DETECTION OF UNDIAGNOSED ELEVATED CARDIAC RISK BIOMARKERS IN HIV-POSITIVE PATIENTS ON ANTIRETROVIRAL THERAPY (ART) FROM SEVEN HEALTH CENTERS IN KIGALI-CITY, RWANDA

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Masters of Science in Medical Biochemistry

DECLARATION

Declaration by the Student

I, Marcus Bushaku declare that the work contained herein is my original work, and that where I have made use of other's ideas, I have referenced accordingly. This work has never been submitted before for any degree or qualification, certificate or publication.

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Declaration by Supervisors

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DEDICATION

I dedicate this work to my sponsors, Moi University staff, my wife UMUHOZA Pascasie, my lovely daughter Juru M. Eleanor and my son Jabo Carl Myles BUSHAKU and all my friends that have been of great support during the entire process of this work.

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ABBREVIATIONS

AIDS:	Acquired Immune Deficiency Syndrome
ACC	American College of Cardiology
AHA	America Heart Association
ANNOVA	Analysis of variance
ART	Antiretroviral therapy
BMI	Body Mass Index
CAD	Coronary artery disease
cART	Combination antiretroviral therapy
CHUK	Centre Hospitalier Universitaire de Kigali
CVD	Cardiovascular Diseases
DM	Diabetes mellitus
ECG	Electrocardiography
ESC	European Society of Cardiology
FBS	Fasting blood sugar
HA	Hypoalphalipoproteinemia
HAART	Highly active antiretroviral therapy

HbA1c	Haemoglobin A1C / glycated haemoglobin
HDL-C	High density lipoproteins cholesterol
HIV	Human Immunodeficiency Virus
hs CRP	High-Sensitive C-Reactive Protein
IREC	Institutional Research and Ethics Committee
LDL-C	Low Density Lipoprotein cholesterol
LMICs	Low-and Middle-Income Countries
MI	Myocardial infarction
NCDs	Non-Communicable Diseases
NNRTIs:	Non-nucleoside Reverse Transcriptase Inhibitors
NRL	National Reference Laboratory
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NT- proBNP	N-terminal pro-B-Type Natriuretic Peptide
PIs	Protease Inhibitors
PLWH	People Living With Human Immunodeficiency Virus
RNEC	Rwanda National Ethics Committee
SSA	Sub-Saharan Africa
SSPS	Statistical Package Social Sciences

TC	Total cholesterol
TG	Triglycerides
WC	Waist circumference
WHO	World Health Organization

DEFINITION OF TERMS

Biochemical marker: This refers to any hormone, enzyme, antibody, or other substance that is detected in the urine, blood, or other body fluids or tissues that may serve as a sign of a disease or other abnormality

Biomarker: A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention according to the National Institute of Health (NIH) Consortium in 2001 (Atkinson AJ, et.al, 2001)

Cardiac markers: These are biomarkers measured to evaluate heart function. They are often discussed in the context of myocardial infarction, but other conditions can lead to an elevation in cardiac marker level.

Cardiac risk: Cardiac risk is the probability of developing a cardiovascular disease within a defined period of time, taking into account several risk factors simultaneously.

Cardiac risk biomarker profile: Cardiac risk biomarker profile is a range of tests that need to be performed to analyze the risk of a person to get CVD related event such as strokes and heart attacks. The tests may indicate the gravity of getting cardiovascular risks whether either low, moderate or high.

Echo cardiogram test: This is a common test that uses sound waves to produce images of your heart. This test allows your doctors to see how your heart beat and pumps blood, from here the doctor can easily identify the presence of defects.

Electro cardiogram test: Electrocardiogram (EKG or ECG) is a useful test, which is used to record the electrical activity of the heartbeat. The test uses electrical impulse (or "wave") that travels through the heart, and from here a cardiologist can interpret under which conditions the heart is; whether healthy or diseased. This test helps to diagnose different kinds of heart disease to include heart attack/ myocardial infarction, an enlarged heart, or abnormal heart rhythms that may result into a heart failure.

WHO HIV Staging: The World Health Organization (WHO) classifies human immunodeficiency virus (HIV) into four stages Stage 1 (HIV infection): The CD4+ cell count is at least 500 cells per micro liter. Stage 2 (HIV infection): The CD4+ cell count is 350 to 499. Stage 3 (advanced HIV disease or AHD): The CD4+ cell count is 200 to 349.

Lipid profile: Lipid profile also referred to as lipid panel, is a pattern of blood tests that serves as an initial screening tool for abnormalities in lipids, such as, HDL, LDL, cholesterol and triglycerides. The results of this test can be used to identify certain genetic diseases and several other diseases to include determining approximate risks for cardiovascular diseases.

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ABSTRACT

Background: The life expectancy of people living with HIV infection in Rwanda has improved due to access to antiretroviral therapy (ART). Both HIV infection and use of ART have been associated with cardiovascular disease (CVD) due to adverse biochemical changes. The effect of these drugs on cardiac biomarkers in HIV-infected individuals has not been well characterized in Rwanda.

Objectives: To determine demographic and life style CVD risk factors among HIV-infected study participants. To assess the relationship between ART use and changes in biochemical markers of CVD risk among HIV infected adults in Kigali city, Rwanda.

Methods: A total of 150 participants (18-45 years) from HIV clinics in public Health Centers in Kigali city included n=30 HIV-uninfected (HIV-) and n=120 HIV-infected (HIV+) adults. Among the HIV+ adults, n=40 participants were ART-naïve. Cross-sectional data were collected on health-related behaviors and biochemical markers of CVD risk (NT-proBNP, hs-CRP, TC, HDL-C, LDL-C, TG, and glucose). We compared CVD-related biochemical markers between HIV-, HIV+ ART-naïve and HIV+ on ART groups

Data analysis: Data obtained from the questionnaire and biochemical assays, coded and stored in Microsoft Access and was analyzed using Statistical packages for social sciences (SPSS) version 27.0. Descriptive analysis from categorical variables were presented using frequencies and proportions while continuous variables were analyzed for means and standard deviation. Chi-square test was used to check for associations between categorical variables and the significance threshold was considered for a p-value of <0.05. The mean of biochemical markers was compared using independent t-test and Analysis of Variance (ANOVA). Significance difference was considered for a p-value <0.05 with corresponding 95% confidence interval. The association between biochemical markers and ART use was assessed using linear regression with Pearson Correlation test and significance threshold set to $p<0.05$.

Results: Majority of participants were women (60%), and HIV uninfected were younger (35±6 vs. 31±6 years). Total cholesterol and triglycerides concentrations were associated with use of ART. Serum triglycerides concentrations were lower in HIV+ ART-naïve compared to HIV+ on ART (61.20±18.30 mg/dl vs. 85.00±38.30 mg/dl; $p< 0.01$). While total cholesterol concentrations was higher in HIV+ on ART than HIV+ ART-naïve (136.00±45.00 mg/dl vs. 119.00±36.00 mg/dl; $p<0.04$). HDL-C was higher in those taking ART (68.70±30.00 mg/dl vs. 55.00±25.70 mg/dl; $p=0.03$) among HIV+ on ART for 0-6 months and 7-12 months respectively. The cardiac specific biomarker of NT-ProBNP were more frequently distributed among HIV positive patients on longer ART treatment (25% versus 23%), 7-12 months as opposed to those on ART from 0-6 months; ($p=0.05$) . hs-CRP levels were not significantly different across the groups.

Conclusion: Cardiac risk biomarker profiles, NT-proBNP and lipid panel (cholesterol and triglycerides) should be more frequently assessed to timely identify undiagnosed elevated levels in ART-treated HIV-positive patients so that duly management and treatments of CVDs among patients in health centers and HIV clinics can be optimally implemented.

Recommendation: There is need for systematic evaluation of cardiac risk biomarker profiles among HIV-positive patients on ART. The current study suggests early alterations in cardiac and other CVD risk markers accompanying ART usage.

Key words: CVD, HIV, Biomarkers, ART, Rwanda

CHAPTER ONE

1.0 Introduction

1.1 Background

The number of people living with HIV infection continues to grow, and was estimated globally at 37.6 million [30.2 million–45.0 million] in 2020 by UNAIDS (Statistics, 2021) with approximately 1.5 million newly infected by the year 2020, and 1 million deaths of HIV-related illnesses each year according to WHO (World Health Organization, 2020). The burden of the HIV epidemic in Sub-Saharan Africa (SSA) has been reported to be greater than anywhere else in the world, with an estimated 69% (23.5 million) of the global burden (Bloomfield & Velazquez, 2013; WHO, 2017).

In Rwanda, a recent population-based HIV impact assessment suggests a sustained general HIV prevalence of 3.0% among adults between 15 and 64 years over the last 4 years (Nsanzimana, 2019). The prevalence is said to be much higher in females (3.7%) compared with males (2.2%) and higher in urban (4.8%) than rural areas (2.5%) areas (Vos et al., 2017). (Ministry of Health, 2019)

Globally, there has been remarkable success in the scale up of HIV treatment, such that by June 2020, 26 million people with HIV infection (HIV+) were receiving highly active antiretroviral therapy (HAART) worldwide (World Health Organization, 2020). Although the use of antiretroviral therapy (ART) has resulted in improved immunologic and virologic outcome and improved the wellbeing of people living with HIV (HIV+), life-long antiretroviral therapy is not without complications (Remick et al., 2014b). The use of ART has been associated with multiple side effects, especially the first-generation

antiretroviral medications which is claimed to present with metabolic derangements (Gaziano, Crowther, 2018; Kramer, Lazzarotto, Sprinz, & Manfroi, 2009). In particular, the extended use of Protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) have been implicated as causes of metabolic disorders such as dyslipidaemia, hypertension and diabetes mellitus (DM), which are risk factors for cardiovascular disease (CVD) (Lambert, Sandesara, 2016) such as coronary heart disease that results in myocardial infarction, heart failure, hypertension, stroke and other derangements (Gaziano et al., 2017)

The etiologies of cardiovascular disease also called heart and blood vessel disease or simply referred to as heart disease are not always well known, however acute events of heart attacks and strokes are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a buildup of fatty deposits on the inner walls of the blood vessels that supply the heart or brain (Lambert, Sandesara, 2016). This fatty deposit is due to elevated levels of cholesterol, triglycerides and/or high blood pressure (Hao, 2014). Blood clot formation called coronary thrombosis, may also lead to total obstruction of blood flow, resulting in a heart attack and/or stroke (myocardial infarction), which may be fatal if not treated. Myocardial infarction (MI) occurs when the supply of blood to a part of the heart is completely interrupted, which causes the cells of the heart muscle affected to die by necrosis (Thygesen et al., 2012). Atherosclerosis is another condition which develops following accumulation of LDL-C, the ‘bad cholesterol,’ in the lining of the blood vessels, which subsequently leads to its oxidization resulting in inflammation (Johan Frosteg, 2001).

This inflammatory response is associated with white blood cells surrounding the LDL and the formation of a fibrous plaque (Lambert, Sandesara, 2016),(Johan Frosteg, 2001).

In the last few decades, it has become obvious that the burden of cardiovascular diseases and the attendant risk factors are on the rise in Africa (Bloomfield & Velazquez, 2013). Whether the prevalence of cardiovascular diseases in people living with HIV on antiretroviral therapy, is different from that in the general population, has been scarcely investigated (Mutimura, Crowther, & Cade, 2015). A recent WHO report revealed that 17.9 million people died from cardiovascular diseases in 2016, contributing for about 31% of all deaths worldwide. Surprisingly, 82% of all cardiovascular diseases related deaths occurred in low and middle-income countries and up to 85% of all cardiovascular diseases deaths are due to heart attack and stroke (WHO and CDC., 2017).

There is a growing concern that HIV infection increases cardiovascular diseases risk (Nirdesh Jain, 2014),(Dau & Holodniy, 2008) Moreover, HIV patients are more prone with the highest risk of developing atherosclerosis than the general population (Fourie et al., 2020),(Jain et al., 2014). In addition, it is avered that HIV infection on its own may lead to chronic inflammation of the blood vessels, which is associated with elevated levels of bad cholesterol (LDL-C) (Samson, Mundkur, 2012). Coupled with high blood pressure, the LDL-C accumulation damages the artery walls (Cerrato, D'Ascenzo, 2015). Earlier studies have also identified an increased incidence of heart diseases in HIV-infected patients, exacerbated with extended use of combined antiretroviral therapy (cART) (Lambert, Sandesara, 2016),(Cerrato, D'Ascenzo, 2015),(Dimala, 2017) Notwithstanding, treatment with antiretroviral therapy has had a positive impact on the extent of HIV-associated cardiomyopathies (Mutimura, Crowther, et al., 2015),(Remick

et al., 2014a). However, the prolonged use of protease inhibitors (PIs), defined as any of the various drugs that inhibit the action of HIV protease, an enzyme necessary for replication of the HIV virus and nucleoside reverse transcriptase inhibitors (NRTIs) that are structural nucleoside analogues of DNA nucleotide which prevent reverse transcription of the HIV genome, thereby inhibiting the action of HIV-1 Reverse transcriptase (RT) and viral replication, leads to metabolic imbalances, resulting in elevated levels of LDL-C and low levels of HDL-C, which contribute to atherosclerosis, the primary cause of cardiovascular disease (Hao, 2014),(Muralikrishna, 2009).

