 (-0.005; 0.012)	0.373
BMI	-0.182 (-0.212; 0.056)	0.245	-0.230 (-0.455; 0.074)	0.153	0.091 (-0.007; 0.013)	0.568
SBP	0.169 (-0.083; 0.261)	0.300	0.056 (-0.283; 0.387)	0.735	-0.044 (-0.015; 0.011)	0.788
FBG	0.198 (-0.080; 0.349)	0.211	0.040 (-0.371; 0.477)	0.802	0.197 (-0.006; 0.026)	0.225
	eGFR (adj. R^2=0.064; p=0.163)					
	β (95% CI)	***P-VALUE***				
Age	-0.188 (-0.304; 0.079)	0.241				
BMI	0.050 (-0.201; 0.280)	0.741				
SBP	-0.178 (-0.482; 0.137)	0.267				
FBG	-0.228 (-0.669; 0.103)	0.146				

CHAPTER 5: DISCUSSION

5.1. SOCIODEMOGRAPHIC CHARACTERISTICS

In the present study, we found that the mean age was significantly higher in the cART-treated group as compared to the control group. The cART-treated group consisted mostly of older adults while the HIV-negative group consisted mostly of young adults. Evidence shows that South African HIV-positive adults nowadays have a near-normal life expectancy as the treatment increases CD4+ T-cell count and therefore prolongs survival due to reductions in HIV-related morbidity and mortality (Meintjes *et al*., 2017; Johnson *et al*., 2013). Our finding showed that $CD4^+$ T-cell count of the cART-treated patients was higher which reflect that the treatment did have a beneficial role in the prolonging survival of our patients. With good adherence to cART, this population will live longer and have low death rates.

We observed that in the cART-naïve group, most participants were in the age group 30–39 years which could mean that infection rates are higher among individuals in their thirties as compared to other age groups. Contrary to our findings, previous studies reported that HIV transmission is more prevalent in the age group 15–24 years which may be because the sample of these studies were taken from a younger group and that younger people are keener to test for HIV than the older population (Naicker *et al*., 2015; Simbayi *et al*., 2019). We speculate that poverty, unemployment, gender-based violence which promote multiple sexual relationships, non-condom use, intergenerational sex, and other unsafe sexual practices may have contributed to the high infection rates we found in the age group 30–39 years. Furthermore, most people in SA get married between the ages of 30–39 years and this group was reported to have lower condom use and high male infidelity rates which encourage risky sexual behaviours and predispose to HIV infection (Hallett and Abu-Raddad, 2012;

Shisana *et al*. 2014; StatsSA, 2021). The age group 30–39 years is the working class which implies that the high HIV infection rate in this group will result in many of them not being able to go to work which will negatively affect the economy.

We found that HIV infection was more prevalent in the female gender in the present study population which may be because females are more likely to test for HIV than men meaning that they were readily available. Another possible reason for the higher HIV prevalence among females may be that the majority were unable to further their tertiary education or dropped out of school at the secondary level due to financial constraints. These social ills drive unemployment and poverty which increase promiscuous or risky sexual behaviours and unsafe sexual practices that result in a higher prevalence of HIV among women. Oliveira *et al*. (2017) and Simbayi *et al*. (2019) reported similar findings where the prevalence of HIV was higher among women as compared to men. Females bear the high burden of HIV which will consequently impact the country's economy. The high prevalence of HIV among females also raises concerns about HIV transmission to new-borns which will have implications on the overall HIV prevalence.

5.2. CLINICAL CHARACTERISTICS

There was a significant difference in $CD4^+$ T-cell count, and it was significantly higher in the cART-treated group as compared to the cART-naïve group which suggest the role of cART suppressing HIV and improving immune function. This is consistent with Behera *et al*. (2021) who reported a significantly higher $CD4^+$ T-cell count among cART-treated patients as compared to cART-naïve patients. This is attributed to the fact that treatment with cART results to viral suppression and restoration of immune function which leads to improvement in $CD4^+$ T-cell count (Smith *et al*., 2014; WHO, 2021). Although we found a statistical significance, there was not a big difference in $CD4^+$ T-cell count which may be attributed poor adherence to treatment and that some of the cART-naïve patients were newly diagnosed with $CD4^+$ T-cell counts above 500 cells/µl. The findings

of this study, therefore, reiterate the importance of cART use among the HIV-positive populations, especially among the newly diagnosed HIV-positive individuals.

5.3. CARDIOVASCULAR RISK FACTORS

5.3.1. Prevalence of obesity

We used BMI to assess obesity, but it is not a reliable measure of health as it does account for body fat percentage or body fat distribution. The prevalence of obesity among our three study groups was similar, which is in line with previous findings of Crum-Cianflone *et al*. (2008) and Nsagha *et al*. (2015). However, our finding contradicts with that of Ogunmola *et al*. (2014) Rwegerera *et al*. (2021) and Hanttu *et al*. 2021). The levels of obesity among our HIV-positive patients seem to have reached similar levels as that of the HIV-negative individuals. This is indicative of inheriting poor lifestyle factors prior HIV infection and the commencement of cART. Furthermore, we speculate that both our HIV-positive and HIV-negative patients may be genetically predisposed to obesity. The prevalence of obesity is rising in the general population and among people living with HIV globally (Hanttu *et al*., 2021), and our finding support this trend of the rising obesity prevalence among HIV-positive cART-treated and cART-naïve patients. The lack of significance in our study, however, does not show the link between the prevalence of obesity, cART and HIV infection. Other studies have reported an association between high obesity levels and cART use among HIV-infected patients (Anuurad *et al*., 2010) and this discrepancy in findings may be attributed to different cART regimens, traditional risk factors, and duration of HIV and treatment. Despite the similar prevalence in this study, obesity seems to be a growing health problem not only in the general population, but also in the HIV-positive population. The rising obesity prevalence raise concerns about the prevalence of diabetes and risk of CVD complications among our populations.