Despite a lack of sufficient data on cardiovascular diseases in people living with HIV in Rwanda, studies at regional level have shown that the most affected are women living with HIV at a proportion of about 58% in Sub-Saharan Africa (Vos et al., 2017). While the information on what drives cardiovascular diseases in HIV infection remains limited, protease inhibitors (PIs) are thought to account for 60% of metabolic changes that contribute to cardiovascular diseases among its users (Kramer et al., 2009),(Dimala, 2017),(Dillon et al., 2017). In the cohort study conducted by Vos et al (Vos et al., 2017) on HIV and risks of cardiovascular diseases in Sub-Saharan Africa, it has been pointed out that in HIV-infected patients the major cause for heart diseases were the traditional risk factors interplaying with other mechanisms such as pro-inflammatory effects of HIV infection, immune activation after initiation of antiretroviral therapy and the confounding effects of different combinations of antiretroviral therapy (cART) (Cerrato, D’Ascenzo, 2015),(Remick et al., 2014b). The mentioned factors may predispose HIV positive individuals on antiretroviral therapy treatments to an accelerated or increased risk of cardiovascular diseases (CVDs).

The effect of antiretroviral therapy on cardiovascular function is a broad topic of interest for research. While recent studies indicate that ART treatment reduces cardiovascular disease risk for short term use, more recent literature revealed an estimate of 26% of myocardial infarction per annum in patients on ART treatment (Muralikrishna, 2009) .

Given the paucity of data on the incidence of cardiovascular diseases in HIV patients on antiretroviral therapy in Sub-Saharan Africa, specifically in the context of potential biomarkers of disease risk, a cross-sectional study to assess biomarkers of cardiovascular risk in HIV+ on antiretroviral therapy, compared to HIV+ antiretroviral therapy -naïve and HIV-uninfected (HIV-) was conducted in health centers in Kigali city, Rwanda involving HIV-infected individuals on ART treatment. It is therefore predictable to consider that even with the availability of effective treatment to reduce cardiovascular diseases complications in the general population, cardiovascular disease risk screening ought to remain a priority in low-and middle-income countries' health care strategies in an era of ART treatment among HIV positive individuals.

1.2. Statement of the problem

There is limited reporting on health problems surrounding HIV and cardiovascular diseases in Sub-Saharan Africa (SSA), perhaps due to discrepancy in the management of the two coexisting epidemics among HIV patients (Bloomfield & Velazquez, 2013). In the people living with HIV in care, much attention is paid to HIV infection as opposed to cardiovascular diseases which is said to be on the rise in Sub-Saharan Africa in an era of highly active antiretroviral therapy scale up mostly, resulting from the generalization of data from the western countries (Alinda, Grobusch, 2017). In Rwanda there is high rate of cardiovascular diseases among HIV patients as reported in a retrospective study that

was conducted at a national referral and University Teaching Hospital, Centre Hospitalier Universitaire de Kigali (CHUK) in Kigali city on 226 cardiovascular diseases cases, the association between cardiovascular diseases and HIV/AIDS infection was found to be 23.9% of the total patients (Amendezo, Twagirimukiza, Sebatunzi, & Kagame, 2008). The incidence of cardiovascular diseases among patients on antiretroviral therapy are thought to be higher as ascertained by dyslipidaemia, hypertension and diabetes among these patients (Dillon et al., 2017; Mutimura, Crowther, et al., 2015). However, there is a paucity of information on the effect of ART on the biochemical markers of cardiovascular risk; NT-proBNP, hs-CRP, lipid panel and glucose which may be strong predictors of cardiovascular diseases among People leaving with Human Immunodeficiency Virus (PLWH) in Rwanda, hence the need for the current study.

1.3. Justification of the study

There are no reports on studies done on biochemical markers in HIV patients in Rwanda. The role of these cardiac risk biomarker profiles which are tests done to analyze the risk of heart disease (NT-proBNP, hs-CRP, lipid panel) , have not been well established to address HIV-ART-related CVDs risk; thus pose a need to assess these serum cardiac risk related biomarkers that are good predictors of CVDs. The results from timely detection of undiagnosed elevated levels of cardiac risk biomarkers in PLWH in this study, will likely have a significant impact on treatment and preventive strategies through targeting early-, mid-, and long-term CVDs in Rwanda. This will accelerate further investigations to elucidate CVD interactions with ART, hence guide in individual drug adjustment to rule out resulting metabolic imbalances. This study will also generate relevant knowledge

that may guide policy and practice for chronic care of HIV-related non-communicable diseases (NCDs) in the region.

1.4. Research questions

i. What is the current distribution of the cardiovascular disease lifestyle risk factors among people living with HIV in Kigali, Rwanda?

ii. What is the distribution of the serum level of biochemical markers of cardiovascular diseases risk among People living with HIV in Kigali, Rwanda?

iii. Does the use of antiretroviral viral therapy result in changes in biochemical markers of cardiovascular diseases risk among People Living with HIV?

1.5. Objectives of the Study

1.5.1. Broad objectives

The main objective of this study was to assess the relationship between antiretroviral viral therapy use and changes in biochemical markers of cardiovascular diseases risk among HIV infected adults attending HIV clinics in Kigali, Rwanda.

1.5.2. Specific objectives

i. To determine demographic and life style CVD risk factors among HIV negative (HIV-) and HIV-infected study participants.

ii. To determine the serum level of cardiac risk biomarker profiles and glucose among antiretroviral viral therapy -treated HIV-positive patients;

iii. To determine the association between antiretroviral viral therapy duration and changes in biochemical markers of cardiovascular diseases in HIV-infected patients.

CHAPTER TWO

2.0 Literature Review

2.1 HIV/AIDS and cardiovascular diseases risk

Cardiovascular disease (CVD) burden remains a global threat to health and sustainable development (Roth et al., 2020). It is estimated that 17.9 million people deaths representing 32% of all the global mortality, results from CVD annually according to WHO (WHO updates fact sheet on cardiovascular diseases (11 June 2021). This therefore makes CVD, number one cause of death worldwide. There is a growing concern that HIV infection increases cardiovascular disease risk and that people living with HIV are said to be at an increased risk of CVD compared with people without HIV (Mesquita et al., 2019). Although the mechanisms lying behind HIV-related cardiovascular diseases may be the same in all populations with HIV, the distribution of cardiovascular disease risk factors varies by geographical location (D.A., C., M., & S., 2003). Sub-Saharan Africa (SSA) has young population, and is said to have a higher prevalence of elevated blood pressure, lower smoking rates and lower prevalence of elevated cholesterol than western Europe and North America according to the litureture. These variations in data mean that the profile of cardiovascular disease differs between low- income and high-income countries (So-Armah et al., 2020). Although data linking HIV and CVDs comes mostly from Europe and North America, it has most recently become obvious that the burden of cardiovascular disease and the attendant risk factors are on the rise in Africa. Of an estimated 37.9 million people living with HIV globally, 25.6 million live in SSA, where less is known about incidence and the burden of risk factors driving cardiovascular disease (Yuyun, Sliwa, Kengne, Mocumbi, & Bukhman, 2020)

2.2 Role of antiretroviral therapy (ART) in HIV- related cardiovascular diseases risk

Highly Active Antiretroviral Therapy (HAART) era has been marked with a remarkable success in the scale up of HIV treatment over the last decade such that by June 2020, 26 million people with HIV infection were receiving HAART worldwide (World Health Organization,2020). Notwithstanding the effective use of ART has resulted in improved immunologic and virologic outcome and improved the well being of people living with HIV. Due to the successful control of HIV viremia and HIV-induced AIDS through ART treatment, CVD has emerged as the leading cause of death among those living with HIV and coronary artery disease (COD) exist as one of the most critical complications of HIV infection and a major cause of morbidity and mortality (Wang et al., 2015). Although the increase of CVD in HIV+ patients remains not fully understood yet, one explanation could be HIV virus-or ART-induced hyperlipidemia and hypercholesterolemia, conditions well known to be atherogenic. Earlier studies have identified an increased incidence of heart diseases in HIV-infected patients, exacerbated with extended use of combined antiretroviral therapy (cART) (Dimala,2017). The use of ART has been therefore associated with multiple side effects, especially the first-generation antiretroviral medications which is claimed to present with metabolic derangements (Gaziano, Crowther, 2018; Kramer et al., 2009). In particular, the extended use of Protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) have been implicated as causes of metabolic disorders such as dyslipidaemia, hypertension and diabetes mellitus (DM), which are risk factors for cardiovascular disease (CVD) (Lambert, Sandesara, 2016; Mutimura, Crowther, et al., 2015) such as coronary heart

disease mentioned earlier, that results in myocardial infarction, heart failure, hypertension, stroke and other derangements (Gaziano et al., 2017). Protease inhibitors (PIs), are defined as any of the various drugs that inhibit the action of HIV protease, an enzyme necessary for replication of the HIV virus and nucleoside reverse transcriptase inhibitors (NRTIs) are nucleoside analogues of DNA nucleotide which prevent reverse transcription of the HIV genome; thereby inhibiting the action of HIV-1 Reverse transcriptase (RT) and viral replication, hence leading to metabolic imbalances, resulting in elevated levels of LDL-C and low levels of HDL-C, which contribute to atherosclerosis, the primary cause of cardiovascular disease (Hao, 2014),(Muralikrishna, 2009).

2.3 Lifestyles behaviors related to cardiovascular diseases (CVDs)

Cardiovascular diseases has emerged as a major threat to public health concern in developing countries (Adhikari, Kp, & Adhikari, 2013). It has become the leading global cause of death accounting to approximately 17.9 million people who died from cardiovascular diseases in the year 2019, representing 32% of all global deaths (Roth et al., 2020). Of these, 85% deaths were particularly due to heart attacks and stroke with an estimated three quarters of these heart diseases deaths occurring in low and middle income countries (Yuyun et al., 2020). However, it is worth noting that as of recent, both developed and developing countries realize the increase in number of lifestyle related cardiovascular diseases risk factors such as excessive smoking, alcohol consumption, a poor dietary habit and physical activity among adults as well as in adolescents (Odunaiya, Grimmer, & Louw, 2015). Cardiovascular diseases risk factors are also said to be on rise in HIV-positive patients which places them at increased risk for cardiovascular disease

(D. S. Nsagha et al., 2015, Ifeyinwa et al., 2016). Although scientific evidence supports guidelines-based pharmacotherapies for achieving cardiovascular risk reduction, lifestyle modification is equally effective in the improvement of many cardiovascular risk (Brinks, Fowler, & Pharmacotherapy, 2016) adjunctive to other risk assessment like regular updated family history, blood pressure, anthropometric measures (body mass index (BMI), waist circumference (WC)) and biochemical biomarker tests, fasting serum lipoprotein profile or total and HDL cholesterol) if fasting is un available), and fasting blood glucose for primary prevention of cardiovascular diseases (Interventions for a healthy heart, 2007),(Wellard-cole et al., 2021). Individuals who have ever experienced a cardiovascular clinical event such as myocardial infarction or stroke are good candidates for risk factor assessment. This would be the only way to plan ahead for cardiovascular (CVD) risk management. Across sectional study was conducted in primary care practice in six European countries' (Croatia, France, Portugal, Slovenia, Spain and Turkey) health settings. The Patients with already established cardiovascular events of coronary heart disease and stroke who were attended in the same primary care setting were selected and assed for a period from 6 months up to 3 years after, from the day they experienced the event. Nine hundred and seventy-three patients (32.4% females) in total were evaluated. Of those assessed, about 14%, were smokers, 32% lived sedentary life, and 30% had nutritionally poor eating habits. The bad cholesterol, (LDL-cholesterol) target value considered below 70 mg/dl was achieved in about only 23% of patients, and in general, women were less cardio-protected by drugs than it was found in men. The observation from this study with given statistics is that many patients silent but with established heart disease who attended in general practice still fail to achieve the lifestyle, risk factor

modification and the therapeutic targets set by European guidelines. The results here are relevant to the general practitioners because these patients have a high risk of subsequent cardiovascular events, including myocardial infarction, stroke, and death (Fernández et al., 2019). It is worth noting that primary care setting is the right place for interventions to reduce behavioral risk factors and recommend preventive measures for both healthy persons as well as those with established diseases. In fact, there is certain intervention practices put forward to improve on the life expectancy and clinical outcomes among people who are already affected with cardiovascular disease (CVD) irrespective of their potential to reduce on the risk of recurrent disease and death however, reports on the implementation of prevention guidelines are discouraging. A survey performed by cardiologists in 27 European countries demonstrated that a large majority of coronary heart diseases patients have unhealthy lifestyles in terms of smoking, diet and living a sedentary life style, which negatively affect major cardiovascular risk factors (Gyberg et al., 2015). Also, results of intervention studies show that further improvements in risk factor management are difficult to achieve. Professional counseling is an alternative to improve on cardiovascular health of individuals especially when it comes to behavioral change for some who may have misinterpreted that life style modification may have come with its own disadvantages that it may worsen their health or that altering their diet may also cause more devastating conditions, those who believe lifestyle change may come with no effect on their health and those who may want to change but don’t know how to go about it. Patients in belief need clear, concise information in coherent and consistent manner in order to realize positive cardiovascular out come. Regular monitoring of individuals’ lifestyles may also give professionals opportunities to identify

difficulties patients are experiencing and facilitate lifestyle change effectively. Health professionals may take advantage and encourage and discuss new with patients new approaches to exercise or practice healthy eating, to manage disruption to daily routines, to overcome potential negative influences of friends and to exploit social networks for their own benefits (Cole, Smith, Hart, & Cupples, 2013)

2.4 Diagnosis of myocardial damage

For the last two decades, biochemical markers, have come into play in disease diagnosis and have therefore significantly played a great role in informing medicine. In cardiology, markers such as creatine kinase and LDH (lactate dehydrogenase) activity have added insurmountable knowledge to clinicians in detection and treatment of cardiac tissue necrosis (Tomáš Janota , 2014). However, little exists in the literature on the role and importance biochemical markers are assuming in the clinical assessment of cardiac function, more especially in an HIV-infected population especially in Sub-Saharan Africa where there is a knowledge gap on the cardiovascular diseases burden in this vulnerable population (Hyle et al., 2017). Most recently, highly sensitive and specific assays for the detection of myocardial damages and heart failure (HF) such as cardiac troponin-I (cTnI) and NT-proBNP have become reliable markers; to help in the diagnosis of heart attack, detect and to evaluate heart injury. Inflammatory responses due to HIV infection may contribute to the heart damage (Reser et.al, 2018). Nevertheless, little has been mentioned on the direct involvement of antiretroviral therapy in heart failure in HIV positive persons. In pursuit of possible cardiovascular diseases risk in HIV infected patients on antiretroviral therapy, cTnI and NT-proBNP are claimed good candidate biomarkers to be evaluated. According to the literature, measuring cTnI and NT-proBNP has been shown

to be of vital importance in ruling out systolic heart failure (HF) besides facilitating in the selection of the patients who should undergo a confirmatory echocardiography for proper diagnosis of myocardial infarction , heart failure and other structural heart diseases such as valve disease and atrial fibrillation, conditions that are characterized by elevated levels of cTnI and NT-proBNP (Domingo, 2013). The diagnostic power the knowledge of elevated levels of cTnI and plasma NT-proBNP (>400 pg/ml) are significant predictive values for myocardial damage and heart failure. Therefore, the inclusion of NT-proBNP and troponins in the diagnosis of cardiovascular diseases would provide an accurate and considerable power.

The joint European Society of Cardiology (ESC) and American College of Cardiology (ACC) committees have reached a consensus for the definition of acute myocardial infarction and the role of biochemical evidence by recommending that the definition be based on biochemical markers of myocardial necrosis such as cardiac troponins which have a higher value of sensitivity to smaller myocardial injury and highly specific to cardiac damage (Mauro Panteghini, 2004; Mendis, Thygesen, 2018; Thygesen, Alpert, 2012).

In a study carried out in India; results from a three months study on cardiac abnormalities in HIV-positive patients, revealed a 67% abnormality in echocardiography of 100 patients enrolled and a confirmed 57 % of non-conduction defects as shown by ECG/EKG. This repolarisation changes according to this study, explains a possible baseline cardiac abnormalities that may exist even in asymptomatic HIV-infected patients. The study further show that, the diastolic dysfunction was highly prevalent in 42.8% of the HIV patients, followed by dilated cardiomyopathy in 17.6% (Nirdesh Jain,

2014). However, the study did not prove why there was a high prevalence of diastolic dysfunction which was said to be independently associated with HIV infection and not affected by traditional CVD risk factors, and also did not show what would be the course of diastolic dysfunction in the HIV infection. The study further suggested risks of developing cardiomyopathy and coronary heart diseases in patients on antiretroviral therapy, but did not make it clear if there was direct effect of antiretroviral treatment (ART) treatment on cardiac function among HIV- infected patients. Although a lot has been achieved in research to affirm evidence in the usefulness of a number of biochemical markers in patients with cardiovascular disease, it has also most importantly emerged with candidate biomarkers in the clinical and research communities, however few of them are less likely to escape the test of time as useful for diagnostic purposes. Varying types of biochemical markers are still used to help in predicting the risk of cardiovascular disease, the prognostic utility of any marker therefore used as a risk assessment tool is dependent on the long- and short-term biological variability that the marker shows in different individuals (Alexander, Kazmierczak, Snyder, Oberdorf, & Farrell, 2013). Guidelines are in place for utilization of biochemical markers in acute coronary syndromes are involved in recommendations for use of biomarkers in the initial evaluation of patients with no traumatic chest symptoms, for diagnosis of myocardial infarction, as well as recommendations for early risk stratification of subsequent death and/or recurrent ischemic event according to a study on prognostic utility of biochemical markers of cardiovascular risk by K. Alexander et.al. Notwithstanding, beside research clinical laboratory tests are done to serve a variety of purposes to include diagnosis, monitoring of disease and risk stratification. With respect to risk stratification, the

prognostic utility of any markers of risk assessment is often based on epidemiologic studies involving hundreds to thousands of study subjects. Although these large epidemiological studies enable the identification of markers associated with increased or decreased risk of CVD, they fail to account for the prognostic utility of the marker when it is applied to a single measurement in a single individual. For any marker of risk assessment to have good prognostic utility in an individual subject, the biological variability of the marker must be low enough to enable appropriate risk stratification using as few serially collected blood samples as possible (Alexander et al., 2013).

2.5 Lipid profile and cardiovascular disease

Lipid profiles or simply lipid panel is a range of blood tests that serves as an initial broad medical screening tool for abnormalities in lipid levels such as cholesterol and triglycerides. The results for these tests can identify a range of diseases, and can determine approximate risks for cardiovascular diseases (Niroumand, Khajedaluee, 2015). Blood lipids are fatty acids and cholesterol that are either free or bound to other molecules. They are mostly transported in a protein capsule; and the density of lipids and type of protein determine the fate of the particle and its influence on metabolism (Samson, Mundkur, 2012). The concentration of blood lipids depends on intake, uptake, and secretion from cells and, excretion from the intestines. Hyperlipidemia is the presence of elevated or abnormal increased levels of lipids and/or lipoproteins in the blood and is a major risk factor for cardiovascular disease (Niroumand, Khajedaluee, 2015; Samson, Mundkur, 2012; Sprinz, Lazzaretti, 2010). In particular, decreased levels of high density lipoprotein cholesterol (HDL-C) also known as the “good” cholesterol, elevated levels of low density lipoprotein cholesterol (LDL-C) the “bad” cholesterol, and/

or elevated levels of total cholesterol (TC) predicts increased risk of cardiovascular diseases. Altered serum lipid levels in inflammatory diseases such as HIV lead to increase in levels of TC, LDL-C and Triglycerides (TG) and reduce HDL-C (Johan Frosteg, 2001; Niroumand, Khajedaluee, 2015; Samson, Mundkur, 2012). Once observed, lipid profile forms an important prognostic marker for cardiovascular disease risk. In one of the current epidemiological studies, the association between lipid panel and cardiovascular disease events in young adults has been observed (Hidehiro et.al). A study in Japan has revealed that LDL-C of ≥ 140 mg/dL, HDL-C < 40 mg/dL, and triglycerides ≥ 150 mg/dL were independently associated with higher incidences of cardiovascular diseases among the general population respectively (Yandrapalli, Nabors, Goyal, Aronow, & Frishman, 2019) . In addition, results from this study has evidenced that the incidences of myocardial infarction (MI), angina pectoris, and heart failure increased significantly with the number of abnormal lipid profiles. Nevertheless, the number of abnormal lipid profiles was not associated with the risk of stroke. Notwithstanding, the analysis has therefore demonstrated very closely the relationship between lipid profiles and subsequent cardiovascular diseases events. Similarly a case control study that included 300 subjects (150 coronary heart diseases (CHD) patients and 150 controls) that were randomly selected and were found to have coronary heart diseases based on WHO guidelines, the levels of lipid profiles among those study subjects were found be associated with cardiovascular disease. The total cholesterol (P=0.001), Triglyceride (P=0.03), LDL-Cholesterol (P=0.001) and HDL-Cholesterol (P= 0.013) were all said to be significant in coronary heart diseases group when compared with the control group (Hussain, Mohammed, & Jeddoa, 2018). The findings above

conclude that abnormal lipids marked with elevated levels of triglycerides (TGs), LDL-Cholesterols, and low levels of HDL-cholesterol are associated with cardiovascular risk. However, it is also worth pointing out that dyslipidemia a condition characterized with imbalances of lipids, is a critical problem among people infected with human immunodeficiency virus (HIV) though it is also agood prognostic biomarkers for the cardiovascular disease prediction. It is worth noting that irrespective of dyslipidemia being a health issue in people living with human immunodeficiency virus (HIV), as ascertained by the recent research data, this population presents with increased risk of developing cardiovascular diseases as opposed to the general population, it is as well inevitable to consider that well established systems of antiretroviral therapy should insure that HIV-infected patients should undergo evaluation and treatment regimens based on the current National Cholesterol Education Program guidelines (where they exist), since the use of antiretroviral therapy especially the protease inhibitors (PI) has been found to highly contribute to metabolic imbalances resulting into abnormalities in lipids and in consequence exacerbating cardiovascular risk conditions among HIV-infected individuals on antiretroviral therapy. In a studies conducted on Lipid lowering therapy in patients with HIV infection on antiretroviral therapy and on Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors, it was found out that among the recipients of protease inhibitors (PI), forty seven percent (47%) at one health care clinic presented with lipid abnormalities, according to the National Cholesterol Education Program (NCEP) guidelines for intervention (Wierzbicki et al., 1998,). It was also reported that that 56 (57%) of 98 protease inhibitors (PI) recipients experienced hyperlipidemia. Among those, 19% had elevated low-density

lipoprotein cholesterol (LDL-C) alone, 44% had hypertriglyceridemia alone, and 37% had both abnormalities. Thus, dyslipidemia is a common problem among HIV-infected individuals receiving treatment (Behrens et al., 1999). Nevertheless, it should not be neglected to insure that the primary intervention should not be pharmacological but rather maintain a stable management of other attendant risk factors of cardiovascular diseases like smoking, sedentary life, diet, and high blood pressure(HBP) since addressing these modifiable lifestyle risk factors, cardiovascular diseases can be slowed or even reversed (Dube & Fenton, 2003). The prevalence of abnormalities in lipid levels among HIV-infected individuals remains high and accentuated among those receiving antiretroviral therapy.

2.6 Markers of inflammation

C-reactive protein (CRP) is one of the most important plasma markers of inflammation. C-reactive protein together with other plasma markers has been widely studied to investigate their possible role in group risk stratification of patients who needs special care. However, C-reactive protein is most pointed for its association of inflammation with heart diseases outcome (Mauro Panteghini, 2004; Payman Zamani; Gregory G. Schwartz, Anders G. Olsson; Nader Rifai; Weihang Bao; Peter Libby; Peter Ganz; Scott Kinlay, 2013; Reser et.al, 2018; Tomáš Janota, 2014). Some studies have found out that patients presenting with unstable angina pectoris and elevated plasma concentrations of C-reactive protein are predisposed to potential risk of death and development of myocardial infarction besides being also exposed to acute coronary syndrome (ACS). A high sensitive C-reactive protein (hs-CRP) test unlike the traditional C-reactive protein is more sensitive. This means that the mentioned high-sensitivity test, can even detect extremely

low increases within the normal range of the standard CRP levels. Although research continue to investigations and discuss on the precise physiological importance of C-reactive protein, the prognostic value of high sensitive C-reactive protein (CRP) as a biological marker for cardiovascular disease risk is now been firmly established. hs-CRP is said to be a good future predictor of cardiovascular disease risk in a generally apparent health population under study to include known healthy individuals without cardiovascular disease, patients presenting with acute coronary syndromes, patients with stable angina pectoris or that are in the stable phase after myocardial infarction (MI), and patients with the metabolic syndrome, diabetes mellitus (DM) and, or renal disease (Bassuk, Rifai, & Ridker, 2004). In prospective epidemiologic studies (3 to 20 years follow up) that intended to associate the predictive value of high sensitive C-reactive protein (hs-CRP) with cardiovascular diseases risk has revealed that a single hs-CRP measurement alone is a strong predictor of myocardial infarction and or other coronary heart disease (CHD) mortality to include peripheral vascular disease, congestive heart failure, atrial fibrillation, and sudden cardiac death in individuals without a history of cardiovascular disease (CVD) (Bassuk et al., 2004). Furthermore, data from aggregate epidemiologic studies pointed out those subjects with baseline hs-CRP levels in the top quartile of the sample distribution are 2 to 3 times more likely to have a future cardiovascular (CV) event than are those in the bottom CRP quartile. Typically the association between CRP and subsequent cardiovascular events exhibits a linear “dose-response” shape and is independent of age, smoking, hypertension, dyslipidemia, and diabetes, the traditional risk factors evaluated in daily practice and included in global cardiovascular prediction algorithms such as that derived from the Framingham Heart