5.3.2. Prevalence of hyperglycaemia

The prevalence of prediabetes among our three study groups was similar, which is consistent with previous studies from Botswana and South Africa (Dave *et al*., 2016; Rwegerera *et al*., 2021). Our finding, however, contradicts that of Maganga *et al*. (2015) and Levitt *et al*. (2016) who showed a significant difference in the prevalence of prediabetes among cART-treated, cART-naïve, and HIV-negative patients. Although no significant difference was found, it is evident that hyperglycaemia is a present metabolic disorder among our HIV-positive and HIV-negative patients. Since our cART-treated patients were not on first-generation ART regimens (indinavir, stavudine, didanosine), the modern cART drugs may have had mild or no effects thus resulting in similar levels of prediabetes. Compared with the current cART regimens, the first-generation cART regimens have major adverse effects on glucose, fat and lipid metabolism (Ledergerber *et al*., 2007; Lagathu *et al*., 2019). In addition, we speculate that the high levels of overweight in the cART-treated patients may have resulted in similar levels of prediabetes as the control group where the prevalence of obesity and abdominal obesity were high. It is known that overweight and obesity are major risk factor for prediabetes (Arafath *et al*., 2018; Nansseu *et al*., 2018). Hyperglycaemia has become an increasingly prevalent comorbidity among HIV populations globally (Arafath *et al*., 2018) and our study supports the presence of this cardiovascular risk factor in the HIV-positive population in our region. The results of our study demonstrate that hyperglycaemia exists in both our general population and HIV-positive population, which imply that these populations are particularly at a higher risk of progressing to type 2 diabetes, raising concerns about CVD and related complications.

5.3.3. Prevalence of hypertension

The prevalence of hypertension in the HIV-positive population has risen over the years (Fahme *et al*., 2018). In the present study, the prevalence of hypertension among our three study groups was similar, which is consistent with previous findings (Bergesen *et al*., 2003; Ogunmola *et al*., 2014; Mashinya *et al*., 2016). Contrary to our finding, Nsagha *et al*. (2015), Pangmekeh *et al*. (2019) and Ekun

et al. (2021) have reported a significantly higher prevalence of hypertension in the cART-treated patients compared to cART-naïve patients. Our finding is indicative of inheriting poor cardiometabolic profile and lifestyle factors (smoking, increased stress levels and salt intake) prior the infection and commencing of cART. We speculate that our HIV-positive patients were already predisposed to high hypertension risk before the infection and initiation of cART. For example, overweight/obesity is known as strong risk factor for hypertension, and it was common in both our cART-treated and control groups. We also speculate that older age in the control group, a major risk factor for hypertension (Masenga *et al*., 2019), may have resulted in similar levels of hypertension as we observed that 11.4% in the control group was ≥60 years compared to 8.4% in the cART-treated and 3.7% in the cART-naïve group. Although cART is associated with a high prevalence of hypertension among HIV-positive populations globally (Masenga *et al*., 2019), the lack of significance in our study does not show the link between hypertension, cART and HIV. This may be attributed to the fact that only specific regimens affect the blood pressure but causing a low to moderate increase. Despite the similar prevalence of hypertension, it is evident this is a comorbidity in our HIV-positive and HIV-negative populations, which is a cause for concern. This cardiovascular risk factor will increase the risk of CVDs and contribute to the high morbidity and mortality in the HIV-positive population.

5.4. THE EFFECT OF COMBINATION ANTIRETROVIRAL THERAPY ON RENAL FUNCTION

5.4.1. The prevalence of renal dysfunction

The prevalence of renal dysfunction among people living with HIV has risen over the years (Heron *et al*., 2020). The prevalence of renal dysfunction among people living with HIV has been extensively studied in the Western countries; however, a lot has not been done in SSA in the rural context, particularly here in SA. In the present study, the prevalence of renal dysfunction was similar in cART-treated, cART-naïve and control patients. There are few studies comparing the

prevalence of renal dysfunction among cART-treated, cART-naïve and HIV-negative patients. We did not find a previous study with similar finding as our study. Contrary to our finding, previous studies from African countries have reported a significance difference in the prevalence of renal dysfunction among cART-treated, cART-naïve, and HIV-negative patients (Kwantwi *et al*., 2017; Nforbugwe *et al*., 2020; Ekun *et al*., 2021). The lack of significance in our study suggests that there is no association between renal dysfunction prevalence, cART and HIV infection in our HIV population. Our study shows that the use of TDF-based regimen in our HIV population is not associated with high renal dysfunction prevalence, which supports the recommendation of the WHO on the use of TDF as first-line regimen (WHO, 2013). It is possible that we did not observe a significance difference because only small percentage of our cART-treated patients (3%) used TDF regimen concomitantly with LPV/r, as 70% of TDF nephrotoxicity is observed with concomitant use of low-dose ritonavir (Izzedine *et al*., 2009). As a result, cART may have caused mild renal effects in our HIV population. The presence of traditional renal risk factors including obesity, prediabetes and hypertension in all our study groups may also account for the similar prevalence of renal dysfunction in this study. The results of our study shows that renal dysfunction exist, and it will become a major health problem particularly among those on cART leading to high mortality rates.

5.4.2. Biomarkers of renal function

The kidney plays an important role in the metabolism and excretion of drugs (Miners *et al*., 2017). We compared cystatin C, RBP4, clusterin and eGFR among the different study groups to evaluate renal function.