Study (Sai Ravi Kiran, Mohanalakshmi, Srikumar, & Prabhakar Reddy, 2017). For instance, an 8-year follow up data from 2 large primary prevention cohorts. The Study, concluded that, after adjustment for traditional risk factors, for each quintile increase in baseline hs-CRP, the risk of a future cardiovascular event increases by 26% for men (95% confidence interval [CI], 11%-44%; Pv =0.005) 4 and 33% for women (95% CI, 13%-56%; Pv = 0.001). A meta-analysis of 14 prospective long-term studies (2557 cases; mean age at baseline, 58 years; mean follow-up, 8 years) of hs-CRP and risk of non fatal myocardial infarction and or coronary heart diseases (CHD) death yielded an adjusted summary relative risk (RR) of 1.9 (95% CI, 1.5-2.3) for the study subjects in the top tertile of baseline hs-CRP as compared with those in the bottom tertile. The association between high sensitive C-reactive protein (hs-CRP) and cardiovascular diseases (CVD) has been observed in the United States and Europe, in the middle-aged and elderly, and in high and usual risk populations. The association is apparent even in studies with follow up periods exceeding 10 years. For instance, elevated levels of high sensitive C-reactive protein (hs-CRP) at baseline were predictive of 17-year coronary heart disease mortality in the Multiple Risk Factor Intervention Trial (MRFIT) and of sudden cardiac death in the Physicians' Health Study (PHS) (Danesh et al., 2000) . By observing closely, among Japanese American men participating in the Honolulu Heart Program, C-reactive protein (CRP) was a strong predictor of myocardial infarction (MI) and thromboembolic stroke up to 20 years after initial blood samples had been taken. Nevertheless, the Honolulu Heart Program is one of only a handful of studies of nonwhite populations; data on the predictive utility of high sensitive C-reactive protein (hs-CRP) in such populations are sparse. Much of the available data on high sensitive C-reactive protein (hs-CRP) and

incident of cardiovascular disease (CVD) have been derived from nested case-control studies, which permit estimation of relative risks (RRs) of disease but not of absolute risks within risk factor strata (Kozarevic et al., 1981). However, event-free survival data from several large cohorts, to include the Women's Health Study, the Air Force/Texas Coronary Atherosclerosis Prevention Study, the multiethnic Atherosclerosis Risk in Communities (ARIC) study in the United States and the monitoring of trends and determinants in cardiovascular diseases. In contrast to the unequivocal, findings on the relation between high sensitive C-reactive protein (hs-CRP) and incident cardiovascular events, epidemiologic studies of general populations unselected for cardiovascular diseases (CVD) status have found inconsistent associations between hs-CRP and measures of subclinical atherosclerosis such as carotid intimal medial thickness (IMT) as measured by Doppler ultra- sound and coronary calcification as measured by electron beam computed tomography (EBCT)

The cause of cardiovascular disease (CVD) is not fully understood. Traditional cardiovascular risk factors, such as increased levels of cholesterol concentrations and blood pressure (BP), are used to assess cardiovascular disease risk. Recently, better understanding of the role of inflammation in atherosclerosis has brought about to many to propose the measurement of various inflammatory biomarkers markers to better identify those who are at increased risk. C-reactive protein (CRP) is found in endothelial atherosclerotic lesions, and evidence suggests that it may play vital role in the formation of fatty plaques in the arteries. Of candidate serum markers that might add information to clinical risk assessment, high-sensitivity C-reactive protein (hs CRP) measurement has the most potential for clinical use for multiple reasons. high hs-CRP is associated with a

twofold to a threefold increase in the prevalence of myocardial infarction, stroke, and peripheral vascular disease, and it predicts incident cardiovascular events in those with and without preexisting cardiovascular conditions; the increased risk associated with high hs-CRP is independent of other established risk factors; hs-CRP augments the predictive capacity of the Framingham Risk Score; hs-CRP assays are standardized, and this analyte is biologically stable over time and lastly various risk-reducing interventions also reduce hs-CRP, and research is underway to assess whether specifically targeting hs-CRP reduces cardiovascular diseases (CVD) risk. Elevated level of the acute phase reactant C-reactive protein (CRP) is a very sensitive marker of acute inflammatory reactions. Using high sensitivity assays for CRP, recent observations indicate that slightly elevated CRP levels which would be in the normal range of conventional assays are a novel marker for an increased risk for cardiovascular events, especially coronary artery disease and myocardial infarction (Aviles et al., 2003). Various large scale prospective trials including the Physicians' Health Study and the Women's Health Study as previously stated, revealed that slightly increased hs-CRP levels at base line in apparently healthy persons are associated with a 2-fold increase in the risk of a future myocardial infarction. The predictive value of hs-CRP was found to be independent from classic risk factors, in particular from elevated serum cholesterol. An increase in hs-CRP levels was also associated with a higher risk to develop peripheral artery disease and with a faster progression of carotid artery disease. As of the recent, treatment with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) has been found to be the only medication to consistently decrease hs-CRP levels, although by about 15% only. Despite the association of elevated hs-CRP levels with future cardiovascular events, routine

measurement of hs-CRP for cardiovascular risk assessment is currently not recommended because of its low accuracy, the lack of a clear cut-off point for elevated hs-CRP levels and the lack of an absolute predictive value. So far, hs-CRP remains an interesting potential risk marker for cardiovascular disease whose definite relevance remains to be established.

2.7 The role of cardiac natriuretic peptides in assessment of cardiac function

Little has been said in research on the role and the importance of biomarkers and biochemical tests in the clinical assessment of cardiac function (Dupreza, Neuhaus, Deeks, Orkin, 2011; Kristoffersen, Lebech, Gerstoft, 2008). Cardiac natriuretic peptides; atrial natriuretic peptides (ANP) and brain natriuretic peptide (BNP) or B-type, are hormones of related peptides with similar peptide chains as well as degradation pathways. Other natriuretic peptides like C-type natriuretic peptides and urodilatin, are not produced and secreted by cardiac tissue but by other tissues. Atrial natriuretic peptide and brain natriuretic peptide are secreted in different sites, ANP is mainly from atrial cardio-myocytes while the BNP is produced and secreted in the left ventricle, the right side of the human heart is also able to synthesize and secrete BNP in response to disease (Clerico, 2002). Besides certain diseases such as renal failure, primary aldosteronism, and congestive heart failure (CHF) may exhibit increased plasma levels of these peptides and can therefore be used to generally detect all cardiac abnormalities though not diagnosis of the underlying cause of myocardial dysfunction. Understanding the nature of NT-proBNP, it is without doubt that the measurement of this biomarker is vital to rule out suspected cases of heart failure (HF) in the clinical settings, be it either in the emergency care department for inpatients consideration or, for outpatient and may assist in identify

the persons with asymptomatic ventricular abnormalities, who will benefit from therapy and preventing the risk of progressing to heart failure (Rademaker & Richards, 2005) . The cardiac natriuretic peptides, ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide), are secreted by the heart in proportion to cardiac transmural pressures and are said to possess a wide range of effects in multiple tissues, paving way to the overall pressure /volume homoeostasis. The close relationship between plasma concentrations of these peptides and the cardiac out, has led to their use as potential biomarkers of cardiovascular health with both diagnostic and prognostic utility in a variety of heart defects affecting the cardiovascular health. BNP and its N-terminal fragment (NT-BNP) are especially sensitive indicators of cardiac dysfunction and ventricular remodeling (Ventura & Silver, 2011). These structural changes which may either result from physiological or pathological reasons are likely said to correlate strongly with the severity of heart injury. Given that cardiac ischaemia is also an important cause for the release of these ventricular peptides, they may likewise play a role in the detection of coronary artery diseases. Notwithstanding, assessment of BNP peptides is also said to help in prediction of subsequent haemodynamic deterioration and adverse events in cardiovascular disease, and can therefore be used to monitor those at high risk and act as a guide to optimization of treatment. The favourable biological nature of the natriuretic peptides has also led to their use as therapeutic agents. (Rademaker & Richards, 2005). A number of studies have demonstrated the clinical importance of the cardiac natriuretic peptides in the cardiovascular disease diagnosis and risk stratification of both acute and chronic heart failure. Most recently, these biomarkers have been found to have diagnostic value beyond standard risk factors for determining outcome prognosis

for cardiovascular morbidity and mortality in patients with chronic heart failure, hypertension (Olsen et al., 2004) acute coronary syndromes,(Morrow et al., 2008) prior myocardial infarction , stable coronary artery disease, vascular disease, or high coronary risk (Kragelund, Grønning, Køber, Hildebrandt, & Steffensen, 2005) and in community-based cohorts.

2.8 Diabetes mellitus (DM)

Diabetes mellitus is a group of metabolic disorders characterized by an increase in sugar levels beyond normal, also referred to as hyperglycemia, which is caused by an absolute or relative insulin deficiency (American Diabetes Association, 2006; Rudruidee Karnchanasorn,1 Jean Huang,2,3 Horng-YihOu,4 Wei Feng,2,3 Lee-Ming Chuang,5,6 Ken C. Chiu,2, 2016). Diabetes mellitus has been defined by the World Health Organization (WHO) basing on laboratory findings, as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140mg/dl) or greater than 11.1mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion of the equivalent of 75 g of glucose, even if the fasting concentration is normal (Davidson, 2001). Recent contributions by the International Expert Committee (IEC) suggests new diagnostic criteria on the basis of the HbA1c measurement, with HbA1c >6.5% being definitive for diabetes and the range of 6.0-6.4% as indicative of high risk of progression to diabetes (Shimodaira, Okaniwa, Hanyu, & Nakayama, 2015). Diabetes is a non communicable and chronic disease that occurs either when the pancreas in body does not produce sufficient insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes which over time leads to devastating health

impact like body damage affecting mostly the heart, blood vessels, eyes, kidneys and nerves. According to the world health organization (WHO), adults with diabetes have a two-to threefold increased risk of heart attacks and stoke. Combined with reduced blood flow accompanied with nerve damage in the feet increases the chance of foot ulcers, followed with infection which is in most cases associated with limb amputations in diabetic people (Emerging & Factors, 2010). Diabetic retinopathy is also the major cause of blindness among people with diabetes. It is estimated that about 1 million people are blind world as a result of long term accumulated damage of the small blood vessels in the retina (Bourne et al., 2021). Besides, diabetes is the leading cause of renal failure (Saran et al., 2015). The prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise by 5.4% in the year 2025. It is said to be higher in developed than in the third world countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The major part of this numerical increase will occur in low developed countries. There will be a 42% increase, from 51 to 72 million, in the developed countries and a 170% increase, from 84 to 228 million, in the developing countries (King, Aubert, & Herman, 2000). The global prevalence of diabetes is predicted to increase greatly in the coming decades as the population grows and ages, in parallel with the rising burden of overweight and obesity, in both developed and developing countries. The statistics therefore provides a provisional picture of the characteristics of the epidemic. The worldwide surveillance of diabetes is therefore a necessary first step towards its prevention and control, which is now recognized as an urgent priority according to the WHO (King et al., 2000). Cardiovascular disease accounts for most of mortality and morbidity cause among people with diabetes,

especially in those with type 2 diabetes mellitus. Adults with diabetes have 2-4 times increased cardiovascular risk compared with adults without diabetes, and the risk rises with worsening glycaemic control. Diabetes has been associated with 75% increase in mortality rate in adults, and cardiovascular disease accounts for a large part of the excess mortality. Diabetes-related macrovascular and microvascular complications, including coronary heart disease, cerebrovascular disease, heart failure, peripheral vascular disease, chronic renal disease, diabetic retinopathy and cardiovascular autonomic neuropathy are responsible for the impaired quality of life, disability and premature death associated with diabetes (Anand et al., 2012). Given the substantial clinical impact of diabetes as a cardiovascular risk factor, there has been a growing focus on diabetes-related complications. While some population-based studies suggest that the epidemiology of such complications is changing and that rates of all-cause and cardiovascular mortality among individuals with diabetes are decreasing in high-income countries, the economic and social burden of diabetes is expected to rise due to changing demographics and lifestyle especially in middle- and low-income countries. In this review we outline data from population-based studies on recent and long-term trends in diabetes-related complications (Dal Canto et al., 2019).

CHAPTER THREE

3.0 Methodology

3.1 Study design

This study adopted a cross-sectional design.

3.2 Study setting

This was a health facility and laboratory based study conducted in seven health centers (HCs) serving as HIV/AIDS care clinics in Kigali, Rwanda. The HCs comprised of Nyarugunga, Remera, Kinyinya, Masaka, Biryogo, Cornum, and Gikondo and laboratory work was carried out at Walk in Walk out (WIWO) an Indian specialized hospital in Kigali. The prime work for the mentioned health centers include treatment and medical psycho social support for people infected with HIV. This care include tri-therapy and counseling on HIV/AIDS with the overall objective to decrease the mobility and mortality of people living with HIV/AIDS (PLWHIV/AIDS) of the outpatients within the clinics. The tertiary health centers of Nyarugunga, Remera, Kinyinya, Masaka, Biryogo, Cornum, and Gikondo, which are also HIV/AIDS care clinics, see a considerable number of HIV patients. Many of these sites are used as centers for the international epidemiologic databases to evaluate AIDS (IeDEA) (http://www.iedea.org) and are strategically located in the areas of Kigali city easily accessible hence together with better services makes them preferred centers for the management of HIV-positive patients.

3.3 Study population

The study population were men and women aged between 18−45 years. These included HIV-uninfected (HIV-) and HIV-infected (HIV+) adults. Among the HIV positive (HIV+) population, the participants were subdivided into the categories of antiretroviral

therapy-treated naïve and HIV+ participants on stable WHO-recommended antiretroviral therapy for 0-6months and 7-12 months found in the selected HIV/AIDS clinics, in Kigali.

3.4 Sample size

There is basically no preliminary data in the literature on N-terminal pro-B-type natriuretic peptide (NT-proBNP) in ART, in early treatment period in Rwanda, making it difficult to estimate what the difference might be between groups. The sample size for the participants in this study that aimed at evaluating the cardiac biochemical markers among stable antiretroviral therapy-treated HIV-positive patients in Kigali-Rwanda, was calculated using the formula as proposed by Charan & Biswas (Charan & Biswas, 2013) using data from a previous observational study on cardiac abnormalities in HIV-positive patients in India (Nirdesh Jain, 2014).

$$\frac{Z^2 P(1-P)}{d^2}$$

Where:

Z: is equals to 1.96, a standard normal variate for the level of significance where p value is ≤ 0.05.

P: Is the expected proportion in the population

d: is the precision

Data from a previous observational study on cardiac abnormalities in HIV-patients indicated elevated levels of serum troponin in 8% of 100 HIV-patients enrolled. There were a confirmed 67% of abnormalities seen in echocardiography with 57% of non-conduction defects as was shown by ECG interpretations. Other cardiac abnormalities to

include; diastolic dysfunction that was found out to be the most prevalent; presenting with 42.8%, followed by dilated cardiomyopathy that was only observed in 17.6% among these HIV-patients, according to the study (Nirdesh Jain, 2014).

Thus, **8**% used to calculate the minimum sample size (N) for the study participants as follows:

$$\frac{(1.96)^2 \times 0.08(1-0.08)}{(0.05)^2} = 113$$

Therefore, the overall sample size (N) of the study participants enrolled for the current study added up to **150** in total.

3.4.1 Sampling procedure

The health centers of Remera, Nyarugunga, Kinyinya, Masaka, Cornum, Biryogo and Gikondo all in Kigali that are also HIV/AIDS care clinics were purposively selected. They are strategically located in the areas of Kigali city easily accessible. In addition to that, most of these health centers and also HIV/AIDS care clinics are used as centers for the international epidemiologic databases to evaluate AIDS (IeDEA) (http://www.iedea.org); hence together with better services makes them therefore preferred centers for the management of HIV, in infected patients. Convenient sampling was employed to recruit the study participants, until the required sample size was achieved. The study participants constituted groups of HIV negative participants as negative controls, newly diagnosed HIV positive patients yet to start HIV antiretroviral therapy medication (ART naïve), HIV+ patients who were known to have been on stable WHO-recommended antiretroviral therapy for 0-6 months, and those on ARV for 7-12

months after treatment initiation. The newly HIV+ tested subjects were recruited from the consultation rooms, in voluntary counseling and testing (VCT) departments and in the organized community outreach programs for HIV testing and that who volunteered to participate in this study. Each group of the study participants comprised of 40, except for the negative control that had only 30 bringing the overall sample size to be 150 participants in total. The study selected to abstract data cross sectionally among the participants with different duration of exposure (0-6 months and 7-12 months) on antiretroviral therapy treatment with an anticipation that the longer you are on the treatment; you should have increased the levels of cardiovascular risk biomarkers profiles (e.g. NT-proBNP levels would be highest in the 7-12 months group, et cetera). The blood samples were then collected for biochemical markers tests from the study participants who consented.

3.5 Inclusion and Exclusion criteria

3.5.1 Inclusion criteria

The study included men and women aged between 18 and 45 years, and were either HIV-uninfected (-), who recruited mainly from voluntary counseling and testing service (VCT) consultations or HIV+ ART-naïve for the newly diagnosed HIV-infected (+) yet to start medication and those on ART treatment. Participants on antiretroviral viral (ART) treatment were expected to be on stable WHO-recommended ART; NNRTIs, NRTIs and PIs based combination ARV therapy (cART) at the selected HIV/AIDS care clinics, for a period of 0-6 months and 7-12 months of treatment after initiation.

3.5.2 Exclusion criteria

The study excluded those who were critically ill, those presenting with active cardiovascular or significant symptoms of heart disease conditions (HTN), diabetes or

renal disease and those found to have had irregular ART adherence within 3 months prior to the recruitment period.

3.6 Method of data collection

3.6.1 Administration of questionnaire

At enrollment, consent was obtained from prospective and if consent was obtained a structured interviewer administered questionnaire was used to obtain details on demographic data (gender, age, marital status, residence, social economic status) from the participants. Traditional cardiovascular disease risk factors present before recruitment including the family history of arterial blood hypertension, diabetes mellitus, history of smoking and alcohol habits plus other pre-existing active cardiovascular diseases conditions or significant symptoms of heart diseases; were also captured in the data extraction sheet/ questionnaire. The data extraction sheet also helped to provide other relevant information regarding HIV infection and the ART regimen initiated by the patients.

In addition, measurements of their blood pressure (BP), weight and height were taken by qualified nurses at the HIV/AIDS care clinics.

3.6.2 Collection of blood Samples

Blood samples were collected in the morning after overnight fasting through venipuncture by qualified nurse. The blood was collected in dry/red topped tubes and coded and transported to WiWo global Indian specialized hospital laboratory in Kigali, for subsequent processing and analysis.

3.7 Laboratory analysis of the samples

For analysis of all biomarkers, NT-proBNP, hs-CRP, TC, HDL-C, LDL-C, TG, and glucose levels were measured. Whole blood was centrifuged at 10,000 relative

centrifugal forces (RCF) for 10 minutes to obtain serum. The centrifuged specimens with a surface lipid layer were transferred into secondary tubes for Cobas C-111 clinical lipid profile analysis (Roche Diagnostics). NT-pro BNP and hs-CRP were assayed using a Maglumi-600 automated machine. In order to ensure proper test performance for NT-proBNP and hs-CRP using Maglumi Fully Auto Analyzer, standard operating procedures (SOPs) were carefully followed while doing the tests. The first step was to ensure that the reagents were kept in proper storage conditions (2-8˚C) before use and that reagents were kept away from direct sunlight. The second step is calibration. Maglumi machine has to recalibrate after each exchange of lot (Reagent integral or Starter reagents). Maglumi machine was recalibrated every time to insure controls run for every test, were in the expected range. Serum samples were kept at (2-8˚C) for those to be tested in 24 hours and surplus serum samples for longer storage were freezed to below -20˚C to allow for repeat testing in case needed. Stored samples before testing had to be thoroughly mixed using a vortex mixer (after melting ice). Some strict precautions were followed before testing. These included not interchanging reagents from different lots. Kits were used after checking that components are not beyond the labelled expiration date. To ensure proper test performance, strict adherence to the operating instructions on the operator's manual/protocol/ SOP of the Maglumi Fully Auto analyzer was done. The analyzer automatically calculates the NT-proBNP and hs-CRP concentrations in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure and results expressed in pictograms per millilitre (pg/ml) for NT-proBNP and nanograms per millilitre (ng/ml) for hs-CRP. Interpretation of results was done using the given reference values on the kit. Regarding the adults between 18 and 55 years, the

value for NT-proBNP was 125 pg/ml. < 125 pg/ml was considered normal and > 125 pg/ml was considered at risk. For hs-CRP, < 1000 ng/ml was considered low risk while 1000 - 3000 ng/ml and > 3000 ng/ml was considered high risk. Cobas C-111 is fully automated chemistry analyzer.

Other than the Maglumi analyzer, what is unique with the low volume bench-top clical chemistry analyzer from Roche Diagnostics (Indianapolis, IN) is that daily start-up and system checks are prompted by the user interface and involve some steps as seen below:

1) A simple fluid's check (water and cleaner bottles full, waste empty)
2) Less than 10 minutes of daily maintenance involving an automated probe cleaning and priming of the fluid system
3) Replacing the incubation cuvette sections as indicated by the system screen
4) Loading the reagent disk and performing system-prompted calibration and quality control (QC) as needed.
5) Weekly maintenance takes about 15 minutes, and there are system-generated prompts for all maintenance interval requirements.
6) In addition to 3 ISE assays, 14 reagents can be loaded on each reagent disk, which is stored refrigerated in a reagent disk container when not in use.
7) Bar-coded or non-bar-coded patient samples can be continuously loaded into the 8 provided positions of the Cobas machine, and a variety of primary tubes and sample cups can be used. Considering all steps on the manual well followed, tests

were run and results interpretation was made using reference ranges from the reagents provided kits.

3.7.1 Operational definitions

Concentrations of serum biomarkers were graded according to the criteria established by American Association of Clinical Endocrinologists (Jellinger et al., 2017). Dyslipidemia, an abnormal amount of lipids in the blood is a condition present in routine lipid panel analysis and is defined as the presence of any of the following lipid abnormalities: hypercholesterolemia, hypertriglyceridemia, hypoalphalipoproteinemia also known as low HDL-C, or increased LDL-C after ruling out all other risk factors among subjects based on individual circumstances and presentations. All of these abnormalities have been implicated as being independently atherogenic (C. Manjunath, Rawal, Irani, & Madhu, 2013), (Natalie E. Kelso, David S. Sheps, 2015). hs-CRP (inflammation) and NT-proBNP (cardiac injury) were predefined based on prior studies associating their elevated levels with CVD. (Bengt et al, 2018; Jellinger & Handelsman, 2017). Diabetes mellitus, according to World Health Organization (WHO) is a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140mg/dl) or greater than 11.1mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion of the equivalent of 75 g of glucose, even if the fasting concentration is normal (American Diabetes Association, 2006; Anoop Dinesh Shah, et al.; Bengt et al., 2018; Davidson et al., 1979). In this study, diabetes was defined as a fasting blood sugar (FBS) of ≥ 126 mg/ dL though the condition is considered after having higher fasting blood sugar levels on two separate tests, hypercholesterolemia was defined as total serum cholesterol of >240 mg/dL, hypertriglyceridemia was defined as serum TG of ≥ 200mg/dl, decreased HDL-C

was defined as serum HDL-C levels of >60mg/dL, increased LDL-C was defined as serum levels of ≥ 160mg/dl, high risk hs-CRP was defined as serum levels of >3000ng/mL and NT-proBNP was considered increased with serum levels >125pg/mL . The BMI for the study participants were categorized as underweight (<18.5 kg/m2), normal (18.5-25 kg/m2), overweight (25-29 kg/m2), and obese (≥30 kg/m2) (Nuttall, 2015). Social economic status was defined based on ubudehe categorization in Rwanda. In the Ubudehe approach, there are 5 categories (ABCD & E) defined by a set of criteria, from the poorest category (without land, facing difficulties to have food) to the richer people (Rwanda Social Protection Intervention, 2011). Middle to high category = monthly income ≥ 65,000 Rwandan francs (RWF) (approximately 66 USD) to ≥ 600,000 RWF (607 USD) and ≥ 780,000 RWF (791 USD) to ≥ 7,200,000 RWF (7,302 USD) annual income. Low Category = ≤ 45,000 RWF (46 USD) to ≤ 65,000 RWF (66 USD) per month and ≤ 540,000 RWF (548 USD) to ≤ 780,000 RWF (791 USD) annual income (Uwamariya & Bosire, 2013). Lifestyle (physical activity, smoking, alcohol consumption, eating habits) and clinical data (HIV clinical stage, high blood pressure (HBP). Elevated or high blood pressure (BP) was defined as the systolic BP of 140 mmHg or higher, and/or diastolic BP of 90 mm Hg, and/or being on hypertensive medications (Ezrina Dn, 2016). Alcohol consumption as taking a drink that contained alcohol for the last 6 months. (Ikeda et al., 2013). Health diet defined based on how often are fruits and vegetables accompanying routine meals per week and unhealthy diet, defined as an impression of usually eating meals prepared with too much oil (Lachat et al., 2013). Where as physical activity participation was defined as vigorous-intensity exercise activities that cause large increases in heart rate like running, pedal cycle or football for at

least 30 minutes continuously 3 times per week (Trapp, Chisholm, Freund, & Boutcher, 2008).