5.4.2.1. Cystatin C

We did not find a significant difference in mean cystatin C levels among the three study groups. To the best of our knowledge, studies comparing plasma cystatin C in cART-treated, cART-naïve, and HIV-negative individuals are lacking. One

previous study had reported conflicting findings where serum cystatin C levels were significantly higher in cART-naïve participants compared to HIV-negative participants (Glaser *et al*., 2016). Jaroszewicz *et al*. (2006) reported a decrease in serum cystatin levels after cART initiation, reflecting renal function improvement. In our study, the lack of significance suggests that there is no association between plasma cystatin C levels, HIV infection and cART. This could mean the cART regimens used by cART-treated patients do not have adverse or profound effect on the glomerular function, which is further supported by the similar prevalence of renal dysfunction we observed. The mean cystatin C levels remained similar among the three groups after controlling for CVD risk factors (age, gender, alcohol consumption, BMI, SBP and FBG). This further indicate that cART may not be associated with significant change in renal function, despite controlling for the impact of these CVD risk factors on plasma cystatin C. Further adjustment for CVD risk factors and $CD4^+$ T-cell count, cystatin C levels were not significant different between the cART-treated and cART-naïve groups. These findings therefore do not reflect increased renal dysfunction with cART use.

5.4.2.2. Retinol-binding protein 4

In terms of RBP4, the mean RBP4 levels were significantly different among the three study groups. To the best of our knowledge, no study has evaluated renal function with plasma RBP4 among HIV-positive (cART-treated and cART-naïve) and HIV-negative patients. The mean RBP4 levels in our study were significantly higher in the cART-treated group as compared to the cART-naïve group, which may reflect that renal dysfunction occur more among cART-treated patients. This may be attributed to the effect of cART on causing reduction in glomerular filtration. The findings also demonstrate the potential of plasma RBP4 as a renal function biomarker among HIV patients. The mean RBP4 remained significantly different and higher in the cART-treated group as compared to the cART-naïve group after controlling for CVD risk factors (age, gender, alcohol consumption, BMI, SBP and FBG). This indicates that even when controlling for the effect of CVD risk factors on plasma RBP4, renal dysfunction is more increased in the

cART-treated group, which suggest the possible role of cART nephrotoxicity. After further adjustments for CVD risk factors and $CD4^+$ T-cell count, there was still a significant difference in the mean RBP4 between the cART-treated and cART-naïve groups. The mean RBP4 levels were significantly higher in the cART-treated group as compared to the cART-naïve group, which therefore reflect renal function dysfunction in the cART-group. These findings demonstrate that plasma RBP4 may serve as an independent biomarker of renal function and be used as an alternative measuring tool of clinical importance in this rural HIV population.

5.4.2.3. Clusterin

We found no significant difference in mean clusterin levels among the three study groups, which means all groups were similar in mean clusterin levels. There is no available literature comparing mean clusterin levels in cART-treated, cART-naïve, and HIV-negative patients. Our finding may indicate that cART that there is not link between clusterin levels and cART. This may suggest that cART does not cause significant damage in the kidneys, thus, the clusterin levels in the cART-treated group are similar levels with those of the cART-naïve and control patients. Even after controlling for CVD risk factors (age, gender, alcohol consumption, BMI, SBP and FBG), the mean clusterin levels were not significantly different among the three study groups. We still found no significant difference in mean clusterin between the cART-treated and cART-naïve groups after controlling for CVD risk factors, $CD4^+$ T-cell count included. These findings further indicate that cART may not be associated with renal disease when possible confounding factors are controlled.

5.4.2.4. Estimated glomerular filtration rate

In the present study, eGFR was estimated using cystatin C which was not significantly different among the three groups. The mean eGFR was, however, significantly different among the three study groups. With further analysis, no significant difference in mean eGFR was found between any two groups, which

is not in agreement with previous findings (Glaser *et al*., 2016). This mean that mean eGFR was similar among the three study groups, which may suggest that cART does not cause a significant damage and decline in renal function in our HIV population. This may be attributed to the fact that only small percentage of our cART-treated patients used TDF concurrently with LPV/r (3%), which is known to account for 70% of reported cases of TDF nephrotoxicity (Izzedine *et al*., 2009). We also observed that the median $CD4^+$ T-cell count in our cART-naïve group was not below 200 cells/µl known as the advanced HIV stage that is associated with renal dysfunction (Sarfo *et al*., 2013), thus resulting to similar levels of mean eGFR. The mean eGFR was not significantly different among the three groups after controlling for CVD risk factors (age, gender, alcohol consumption, BMI, SBP and FBG). After further adjustment for CVD risk factors and $CD4^+$ T-cell count, the mean eGFR remained similar among the three different groups. There seem to be no strong association between mean eGFR levels, cART and HIV infection in our HIV population, despite controlling for the impact of CVD risk factors. cART does not seem to be associated with reductions in renal function among HIV-positive patients in this region. The findings of this study therefore do not encourage discontinuation or switching from TDF-based regimens.

5.4.3. The effect of lines of regimen on biomarkers of renal function

The levels of cystatin C, RBP4, clusterin and eGFR were compared among patients on first-line regimen, second-line regimen and cART-naïve. The first-line regimen consisted of TDF+FTC+EFV (95%), AZT+3TC+EFV (3%) and ABC+3TC+EFV (2%). The second-line regimen consisted of AZT+3TC+LPV/r (94%) and TDF+FTC+LPV/r (6%). There was no significant difference in mean cystatin C levels among the three different groups, which suggest that there is no link between the line of regimen and renal dysfunction. In terms of RBP4 levels, there was a significant difference and patients in the second-line regimen had significantly higher mean RBP4 levels as compared to those in first-line regimen and cART-naïve group. This is reflective of increased renal dysfunction among

patients on second-line regimen, possibly due to TDF and LPV/r nephrotoxicity. As explained by previous studies, cART-treated patients on TDF/LPVr combination more likely experience renal dysfunction than those on TDF/NNRTIs combination (Albini *et al*., 2012; Young *et al*., 2012). The mean eGFR levels were not significantly different among the different groups, which suggest that there is no association between eGFR level and line of cART regimen.