3.8 Data management plan

Statistical packages for social sciences SPSS version 27.0 was used for all data related management and analysis.

3.8.1 Data collection, entry and storage

The data obtained from both the questionnaire and biochemical assays, was cleaned, coded and stored in Ms Access. The data were imported into statistical packages for social sciences (SPSS) for analysis (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Hard copies were kept in a locked cupboard at Regional Alliance for Sustainable Development (RASD) Rwanda. Soft data were kept in a folder that is secured with password and external hard disc will be used to store back up data.

3.8.2 Data analysis and presentation

Statistical package for social sciences (SPSS) version 27.0 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) was used for data analysis. Descriptive analysis results from categorical variables were presented using frequencies and proportions, continuous variables were presented using mean and standard deviation. Chi-square test was used to compare proportions of categorical variables and the significance threshold was considered for a p value <0.05. The mean of biochemical markers was compared using independent sample t-test and Analysis of Variance (ANOVA). Significance difference was considered for a p-value <0.05 with corresponding 95% confidence interval. The association between biochemical markers

and ART use was assessed using linear regression with Pearson Correlation test and significance threshold set to $p<0.05$

3.9 Ethical consideration

Ethics approvals were obtained from IREC (Institutional Research and Ethics Committee) at MTRH (Moi Teaching and Referral Hospital), Approval Number: 0003163, and from Rwanda National Ethics Committee (RNEC), FWA Assurance No.00001973; IRB 00001497 of IORG0001100. Written informed and signed consent was obtained from all study participants after explaining to them about the aims and benefits of the study. Among the things explained to the participants to include were sample collection procedure, analysis and strict confidentiality of their identity. Participants were informed that only codes will be used as unique identity for participants during the course of this study. It was also made clear to the participants that the results of the study were to be published anonymous of their identity.

CHAPTER FOUR: RESULTS

4.1. Introduction

This chapter presents research findings of the 150 participants. The findings are presented according to specific objectives of the study and include general characteristics of the study participants, distribution of cardiovascular disease life style risk factors and biochemical markers of cardiovascular disease risk, mean comparison based on serostatus (HIV- and HIV+ participants) and antiretroviral therapy status (ART) use status, and risk of cardiac biochemical markers considering the ART use status using logistic regression.

4.2 General Characteristics of study participants

The majority of the participants were females (60%). The HIV-un infected (HIV-) participants were younger than HIV-infected (HIV+) participants (35±6 vs. 31±6) and 44%) of HIV-participants were young aged between 26 and 33 years with mean age of 32 (±6) years. Most participants were city dwellers (83.3% vs. 16.7%). There was no difference between the proportion of participants between those with low or middle to high social economic status (Table 4.1). Table 4.1 also summarizes the clinical and lifestyle characteristics of study participants. Majority of participants were in HIV stage I of the WHO HIV classification compared to that in HIV Stage 2 and above (82.5% vs. 17.5%) and none were either in HIV stage 3 or Stage 4 of illnesses. Although the majority of participants had a normal BMI of 18.5-24.9 in Kg/M^2 of 70% compared with the rest of participants, the HIV+ participants group had a significantly higher proportion of those with BMI in Kg/M^2 less than 18.5 (Table 4.1). The HIV- participants were less likely to smoke compared to HIV+ (100% vs. 73.3%), and were less likely to consume alcohol (50% vs. 30.8%) with the overall majority of the participants reported to have had very limited physical activity (63.3% vs. 36.7%) (Table 4.1)

Table 4. 1: General Characteristics of study participants

Variable	HIV-Ve (n=30) n (%)	HIV+Ve HAART Naïve (n=40) n (%)	HIV+Ve HAART 0- 6 months (n=40) n (%)	HIV+Ve HAART 7- 12 months (n=40) n (%)	Total n (%)
Sociodemographic characteristics					
Gender					
Male	14 (46.70)	17 (42.50)	15 (37.50)	14 (35.00)	60 (40.00)
Female	16 (53.30)	23 (57.50)	25 (62.50)	26 (65.00)	90 (60.00)
Age in years					
Mean ± SD	35±6	30±6	31±6	33±7	32±6
25 and below	2 (6.70)	10 (25.00)	7 (17.50)	5 (12.50)	24 (16.00)
26-33	8 (26.70)	18 (45.00)	21 (52.50)	19 (47.50)	66 (44.00)
34-41	17 (56.60)	9 (22.50)	10 (25.00)	9 (22.50)	45 (30.00)
42 and above	3 (10.00)	3 (7.50)	2 (5.00)	7 (17.50)	15 (10.00)
Marital Status					
Single	12 (40.00)	26 (65.00)	20 (50.00)	16 (40.00)	74 (49.40)
Married	18 (60.00)	14 (35.00)	20 (50.00)	24 (60.00)	76 50.60)
Residence					
Rural	2 (6.70)	11 (27.50)	4 (10.00)	8 (20.00)	25 (16.70)
Urban	28 (93.30)	29 (72.50)	36 (90.00)	32 (80.00)	125 (83.30)
Social Economic status*					
Low	11 (36.70)	26 (65.00)	24 (60.00)	22 (55.00)	83 (55.40)
Middle to high	19 (68.30)	14 (35.00)	16 (40.00)	18 (45.00)	67 (44.70)
Occupation					
Government/Non-government employee	18 (60.00)	5 (12.50)	2 (5.00)	3 (7.50)	28 (18.70)
Self employed	8 (26.70)	20 (50.00)	27 (67.50)	25 (62.50)	80 (53.30)
Unemployed	3 (10.00)	14 (35.00)	10 (25.00)	9 (22.50)	36 (24.00)
No response	1 (3.30)	1 (2.50)	1 (2.50)	3 (7.50)	6 (4.00)
Clinical characteristics					
WHO HIV stage					
Stage 1		36 (90.00)	32 (80.00)	31 (77.50)	99 (82.50)
Stage 2+		4 (10.00)	8 (20.00)	9 (22.50)	21 (17.50)
BMI /kg/m2					
<18.5	1 (3.30)	5 (12.50)	4 (10.00)	3 (7.50)	13 (8.70)
18.5-24.9	21 (70.10)	25 (62.50)	28 (70.00)	31 (77.50)	105 (70.00)
25.0-29.9	4 (13.30)	9 (22.50)	5 (12.50)	6 (15.00)	24 (16.00)
>=30	4 (13.30)	1 (2.50)	3 (7.50)	0 (0.00)	8 (5.30)
Blood pressure					

(BP)					
Normal	29(96.70)	39(97.50)	34(85.00)	37(92.50)	139 (92.70)
High **	1(3.30)	1(2.50)	6(15.00)	3(7.50)	11 (7.30)
Lifestyle associated risks					
Smoking status					
Yes	0(0.00)	14(35.00)	8(20.00)	10(25.00)	32 (21.30)
No	30(100.00)	26(65.00)	32(80.00)	30(75.00)	118 (78.70)
Alcohol consumption ***					
Yes	15(50.00)	29(72.50)	26(65.00)	28(70.00)	98 (65.30)
No	15(50.00)	11(27.50)	14(35.00)	12(30.00)	52 (34.70)
Diet****					
Unhealthy diet	22(73.30)	31(77.50)	26(65.00)	27(67.50)	106 (70.70)
Healthy diet	8(26.70)	9(22.50)	14(35.00)	13(32.50)	44 (29.30)
Level of Physical activity*****					
Adequate	9(30.00)	24(60.00)	12(30.00)	10(25.00)	55 (36.70)
Inadequate	21(70.00)	16(40.00)	28(70.00)	30(75.00)	95 (63.30)

In the Ubudehe approach, there are 5 categories (ABCD & E) defined by a set of criteria, from the poorest category (without land, facing difficulties to have food) to the richer people (Rwanda Social Protection Intervention, 2011)

-Middle to high category = monthly income ≥ 65,000 Rwandan francs (RWF) (approximately 66 USD) to ≥ 600,000 RWF (607 USD) and ≥ 780,000 RWF (791 USD) to ≥ 7,200,000 RWF (7,302 USD) annual income.
-Low Category = ≤ 45,000 RWF (46 USD) to ≤ 65,000 RWF (66 USD) per month and ≤ 540,000 RWF (548 USD) to ≤ 780,000 RWF (791 USD) annual income.

**High blood pressure (BP): Defined as the systolic BP of 140 mmHg or higher, and/or diastolic BP of 90 mm Hg, and/or being on hypertensive medications (Barua, Faruque, & Banik, 2019) (
***Alcohol consumption: Taking a drink that contained alcohol for the last 6 months. (Ikeda et al., 2013)
****Health diet: Defined based on how often are fruits and vegetables accompanying routine meals per week Unhealthy diet: Defined as an impression of usually eating meals prepared with too much oil. (Lachat et al., 2013)
*****physical activity participation: Defined as vigorous-intensity exercise activities that cause large increases in heart rate like running, pedal cycle or football for at least 30 minutes continuously 3 times per week.(Trapp et al., 2008)

4.3. Diet and physical activity lifestyle covariates in the study participants

Physical activity classified by WHO category is low or adequate physical activity. The analysis of inflammatory biomarkers versus physical activity revealed that the inflammation is always lower in a group of participants with adequate physical activity but not statistically significant. The diet habits classified by health diet, defined based on how often are fruits and vegetables accompany routine meals per week and unhealthy diet defined as an impression of usually eating meals prepared with too much oil. The analysis of biomarkers versus diet revealed that the HDL-C levels were lower in a group of unhealthy diet feeding habit but not statistically significant

4.4 Biochemical markers of cardiovascular disease risk among study participants

A big percentage of the study participants presented with increased levels of fasting blood sugar (FBS) ≥ 126 mg/d, (55.3%).The difference between the four arms (HIV serostatus and ART treatment) was not statistically significant with p= 0.973. About (54%) were reported with high hs-CRP (ng/ml), (>3000ng/mL) and the highest proportion was observed between ART naïve participants (62.5%). However, the difference in proportion between the four groups was not statistically significant p= 0.459.

Quite often low HDL-C appeared among HIV+ ART-treated participants from 7 to 12 months, (40%) and the differences among the 4 study groups were significant, p=0.012. Classically, HDL-C levels are considered low while <40 mg/dL in men or <50mg/dL in women and high when >60mg/dL.

Elevated levels of triglycerides was not common among the study participants; only 7.3% was reported and the highest proportion being in the HIV- study group (16.7%). Increased levels predominantly appeared among the participants on ART between 7-12 months. Triglycerides are a type of fat (lipid) found in the blood stream. Normal triglyceride levels in blood are less than 150 (mg/dL), and high levels are greater than 200 mg/dl. Total cholesterol was the least reported, only 5.3% of the participants and the serum plasma level of LDL-C was normal in almost all participants 99.3%. There were no a statistically significant differences in hs-CRP, LDL-C, TC, and glucose levels among all study participants. Only 29.3% of NT-proBNP appeared among the participants. NT-proBNP should be on average less that 125pg/mL and interpreted high when >125pg/mL and individuals with this much high levels are said to at risk of heart disease

Table 4.2 : Distribution of respondents in biochemical markers among the study participants

Parameter	**HIV- (n=30) n (%)**	**HIV+ HAART Naïve (n=40) n (%)**	**HIV+ HAART 0-6 months (n=40) n (%)**	**HIV+ HAART 7-12 months (n=40) n (%)**	**Total (n=150) n (%)**	**P-value**
Frequency of Fasting blood glucose status						
Normal	13 (43.30)	19 (47.50)	18 (45.00)	17 (42.50)	67 (44.70)	0.973
Hyperglycemia	17 (56.70)	21 (52.50)	22 (55.00)	23 (57.50)	83 (55.30)	
Frequency of HDL-C status						
Normal	27 (90.00)	31 (77.50)	34 (85.00)	24 (60.00)	116 (77.30)	0.012
Low	3 (10.00)	9 (22.50)	6 (15.00)	16 (40.00)	34 (22.70)	
Frequency of LDL-C status						
Normal	30 (100.00)	40 (100.00)	39 (97.50)	40 (100.00)	149 (99.30)	0.429
High	0 (0.00)	0 (0.00)	1 (2.50)	0 (0.00)	1 (0.70)	
Frequency of Total cholesterol status						
Normal	28 (93.30)	39 (97.50)	36 (90.00)	39 (97.50)	142 (94.70)	0.376
High	2 (6.70)	1 (2.50)	4 (10.00)	1 (2.50)	8 (5.30)	
Frequency of Triglyceride status						
Normal	25 (83.30)	40 (100.00)	39 (97.50)	35 (87.50)	139 (92.70)	0.019
High	5 (16.70)	0 (0.00)	1 (2.50)	5 (12.50)	11 (7.30)	
Frequency of Serum reactive protein (hs-CRP) status						
Normal	17 (56.70)	15 (37.50)	19 (47.50)	18 (45.00)	69 (46.00)	0.459
High	13 (43.30)	25 (62.50)	21 (52.50)	22 (55.50)	81 (54.00)	
Frequency of Serum plasma of NT-ProBNP status						
Normal	15 (50.00)	30 (75.00)	31 (77.50)	30 (75.00)	106 (70.70)	0.05

High	15 (50.00)	10 (25.00)	9 (22.50)	10 (25.00)	44 (29.30)	

4.5 Comparison of mean levels of biochemical markers in study participants

ANOVA t-test was used to compare the means between the four groups of study participants and help therefore to assess the possible effect of ART on cardiac risk biomarker profiles changes. When considering the 3 groups of HIV positive participants, differences in means between the ART naïve and ART treated participants suggest elevated levels associated with ART use; Serum levels of triglycerides were 61.20±18.3SD mg/dl among ART naïve participants compared to 81.50±31.9SD mg/dl and 88.5±43.9SD mg/dl in ART treated participants for 0 to 6 months and 7 to 12 months, respectively (Table 4.3). These differences are statistically significant $p<0.001$. Again, the mean of FBG was found to be 105.10±22.0SD mg/dl in ART naïve participants against 107.00±22.3SD mg/dl and 109.00±19.9SD mg/dl among ART treated participants for 0 to 6 months and 7 to 12 months respectively.. Means were found to increase with longevity of exposure on HIV medication. The serum level of total cholesterol were lower, 119.0±36.6SD mg/dl in ART naïve participants and increased as compared to 143.9±45.9SD mg/dl and 128.1±43.3SD mg/dl among ART treated participants for 0 to 6 months and 7 to 12 months respectively, ($p=0.015$). Similar results were found for the plasma level of HDL-C where the mean was 53.5±22.7SD mg/dl in ART naïve participants compared to 68.7±30.0SD mg/dl and 55.0±25.7SD mg/dl among ART treated participants for 0 to 6 months and 7 to 12 months respectively ($p=0.028$) (Table 4.3).

Table 4. 3: Biochemical characteristics of study participants on ART and control

Parameters	**Reference range**	**HIV- (n=30)** ***Mean±SD [95% CI]***	**HIV+ HAART naïve (n=40)** ***Mean±SD [95% CI]***	**HIV+ HAART 0 - 6 months (n=40)** ***Mean±SD [95% CI]***	**HIV+ HAART 7 - 12 months (n=40)** ***Mean±SD [95% CI]***	
Fasting blood glucose mg/dl	<100 mg/dl	107.4.0 ±21.10 [99.5 - 115.3]	105.10±22.00 [98.0 - 112.1]	107.00±22.30 [99.7 -114.0]	109.00±19.90 [102.5 - 115.3]	0
CD4 measurements in cells/mm3 at enrolment	500 – 1500		419.80±235.30 [344.6 - 495.0]	394.90±210.70 [327.5 - 465.9]	405.70±188.50 [345.4 - 465.9]	0
Serum plasma level of HDL-C in mg/dl	≥40 and < 60 mg/dL	62.80±20.40 [55.2 - 70.4]	53.50±22.70 [46.2 - 60.7]	68.70±30.00 [59.1 - 78.3	55.00±25.70 [46.8 - 63.2]	0
Serum plasma level of LDL-C in mg/dl	≤ 130mg/dl	61.40±23.00 [52.8 - 70.0]	54.30±18.60 [48.3 - 60.2]	59.40±27.60 [50.6 - 68.3]	57.20±20.70 [50.6 - 63.8]	0
Total cholesterol in mg/dl	≤ 200mg/dl	144.6±31.4 [132.8 - 156.3]	119.00±36.60 [107.3 - 130.7]	143.90±45.90 [129.2 - 158.6]	128.10±43.30 [114.3 - 142.0]	0
Serum plasma level of triglycerides mg/dl	≤ 150mg/dl	97.20±48.90 [78.9 - 115.4]	61.20±18.30 [55.4 - 67.1]	81.50±31.90 [71.4 - 91.7]	88.50±43.90 [74.5 - 102.5]	<
Serum level of cell reactive protein (hs-CRP) ng/ml	<1000 ng/ml	2038.20±2881.70 [962.1 - 3114.2]	18060.90±33170.20 [7452.6 - 28669.3]	10160.30±20472.30 [3613.0 - 16707.7]	10707.20±27378.80 [1951.1 - 19463.4]	0
Serum plasma level of NT-pro BNP in pg/ml	< 125 pg/ml	137.60±82.10 [106.9 - 168.3]	92.20±100.80 [15.9 - 59.9]	105.40±148.30 [58.0 - 152.9]	93.00±81.20 [67.0 - 118.9]	0

SD: Standard Deviation, **CI**: Confidence Interval

4.6 Association between HIV status, use and duration of ART and changes in biochemical markers of study participants.

While evaluating the association between HIV status, use and duration of ART, and changes in biochemical markers, a positive association was observed between all biomarkers and the use of ART (Table 4.4). Differences in means was observed in triglycerides, and in total cholesterol with a strong significant positive association between triglycerides among HIV-infected ART-naïve participants versus HIV-infected on ART (61.20 ±18.30 vs. 85.00±38.30), $p<0.01$ and (119.00 ± 36.00 vs. 136.00± 45.00) with a $p<0.04$ in the later (Table 4.4b). HDL-C was also found to be significantly associated with longevity of exposure on ART therapy. Lower levels were found in HIV+ participants on ART for 7-12 as opposed to those on ART for 0-6 months **(**68.70±30 vs. 54.90±25.70), $p=0.02$ (Table 4.4c)

Table 4. 4: Differences in participants according to HIV status (HIV+ Vs HIV-), use (HIV+ ART naïve Vs on ART) and duration of ART (ART 0-6 months Vs 7-12 months)

Table 4.4a: Comparison between HIV+ and HIV- participants			
Variables	**HIV+ (n=120) mean**±SD	**HIV- (n=30) mean**±SD	***p*-value**
HDL-C (mg/dl)	62.80±20.40	59.10±26.90	0.47
LDL-C (mg/dl)	61.40±23.00	56.90±22.60	0.34
Total Cholesterol (mg/dl)	144.60±31.40	130.30±43.00	0.09
CRP (ng/dl)	79.10	61.10	0.04*
FPG (mmol/dl	2.60±0.50	2.60±0.50	0.87
Triglycerides(mg/dl)	77.10±34.80	97.20±48.90	0.01*
Serum plasma level of NT-ProBNP in pg/ ml	69.30	100.20	0.001
Table 4.4b: HIV-infected ART-naïve versus HIV-infected on ART			
	HIV+ ART-naïve (n=40) mean±SD	**HIV+ on ART (n=80) mean**±SD	***p*-value**
HDL-C (mg/dl)*	53.50 ± 22.70	61.80±28.60	0.11
LDL-C (mg/dl)	54.30±2.90	58.30±2.70	0.36
Total Cholesterol (mg/dl)	119.00 ± 36.00	136.00± 45.00	0.04*
CRP (ng/dl)*	2.10± 0.90	1.85±0.90	0.24
FPG (mmol/dl)*	2.50±0.50	2.60±0.50	0.70
Triglycerides(mg/dl)	61.20 ±18.30	85.00±38.30	<0.01*
Serum plasma level of NT-ProBNP in pg/ ml*	92.20±100.80	99.20±118.90	0.75
Table 4.4c: Comparison between HIV+ on ART for 0-6 months vs. HIV+ on ART for 7-12 months			
	HIV+ on ART (0-6 months) (n=40) mean±SD	**HIV+onART (7-12 months) (n=40) mean**±SD	***p*-value**
HDL-C (mg/dl)*	68.70±30	54.90±25.70	0.03*
LDL-C (mg/dl)	59.40± 27.60	57.20±20.70	0.68
Total Cholesterol (mg/dl)	143.90 ±45.90	128.10±6.80	0.12
Triglycerides(mg/dl)	81.50±31.90	88.5± 6.9	0.42
CRP (ng/dl)	39.70	41.3 0	0.77
FPG (mmol/dl)*	2.60±0.50	2.60±0.50	0.82
Serum plasma level of NT-ProBNP in pg/ ml	39.50	41.50	0.69
*Data are mean rank (the data are not normally distributed)			

CHAPTER FIVE: DISCUSSION

5.1 Introduction

To date, the cardiovascular risk associated with metabolic biomarkers that may affect cardiovascular function with use of antiretroviral therapy (ART), has not been well characterized in Rwanda, similarly to many other resource limited settings in African countries particularly in Sub-Saharan Africa (SSA). The findings from this analysis have broadened our understanding that the use of antiretroviral therapy (ART) in HIV+ infected patients, constitute risk factors for cardiovascular disease, a consequence of adverse changes in some biochemical biomarkers, causing dyslipidemia and other metabolic derangements and that lifestyle modification is equally important in the improvement of many cardiovascular risk factors. Analysis of inflammatory biomarkers versus physical activity also has revealed that the inflammation is always lower in the group of participants with adequate physical activity. In addition, analysis of biomarkers versus diet also has demonstrated that the HDL-C levels were lower in a group of unhealthy diet feeding habits. The diet habits classified by health diet, defined based on how often are fruits and vegetables accompany routine meals per week and unhealthy diet defined as an impression of usually eating meals prepared with too much oil.

5.2 DEMOGRAPHIC CHARACTERISTICS AND CARDIOVASCULAR DISEASE LIFE STYLE RISK FACTORS OF THE STUDY PARTICIPANTS

The present study was undertaken to assess the relationship between antiretroviral viral therapy use and changes in biochemical markers of cardiovascular disease risk among HIV-infected adults attending HIV clinics in Kigali, Rwanda. Furthermore, risk factors for heart disease and other CVDs among study participants were also examined to rule out the role ART may play in CVD risk. Notably, more than a half of the study participants were female. Although females may be presenting more frequently in HIV clinics and seeking healthcare services more than males, women between 24-55 years in sub-Saharan Africa are twice more likely to be living with HIV infection than men in the same age group. Our findings concur with those reported by global systematic review (Silva, Peixoto, Luz, Moraes, & Peres, 2019), and by other sub-Saharan Africa data (Dillon, 2016b).

The majority of participants were in young age range between 26 and 33 years, and the mean age was similar among all study groups. Younger age constitutes a lower risk of cardiovascular disease in various studies conducted in Africa, Europe and other countries where increased age is a predictor of cardiovascular diseases mortality, and higher prevalence of metabolic associated diseases (Tsai, Lee, Liu, Tseng, & Chien, 2020), (MOH STEP Survey, 2015). Most study participants resided in an urban setting. Individuals living in most African urban settings are more likely to consume unhealthy foods containing high animal fats with low fiber and vitamins resulting in increased the risk of CVD. Among study participants 16% and 5.3% were overweight and obese, respectively. These figures are higher than those reported in the previous STEP Survey in

Rwanda, which reported 14.3% and 2.8% as overweight and obese (MOH STEP Survey, 2015). Weight gain is common in HIV-infected adults who initiate ART treatment, which if not controlled may result in adipose tissue changes. Studies from developed countries indicate that excess adiposity and HIV infection contribute to metabolic complications (Ministry of Health (Rwanda), 2015. Additionally, this study shows potential difference in patients profiles (BMI, Lifestyles) in relation to cardiovascular risk between the high income countries (HICs) and low-and-middle income countries (LMICs), where in this study patients starting ART are lean and have relatively lower body mass index (BMI). This finding contrast with reported data from high income countries (HICs) (Mounzer et al., 2021). The majority of participants over 60% lived a sedentary life style. Although this figure is higher than that reported in the STEP Survey among the general population, it is similar to that reported in Kigali in the STEP Survey, which showed that only 46.7% were highly active (MOH STEP Survey, 2015). Most of study participants had an unhealthy diet with fat saturated rich animal foods with low fiber and vitamins. These results concur with the findings of the STEP Survey, which showed that most participants did not consume fruit and vegetables (MOH STEP Survey, 2015).

5.4 Association between HIV Status, Use and Duration of ART and Changes in Biochemical Markers of the Study Participants

Our analysis evaluated the association of abnormal changes in biochemical markers of cardiovascular risk among HIV-infected adults on antiretroviral therapy compared to HIV-infected adults who were antiretroviral therapy-naïve. Furthermore, we assessed whether duration of antiretroviral therapy use was associated with adverse abnormal changes in biochemical markers of cardiovascular risk for a duration of 0-6 months

compared to 7 -12 months. This study did not investigate individual ART molecule, individual drug or individual therapy regimen. However, the study showed that ART treated patients had significantly high levels of total cholesterol and triglycerides as compared to ART-naïve patients. The proportion of patients with dyslipidaemia among our ART treated participants was higher than the rate reported in a study from Cameroon and rural Uganda (Pefura Yone, Betyoumin, Kengne, Kaze Folefack, & Ngogang, 2011) (Buchacz et al., 2008). There are suggestions that the magnitude of first-line ART-induced lipid imbalances could vary across populations and settings. Based on LDL-C cut-off values, dyslipidemia in our study was common and higher than that reported in a study from Cameroon (Pefura Yone et al., 2011), Western India (30%) (Kalyanasundaram, Jacob, Hemalatha, & Sivakumar, 2012) and Uganda (6%) (Buchacz et al., 2008) But the prevalence of high LDL-C prior to ART (5%) was almost same as reported in Uganda and Western India (4%) (Indumati et al., 2014) but less than that reported in Cameroon (21%) (Pefura Yone et al., 2011). Similar to the Cameroon study (Pefura Yone et al., 2011) , our study showed no changes in HDL-C levels after ART, which is not in accordance with the findings in Western India (Indumati et al., 2014), which showed significant increase in HDL-C after 18 months of treatment with first-line antiretroviral therapy (ART) regimen.