5.4.4. The effect of duration of combination antiretroviral therapy on biomarkers of renal function

We observed a significant positive association between the duration of cART and cystatin C, which may indicate that the longer the duration on cART is associated with increase in cystatin C levels and reflect gradual decline in renal function. The finding that cystatin C increases with a longer duration on cART has not been previously reported and contradicts that of Jaroszewicz *et al.* (2006) who reported that longer duration on cART is associated with a decrease in cystatin C levels. This discrepancy may be attributed to different cART regimens and viral loads. We found no association between the duration of cART and RBP4. There was also no association between duration of cART and clusterin. There was a significant negative association between the duration of cART and eGFR, which is in line with previous findings (Kanabusa, 2009; Nishijima *et al*., 2017). However, contradicts with the finding of Nforbugwe *et al*. (2020) who reported no association between the duration of cART and eGFR. Our finding indicate that longer duration of cART is significantly associated with decline in renal function over time in the treated HIV-positive patients. Prolonged exposure to cART may be causing gradual loss of renal function which can lead to renal dysfunction.

5.5. CARDIOVASCULAR RISK FACTORS AND RENAL FUNCTION

In an HIV population, cardiovascular risk factors are prevalent and may further increase the risk of renal dysfunction. We assessed the association between cardiovascular risk factors and renal function.

5.5.1. cART-treated group

We observed that age significantly negatively associated with eGFR which indicate that age is independently associated with renal function decline among cART-treated patients. This suggests that age is an independent cardiovascular risk factor for renal dysfunction among cART-treated patients, which is in line with previous findings (Calza *et al*., 2019 and Sun *et al*., 2019). Renal function declines with age among our cART-treated patients which is a common trend also in the general population.

The duration of cART was significantly negatively associated with cystatin C while significantly negatively with eGFR which indicates that longer duration of cART is independently associated with renal dysfunction in this rural black cART-treated HIV population. Our findings suggest that duration of cART is an independent predictor for renal dysfunction among cART-treated patients, which is in line with previous findings (Crum-Cianflone *et al*., 2010; Nishijima *et al*., 2017). Continuous use of cART is associated with gradual loss of renal function, which can progressively lead to end-stage renal disease. This clearly indicates that cART may have cumulative deleterious effect on the kidney among our HIV population on cART.

The $CD4^+$ T-cell count showed a significant positive association with eGFR which indicate that $CD4^+$ T-cell count is independently associated with renal function improvement. Kwantwi *et al*. (2017) reported similar findings where the $CD4^+$ T-cell count was significantly associated with eGFR improvement. This was attributed to cART in its role of suppressing the viral load which subsequently attenuates the HIV-induced renal damage (Okuonghae *et al*., 2011). In terms of cystatin C, we observed that $CD4^+$ T-cell count showed a significant positive association with cystatin C which indicates that $CD4^+$ T-cell count is independently associated with renal function improvement. These findings suggest that $CD4^+$ T-cell count may be an independent cardiovascular predictor of renal function among this South African HIV population on cART.

5.5.2. HIV-positive group

In the HIV-positive group, age significantly associated negatively with eGFR which indicates that age is independently associated with renal dysfunction among our HIV-positive population regardless of the cART status. The duration of cART was significantly positively associated with cystatin C while significantly negatively associated with eGFR which is an indication that longer duration of cART in this rural black HIV population is independently associated with renal dysfunction.

We observed a significant positive association between cART use and RBP4, which is an indication that cART is independently associated with renal dysfunction among our HIV-positive population. cART seem to be strongly associated with renal dysfunction among our HIV population which is a cause for concern.

There was a significant positive association between $CD4^+$ T-cell count and eGFR which indicates that $CD4^+$ T-cell count is independently associated with renal function improvement. This suggest that $CD4^+$ T-cell count may serve as an indicator of better renal function status among this HIV-positive population.

CHAPTER 6: CONCLUSION

6.1. CONCLUSION

The focus of this study centred on the effects of cART on cardiovascular risk factors, renal function, and the association between cardiovascular risk factors and renal function among a rural black South African population. Combination antiretroviral therapy is known to be associated with cardiovascular risk factors among HIV-positive populations globally. In the present study no difference was observed in the prevalence of obesity, prediabetes and hypertension among the cART-treated, cART-naïve and control participants. This may be reflective of inheriting poor lifestyle factors prior to HIV infection and cART initiation.

The prevalence of renal dysfunction among HIV-positive and HIV-negative patients is similar in the Mankweng District. Renal biomarkers which include plasma cystatin C, RBP4 and clusterin were measured. Plasma RBP4 was significantly higher in the cART-treated group compared to the cART-naïve and control participants. Furthermore, eGFR was the lowest in the cART-treated group after controlling for CVD risk factors. This study therefore gathered sufficient evidence that renal dysfunction is present among the cART-treated participants. In the present study we further demonstrated that RBP4 may serve as alternative biomarker for eGFR, or renal function and it may be of clinical importance as a measuring tool in this HIV-positive population. Further studies are needed to elucidate on cystatin C as a promising renal biomarker in this HIV positive population.

The present study demonstrated that long-term exposure to cART is associated with progressive loss of renal function in our HIV population which is a serious concern. We further demonstrated that RBP4 strongly correlated with the second-line regimen (TDF+LPV/r combination). This study supports previous findings that

TDF+LPV/r combination is associated with nephrotoxicity and can adversely affects renal function. Despite conflicting findings regarding the effect of cART on renal function, this study succeeded in providing information on the impact of cART on renal function in HIV-positive patients living in the Mankweng District.

The present study demonstrated that age, cART use, duration of cART and $CD4^+$ T-cell count were the only CVD risk factors that independently associated renal function among other CVD risk factors in this HIV-positive population. We found that age, cART use, duration of cART emerged as the strongest predictors for renal dysfunction, which corroborates previous findings. The $CD4^+$ T-cell count is a strong CVD predictor of better renal function outcome presently in this HIV-positive population. We highlighted that a higher $CD4^+$ T-cell count in our HIV-positive population can serve as an indicator of normal (healthy) or improved kidney function. Further studies are needed to elucidate on this finding. Our study provide evidence that multiple factors independently contribute to renal dysfunction in this aging cART-treated HIV population.

6.2. LIMITATIONS OF THE STUDY

This study is limited by its cross-sectional nature which means conclusions on the causation cannot be drawn. There was a disparity in the sample size among our study groups which could have influenced the outcome of this study. Our study was conducted in a single district of Mankweng and therefore the findings cannot be generalisable to the province of Limpopo. The results of this study were only limited to black South Africans. The eGFR in this study was measured once and not three consecutive times as recommended. Thus, our definition of renal dysfunction (CKD) is limited to a single eGFR measurement. Our definition criterion of renal dysfunction was limited to eGFR measurement and did not include proteinuria.

6.3. RECOMMENDATIONS

Based on the limitations, future studies should employ a larger sample size with equal number of participants among study groups of interest. We recommended inclusion of cohorts from multiple districts across the Province to be able to generalise the findings to the whole province of Limpopo. We also recommend diversity in racial inclusion to establish variation of the outcome. It is highly recommended that at least two eGFR measurements should be used to determine renal dysfunction in future studies. The definition criteria for renal dysfunction may also include proteinuria analysis. Obesity, prediabetes, and hypertension are growing health problems in this HIV-positive population which points out the need for strategic interventions to monitor weight, blood pressure and glucose levels.

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APPENDICES

Appendix A. Consent letter (English and Sepedi version)

Department of Physiology and Environmental Health University of Limpopo

Private Bag X1106

Sovenga

0727

Date: _____

Dear Participant

This letter is an invitation to participate in the study that investigate renal function and its association with cardiovascular risk factors among HIV-infected patients. We assure you that your responses to the interview will remain strictly confidential.

The researcher will attempt not to identify you with the responses you give during the interview or disclose your personal details as a participant in the study. Please note that your participation in this study is voluntary and you have a right to withdraw from participating at any time should you wish to do so. Kindly answer all questions as honestly possible. Your participation in this study is very important

Thank you for your time and cooperation.

Yours

_____

Student

_____

Supervisor

Department of Physiology and Environmental Health

University of Limpopo

Private Bag X1106

Sovenga

0727

Letšatšikgwedi: _____

Thobela Motšeakarolo

Lengwalo le ke memo go wena go tsea karolo mo thutong malebana le go nyakišiša ‘renal function and its association with cardiovascular risk factors among HIV-infected patients’. Rego netebatša gore dikarabo tša gago go diputšišo tše di tla tshwarwa ka mokgwa wa sephiri.

Monyakišiši o tla leka go se amanye dikarabo tša gago tšeo o tla di fago mo nyakišišong ye, le go se utulle leina la gago bjalo ka motšeakarolo mo nyakišišong ye. O tsebišwa gore go tšea karolo mo nyakišišong ye ke ka boithaopo, le gore o nale tokelo ya go ikgogela morago nako efe goba efe ge o nyaka. O kgopelwa go araba diputšišo tše ka botshephegi bjo bogolo.

Go tšea karolo ga gago go mo nyakišišo e go bohlokwa kudu.

Ke leboga nako ya gago le tšhomišano mmogo.

Wa lena

_____

Morutwana

_____

Mohlahli

Appendix B: Consent form (English and Sepedi version)

I_____ hereby agree to participate in a master's research project that focuses on investigating renal function and its association with cardiovascular risk factors among HIV-infected patients.

The purpose of this study has been fully explained to me. Furthermore, I understand that I am participating freely and without being forced in any way to do so. I also understand that I can terminate my participation in this study at any point should I wish to do so, and that this decision will not affect me negatively in any way.

I understand that this is a research project, whose purpose is not necessarily to benefit me personally. I understand that my details as they appear in this consent form will not be linked to the interview schedule and that my answers will remain confidential.

Signature: _____

Date: _____

Foromo ya tumelelo

Nna _____ ke dumela go tšea karolo mo morerong wa nyakišišo ye itebanyago le go investigating renal function and its association with cardiovascular risk factors among HIV-infected patients.

Ke hlaloseditšwe ka maikemišetšo a nyakišišo e, ebile ke kwešiša gore ke tšea karolo ka go ithaopa ka ntle le go gapeletšwa. Ke kwešiša gape le gore nka ikgogela morago go tšea karolo nako efe le efe ge nka kwa ke sa nyake go tšwela pele, le gore kgato yeo e ka se nkame ga mpe.

Ke kwešiša gore se ke morero wa nyakišišo, o maikemišetšo a gona e sego go nthuša ka bo nna, ke kwešiša le gore leina laka le ge ikaba ditaba tšeo di filwego ka nna bjalo kage di tšwelela mo foromong ya tumelelo di kase utullwe di tla šireletšwa.

Signature: _____

Letšatšikgwedi: _____

Appendix C : Questionnaire

SOCIO-DEMOGRAPHICS				
Age (years)				
Gender (***indicate with an "X"***)	Male		female	
Ethnicity	Pedi		Ndebele	
	Zulu		Sotho	
	Tswana		Zimbabwean	
	Venda		Tsonga	
	If other, specify			
Marita status	Married			
	Living together			
	Never married			
	Divorced			
	Partner deceased			
Education level	No formal education			
	Primary			
	Secondary			
	Tertiary			
Employment status	Employed			
	Unemployed			
	Self-employed			
	Casual jobs			
	Pensioner			
Smoking (***indicate yes or no***)				
Alcohol consumption (***indicate yes or no***)				

SOCIO-DEMOGRAPHICS				
Age (years)				
Gender (***indicate with an "X***")	Male		female	
Ethnicity	Pedi		Ndebele	
	Zulu		Sotho	
	Tswana		Zimbabwean	
	Venda		Tsonga	
	If other, specify			
Marita status	Married			
	Living together			
	Never married			
	Divorced			
	Partner deceased			
Education level	No formal education			
	Primary			
	Secondary			
	Tertiary			
Employment status	Employed			
	Unemployed			
	Self-employed			
	Casual jobs			
	Pensioner			
Smoking (***indicate yes or no***)				
Alcohol consumption (***indicate yes or no***)				
MEDICAL INFORMATION				
HIV Status (***positive or negative***)				

Do you take cART medication? (**indicate with "X")**	YES		NO	
Specific ART combination				
For how long you have been using cART?				
Specific Cart				
Do you take your medication every day?	YES		NO	
If no specify why				
Most recent CD4+ count				
Viral load				
Year of HIV diagnosis				
Potential side effects				
Other medications				

MEDICAL HISTORY				
Have you suffered or do you suffer from TB or UTI?	YES		NO	
If yes, specify the type of infection and the medication				
Were you diagnosed of any renal disease?	YES		NO	
If yes, specify				
Have you ever had kidney transplant or dialysis?				
Any history of cardiac complications?	YES		NO	
If yes, specify				
Breast feeding or Pregnant	YES		NO	
Have you reached menopause?	YES		NO	
ANTHROPOMETRIC MEASUREMENTS				
Weight				

Height	
Waist circumference	
BLOOD PRESSURE	
Systolic	
Diastolic	

Appendix D: Registered nurse license

Appendix E: Department of Medical Science permission letter

University of Limpopo
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 2273, Fax: (015) 268 2272, Email:Solomon.Choma@ul.ac.za

To: Mr S Hansor

Principal Investigator: Renal Function and its association with cardiovascular risk factors among HIV infected patients

From: Choma SSR

HUD: Pathology and Medical Sciences

Date: 22 March 2019

RE: ANALYSIS OF BLOOD SAMPLES

This memo serves to confirm that the Department of Pathology and Medical Sciences has the laboratory equipment to run the Renal Function tests such as Creatinine, Urea, Sodium and Pottasium. Storage space is also available for all the blood samples that will collected.

Furthermore it has personnel who can perform rapid HIV testing.

Please note that as per verbal communication, the department is willing to collaborate with your research group and will thus run these tests on your behalf at no cost except for

(i) Co-authorship of research articles utilising results from these tests and
(ii) Your department purchases reagents required for running these tests and calibration of related equipment.

Please note that it is not a requirement of the HPCSA to use an accredited laboratory for running medical tests for research purposes.

Yours Sincerely,

Chom R (Si)

Finding solutions for Africa

Appendix F: University of Pretoria permission letter

NATIONAL HEALTH LABORATORY SERVICE

Communication, Marketing & PR
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0398 Fax: +27 (0)11 386 6586
Reference:

SERVICE LEVEL AGREEMENT (SLA)
Between
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES (NICD)

A division of the National Health Laboratory Service, being a schedule 3A public entity of the National Department of Health, duly established as such in terms of National Health Laboratory Service Act, 37 of 2000, and Public Finance Management Act, 01 of 1999; having its principal place of business at 01 Modderfontein Road, Sandringham, Johannesburg, 2131, South Africa; duly represented by **Dr. Karmani Chetty**, in her capacity as Acting **Chief Executive Officer**, through its **Centre for Vaccines and Immunology, National Institute for Communicable Diseases**

And
Department of Physiology and Environmental Health, University of Limpopo

1. **Agreement overview**

   This Agreement represent a Service Level Agreement "SLA" or "Agreement" between **Centre for Vaccines and Immunology (CVI), National Institute for Communicable Diseases** and **Department of Physiology and Environmental Health, University of Limpopo** for the purposes of promoting their co-operation in academic education and research.

   This agreement remains valid until superseded by a revised agreement mutually endorsed by the stake holders.

   This agreement outlines the parameters the roles and responsibilities, as they are mutually understood by the stakeholders. This agreement does not supersede current processes and procedures unless explicitly stated herein.

2. **Scope**
   The goal and objectives of this SLA is to clearly identify the roles and responsibilities of each party as they conduct research on the Luminex 200 instrument at the Centre for Vaccines and Immunology at the National Institute for Communicable Diseases.

3. **Purpose**
   The purpose of this SLA is to establish a process where Centre for Vaccines and Immunology will be able to periodically invoice Department of Physiology and Environmental Health, University of

Limpopo at an hourly basis for Luminex 200 use. This fee includes sheath fluid and routine equipment maintenance but excludes consumables such as Luminex Calibration and Verification beads.

4. **Pre-conditions**
A pre-condition for working in the laboratory will be testing of immunity to Measles, Rubella, Hepatitis B and Polio.

5. **Financial Arrangements**
Use of the Luminex 200 system will be billed based on an hourly rate of R350 per hour for the duration of the contract. Additional costs, such as usage of Luminex Calibration and Validation Kits will be billed according to their daily usage at R340 and R380, respectively. These fees may be reviewed, and subject to Consumer Price Index, may change during the duration of the contract.

6. **Reporting Usage**
Use of equipment is to be manually logged by Department of Physiology and Environmental Health, University of Limpopo staff using the logs sheets at the Luminex 200 instrument bench and signed off by an authorised staff of the Centre for Vaccines and Immunology. Hourly usage will be quarterly collated and a valid tax invoice will be issued to the Department of Physiology and Environmental Health, University of Limpopo for payment into a designated NICD account.

7. **General Coordinators**
Each Party shall designate an administrative office to oversee and facilitate the implementation of any agreements arising out of this SLA. These offices are:

Centre for Vaccines and Immunology (CVI):	**Department of Physiology and Environmental Health, University of Limpopo:**
Dr Heather Hong	Mr Sidney Hanser
E-mail: heatherh@nicd.ac.za	E-mail: sidney.hanser@ul.ac.za
Tel: +27 11 386 6461	Tel:

8. **Liability**
Except for loss or damages caused through gross negligence or intent, the Parties shall have no liability to each other hereunder.

9. **Legal Relationship**
This SLA shall be construed as a statement of purpose to promote a genuine and mutually beneficial collaboration between the Parties. This SLA shall create any legal relationship between the Parties.

10. **Acknowledgements**

1 Modderfontein Road, Sandringham, Johannesburg, South Africa
Prof Eric Buch Dr Kemoni Chetty
Private Bag X8, Sandringham, 2131, South Africa
+27 (0) 11 386 6000/ 0860 00 NHLS(6457)

NATIONAL HEALTH LABORATORY SERVICE

Please note that we expect you to acknowledge the unit when publishing results using any of the CVI unit instruments or services. e.g. "Luminex analysis was performed at the Centre for Vaccines and Immunology, National Institute for Communicable Diseases." Kindly inform the Flow unit personnel about your publication.

11. **Commencement, Renewal, Termination**

This SLA will be effective from the date of the last signature hereto and will remain in force for a time period of one (1) years, with a possibility for renewal at the end of the period, subject to the Parties' written agreement. Either Party may terminate this SLA by giving three (3) months' notice in writing to the other Party.

This SLA has been drawn up in two (2) original copies in the English language, each Party receiving one duly signed copy hereof.

Signed on behalf of NHLS (NICD) through its Centre for Vaccines and Immunology

Full Name:	Karmani Chetty
Designation & Position:	Acting Chief Executive Officer
Address:	01 Modderfontein Road, Sandringham 2031
Signature & Date:	

Signed on behalf of Department of Physiology and Environmental Health, University of Limpopo:

Full Name:	Dr M van Staden
Designation & Position:	Head of Department
Address:	University of Limpopo, University road Manakeng
Signature & Date:	13/03/2019

01 Modderfontein Road, Sandringham, Johannesburg, South Africa
Prof Eric Buch Dr Karmani Chetty
Private Bag X6, Sandringham, 2131, South Africa
+27 (0) 11 386 6000 0860 00 NHLS(6457)

5200366

Appendix G: Classification criteria

South African Hypertension practice guidelines (Seedat *et al*., 2014)

BP category	SBP (mmHg)	DBP (mmHg)
Normal	<120	<80
Optimal	120-129	80-84
High normal	130-139	85-89
Grade 1	140-159	90-99
Grade 2	160-179	100-109
Grade 3	≥180	≥110

International Classification of adult underweight, overweight and obesity according to BMI (WHO, 1995; NICE, 2006)

Classification	BMI (kg/m^2)
Underweight	<18.5
Health weight	18.5-24.9
Class 1 obesity, overweight	25.0-29.9
Class 2 obesity, obesity	30.0-39.9
Class 3 obesity, morbid obesity	≥40

World Health Organization cut-off points and risk of metabolic complications (WHO, 2008)

Indicator	Cut off points (cm)	Risk of metabolic complications
Waist circumference	>94 (M), > 80 (W)	Increased
Waist circumference	>102 (M), > 88 (W)	Substantially increased

Waist circumference and waist–hip ratio: report of a WHO expert consultation, Geneva, 8–11 December 2008. M= men W= women

Classification and diagnosis of diabetes: Standards of medical care in diabetes (ADA, 2019)

Category	**FBG (mmol/l)**
Normal	< 5.6
Prediabetes (IFG)	5.6-6.9
Diabetes	≥ 7.0

National kidney foundation (NKF) guidelines for classification of CKD: GFR categories in CKD (NKF, 2002)

Category	**GFR (ml/min/1.73m^2)**
Normal or high	≥90
Mildly decreased	60-89
Mildly to moderately decreased	45-59
Moderately to severely decreased	30-44
Severely decreased	15-29
Kidney failure	<15

Appendix H: School approval letter

School of Molecular and Life Sciences

10/04/2019

NAME OF STUDENT: JM CHOSHI
STUDENT NUMBER: 201402905
DEPARTMENT: PHYSIOLOGY & ENVIROMENTAL HEALTH
QUALIFICATION CODE: MSCA01

Dear JM Choshi

SCHOOL APPROVAL OF PROPOSAL (REF No: SMLS/SRC/2019/03)

I have the pleasure of informing you that your Master's research proposal that served at the School Research Committee (SRC-SMLS) was approved on the 10 April 2019 as follows:

Title of the study: Assessing renal function and its association with cardiovascular risk factors among Human Immunodeficiency virus-infected patients.

Supervisor: Mr S Hanser
Co-Supervisor: Dr B Flepisi (UWC)

Yours faithfully,

Dr KLM Moganedi
Chairperson: School Research Committee

CC: Supervisor
HOD-PEH
Director-SMLS

Appendix I: Faculty approval letter

13/08/2019

NAME OF STUDENT:	Choshi JM
STUDENT NUMBER:	201402905
DEPARTMENT:	Physiology and Environmental Health
SCHOOL:	Molecular and Life Sciences
QUALIFICATION:	MSCA01

Dear Mr Choshi

FACULTY APPROVAL OF PROPOSAL (PROPOSAL NO. 92 OF 2019)

I have pleasure in informing you that your **masters** proposal served at the Faculty Higher Degrees Committee meeting held on **16 May 2019** and your title was approved as follows:

"Assessing renal function and its association with cardiovascular risk factors among human immunodeficiency virus-infected patients"

Note the following: The study

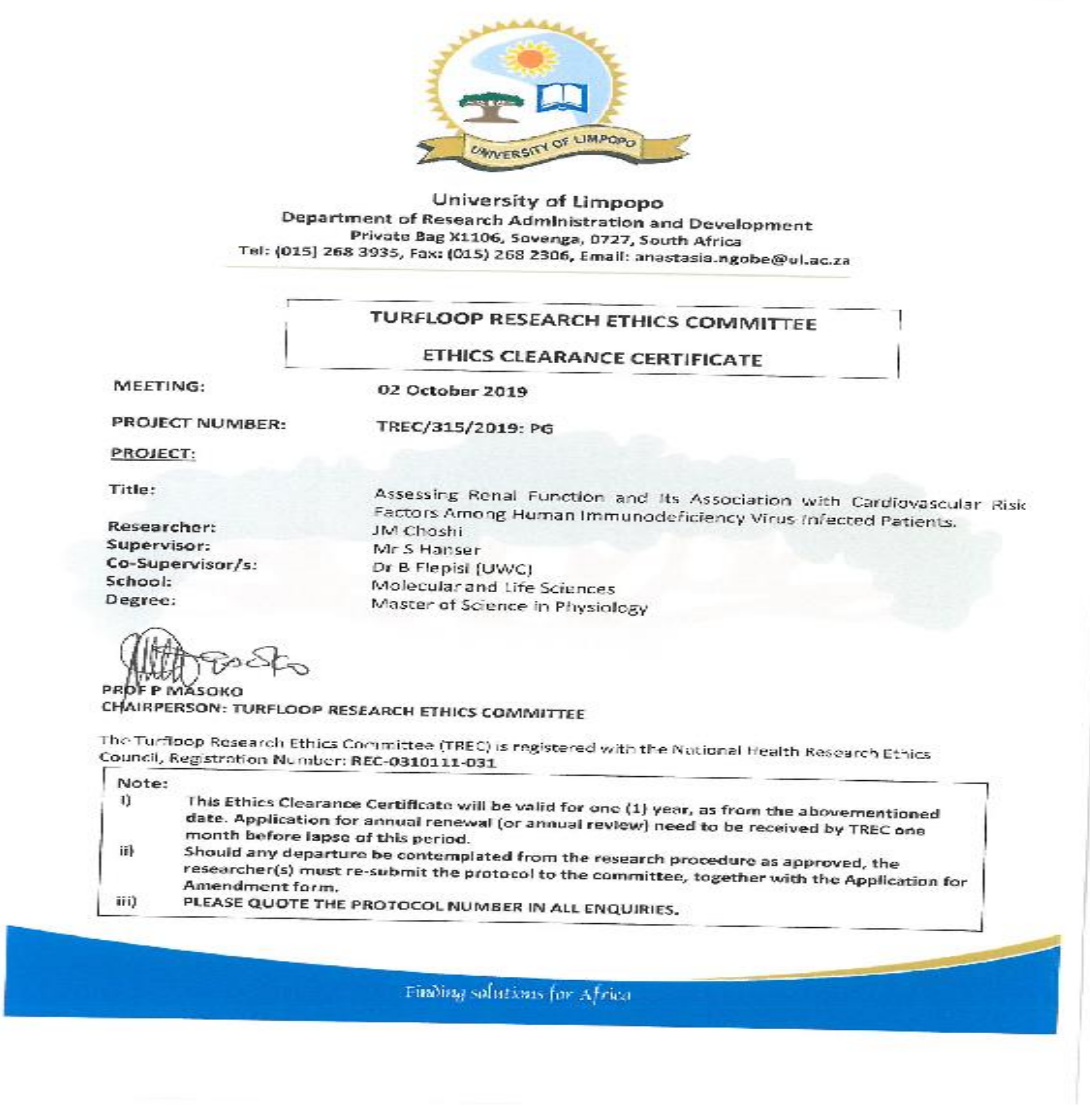
Ethical Clearance	Tick One
Requires no ethical clearance Proceed with the study	
Requires ethical clearance (Human) (TREC) (apply online) Proceed with the study only after receipt of ethical clearance certificate	√
Requires ethical clearance (Animal) (ARLC) Proceed with the study only after receipt of ethical clearance certificate	

Yours faithfully

Prof P Masoko
Secretariat: Faculty Higher Degrees Committee

CC: **Mr S Hanser**
Dr M Van staden
Prof L.J Mampuru

Appendix J: Ethical clearance approval letter



University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 3935, Fax: (015) 268 2306, Email: anastasia.ngobe@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING:	**02 October 2019**
PROJECT NUMBER:	**TREC/315/2019: PG**
PROJECT:	
Title:	Assessing Renal Function and Its Association with Cardiovascular Risk Factors Among Human Immunodeficiency Virus Infected Patients.
Researcher:	JM Choshi
Supervisor:	Mr S Hanser
Co-Supervisor/s:	Dr B Flepisi (UWC)
School:	Molecular and Life Sciences
Degree:	Master of Science in Physiology

PROF P MASOKO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:

i) **This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.**

ii) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.**

iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

Finding solutions for Africa

Appendix K: Letter of request for permission to Department of Health

SENIOR MANAGER

LIMPOPO DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

PRIVATE BAG X9302

POLOKWANE

0700

Dear sir/madam

As an MSc student under supervision of Dr Sidney Hanser and Dr Brian Flepisi, I write to you to request permission to conduct the study we have undertaken at university of Limpopo to investigate the effect of antiretroviral drugs on kidney function and cardiovascular risk among HIV-positive patients of the Mankweng district. This study has been approved by Turfloop Research Ethics Committee (project number TREC/315/2019: PG). Furthermore, this study will provide important health information to the HIV patients and may help guide various stakeholders about the use of more reliable markers for diagnosing renal abnormalities.

We hope you take it into consideration and grant an approval for us to continue with the study. For detailed information about the study, the proposal and ethical certificate are provided with this letter.

Yours faithfully,

JM CHOSHI

Appendix L: Department of Health approval letter

Department of
Health Approval lett