The association between HIV infection and use of antiretroviral therapy with cardiometabolic disease and biochemical biomarkers of cardiovascular risk has been documented in several settings in developed countries (Mutimura, Stewart, Rheeder, & Crowther, 2007) and in Rwanda (Mutimura, Hoover, et al., 2015). However, according to the literature, this is the first study in Rwanda to explore the relationship between ART

and biochemical biomarkers of CVD risk in patients with HIV infection in comparison with HIV-uninfected adults.

Current data suggest that both HIV infection and antiretroviral therapy are associated with, and may increase the risk of cardiovascular disease, a consequence of changes in specific biochemical biomarkers of CVD risk, mainly associated with dyslipidemia (Silva et al., 2019),(Dillon, 2016b). Our findings are consistent with a systematic review and meta-analysis that reported that among antiretroviral therapy treated HIV-infected adults in sub-Saharan Africa, use of ART was associated with higher LDL- Cholesterol and low HDL-Cholesterol (Dillon, 2016a). Thus, similar biochemical markers of cardiac risk were reported, and the study suggested that the differences in cardiometabolic traits between HIV-infected and uninfected individuals were associated with HIV infected and use of ART (Dillon, 2016a). Similarly, in agreement with our findings, one study in Tanzania reported that HIV-infected adults on antiretroviral therapy who presented a cluster of biomarkers of cardiovascular risk had a higher lifetime cardiovascular risk than HIV-uninfected adults (Kingery et al., 2016).

Comparison of biochemical markers between HIV-infected and HIV-Uninfected adults participants suggest abnormal serum levels of HDL- cholesterol, LDL- cholesterol, total cholesterol and triglycerides. Low HDL levels were observed in ART-treated participants between 7 to 12 months compared to ART-treated participants between 0 and 6 months. HDL-C is considered a cholesterol scavenger that removes excess cholesterol from the blood. Low HDL-C constitutes a risk of hypercholesterolemia and this constitutes an increased risk of CVD. Our findings are similar to one study in Cameroon which assessed the prevalence and characteristics of lipid profile derangements associated with

antiretroviral therapy (Pefura Yone et al., 2011). In this study, it was observed that use of ART was significantly and positively associated with high total cholesterol, LDL-cholesterol and HDL-cholesterol (Pefura Yone et al., 2011)

The changes in HDL-C were also observed in a Tanzanian study (Ombeni & Kamuhabwa, 2016), as well as other studies in the US and Europe (D. S. Nsagha et al., 2015),(Aurpibul et al., 2020) indicating the need for routine clinical monitoring and care of HIV patients to ensure timely prevention and management of any cardiovascular disease associated derangements and risks. Hypercholesterolemia and hypertriglyceridemia were characteristic of dyslipidemia in the present study with higher serum plasma levels in ART treated patients and statistically significant. Similar findings were reported from an earlier study (Zhou et al., 2016),(D. S. Nsagha et al., 2015) There was no statistically significant differences observed for serum plasma levels of HDL-C, LDL-C, hs-CRP, NT-ProBNP and glucose, between ART naïve and ART treated participants. There was a significant positive correlation between triglycerides ($p<0.01$), cholesterol ($p<0.04$) and use of antiretroviral therapy. Similar results were reported elsewhere (N. A. et. a. Nsagha, 2015). In line with our findings, though many studies have failed to demonstrate that ART is the main cause of most derangements in lipid metabolism, a study by Cunha et.al,(2015) demonstrated that PI-based ART combination impairs normal lipid metabolism resulting in increased triglyceride levels and lowered HDL-C (Cunha et al., 2015)

Several developed countries and low- and middle-income countries (LMICs) such as Brazil as well as Rwanda have registered tremendous HIV treatment progress in terms of immunologic and virologic outcome after access to potent ART (Benzaken et al., 2019),

(Ford et al., 2018) The efficacy and safety of ART has substantially improved the well being of people living with HIV infection after the introduction of newer drug classes of antiretroviral medications that are now available to patients and HIV care providers. Thus, constant evaluation and assessment of potential metabolic derangements is required in order to curtail potential adverse impact of HIV treatment and functional cure towards the economic sustainability of patients living with HIV infection.

5.6 Strength of the study

A few studies done before, have only determined the lipids and blood sugar levels among HIV infected individuals; however, according to the literature this is the first study in Rwanda to explore the relationship between ART and biochemical biomarkers of CVD risk in patients with HIV-infection in comparison with HIV-uninfected.

5.7 Limitations of the study

Our study has some limitations. First, it is a cross sectional study which limits temporal trends and patterns in association that would provide more evidence to causation. Second, our participants in the study were conveniently enrolled from Health Care Clinics in urban areas of the City of Kigali province limiting inclusion of diversity of participants from rural areas of Rwanda. Third, our sample size is relatively small, which makes it rather explorative and calling for a lager study to confirm our conclusions.

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND FURTHER DIRECTIONS

6.1. Conclusion

This study has indicated that changes in serum levels of cardiac risk biomarker profiles (total cholesterol and triglycerides) were associated with use of antiretroviral therapy in HIV-infected young adults on treatment in Rwanda. Compared to data from developed countries, these biochemical markers of cardiovascular risk changes were within the upper limits of normal ranges. These findings suggest early alterations in biomarkers of cardiovascular disease risk accompanying antiretroviral therapy (ART) usage. Cardiac risk biomarkers should therefore be more frequently assessed to timely identify their undiagnosed elevated levels in ART-treated HIV-positive patients so that duly management and treatments of CVDs among patients in health centers and HIV clinics can be optimally implemented.

6.2. Recommendations and further directions

6.2.1. Recommendations

There is need for systematic evaluation of cardiac risk biomarker profiles among HIV-positive patients on ART. The current study suggests early alterations in cardiac and other CVD risk markers accompanying ART usage. In countries faced with the scourge of HIV infection, routine clinical monitoring of cardiac risk biomarker profiles should be under the treatment guidelines to be monitored in patients on antiretroviral therapy so that any adverse effects of HIV treatments more specifically CVD, could be optimally managed. Our study suggest the serious need for early detection of lipid profiles as biomarkers of cardiac risk, to effectively monitor how antiretroviral therapy may contribute to cardiac risk progression into CVD, and deter treatment programs in Rwanda and other African countries.

The health service providers in HIV/AIDS clinics providing nutrition support for people living with HIV and on ART treatment needs also to closely monitor the BMI of the HIV+ patients before and during national interventions to prevent them from falling into overweight and or obesity conditions which can lead to elevated lipid profiles and hence contributing to cardiovascular diseases risk.

6.2.2. Suggestions for further studies

It is important to make it clear that due to time and financial constraints, this cross-sectional study did not cover all aspects of biochemical biomarkers of cardiovascular diseases risk, other cardiovascular diseases life style risk factors and the effects of particular HIV antiretroviral (ARV) regimens on the serum cardiac biochemical markers of ART treated HIV positive patients. However, the results of this study call for further research. We recommend the implementation of well controlled cohort studies to assess the effect of ART on potential biochemical biomarkers of CVD risk. Longitudinal studies will shed further light into CVD risk in HIV-infected patients on ART therapy as well as studies using echocardiography in addition to the use of biomarkers need to prove whether cardiac markers alone or in association with other diagnostic procedures may be useful to predict cardiac and/or cardiovascular risk and to allow appropriate intervention

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APPENDICES

APPENDIX 1: STUDY TIME FRAME

The table below summarizes the outlines of all detailed activities scheduled from January, 2018 – June, 2019:

S/N	**Detailed activities**	**Timeline**
1	Proposal development	January-April, 2018
2	IREC approval	August – October, 2018
3	Rwanda ethics committee approval	October- December, 2018
4	Data collection	January- March, 2019
5	Data analysis and thesis development	April-May, 2019
6	Journal Manuscript preparation and submission	May, 2019
7	Mock defense	May, 2019
8	Submission of thesis	June, 2019

APPENDIX 2: ESTIMATED BUDGET AND JUSTIFICATION

No.	**Product Description**	**Unit/Pack**	**Quantity**	**Unit price**	**Total**

				(Frw)	**price(Frw)**
1.	NT-proBNP (Maglumi-600)	100	2	250,000	500,000
2.	hs-CRP (Maglumi-600)	100	2	300,000	600,000
3.	Insulin (Maglumi-600)	100	2	250,000	500,000
4.	Glucose 2×125ml Kit	275	1	10,000	10,000
5.	Total Cholesterol 2×125mL Kit	197	2	17,500	35,000
6.	HDL-Cholesterol 120+40mL Kit	120	2	54,500	109,000
7.	LDL-Cholesterol 120+40mL Kit	120	2	72,600	145,200
8.	Triglyceride(TRIGL)12×201mL Kit	483	1	52,000	52,000
9.	Light check	5mL	2		16,000
10.	Starter kit (1+2)	3 pairs	1	156,000	156,000
11.	Reaction modules	64pc/pc	3	21,900	65,700
12.	Wash concentrate	714mL/pc	2	28,100	56,200
13	Dry tubes 5mL	100/pc	2	6,000	12,000
14.	Biostatistician	1	1	250,000	250,000
15.	Transport for sample shipment (Self)	7 HCs	40 days	10,000	400,000
16	Motivation (Nurses) for recruitment	200	12 Health facilities	33,333/Health facility	400, 000
Total					**3,306,900**
Amount in words:					

Three million three hundred and six thousands nine hundred

Proposal writing and result dissemination

Proposal writing involves expenses in printing, photocopying, binding and purchasing other stationeries such as papers, stapler, note books and pens. Result dissemination involves printing of the required 4 copies of the proposal.

Laboratory Work

This will involve purchase of equipments for safety laboratory practice, reagents like for cardiac marker test kits (BNP and N-terminal pro-B-type natriuretic peptide (NT-proBNP)), and lipid profiles test kits, plus performing echo tests for participants as detailed in the tables above.

Other costs

This includes costs for transport allowances, transport of participants for echocardiogram test and daily encouragement for the nurse assistants during the period of data collection

Contingency

This is 10% of the total budget, intended to cover for any unforeseen expenses, should it arise during the course of the study.

APPENDIX 3: INFORMED CONSENT

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES / MOI TEACHING AND REFERRAL HOSPITAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC) INFORMED CONSENT FORM (ICF)

Study Title: EVALUATION OF BIOCHEMICAL MARKERS OF CARDIAC RISK IN HAART-TREATED HIV-POSITIVE PATIENTS IN KIGALI, RWANDA

Name of Principal Investigator(s): Marcus BUSHAKU

Co Investigators: -

Name of Organization: Regional Alliance for Sustainable Development (RASD Rwanda)

P.O.Box 1544 Kigali

KK 11 Avenue Kamashashi- Mulindi

E-mail: rasdrwanda@gmail.com

Website: www.rasdrwanda.org

Name of Sponsor: Regional Alliance for Sustainable Development (RASD Rwanda) a national non-governmental organization.

Informed Consent Form for: WHO-recommended HAART-Treated HIV-positive patients, men and women with matching groups of age 18−45 years attending HIV care services at a national reference and large inner city, University Teaching Hospital of Kigali (CHUK) HIV/AIDS clinic and the HIV/AIDS clinics of Rwanda Military Hospital (national referral and teaching Hospital) and the surrounding health centers Nyarugunga, Kinyinya, Masaka, Gatenga, Biryogo, Cornum, Muhima, Gihogwe, Gikondo and Remera, also HIV/AIDS clinics in Kigali

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you choose to participate)

You will be given a copy of the signed Informed Consent Form

Part I: Information Sheet

Introduction:

You are being asked to take part in a research study. This information is provided to tell you about the study. Please read this form carefully. You will be given a chance to ask questions. If you decide to be in the study, you will be given a copy of this consent form for your records.

Taking part in this research study is voluntary. You may choose not to take part in the study. You could still receive other treatments. Saying no will not affect your rights to health care or services. You are also free to withdraw from this study at any time. If after data collection you choose to quit, you can request that the information provided by you

be destroyed under supervision- and thus not used in the research study. You will be notified if new information becomes available about the risks or benefits of this research. Then you can decide if you want to stay in the study.

Purpose of the study:

The purpose of this study is to find out whether cART predispose to increased risk of cardiovascular disease among HIV-positive patients on the HAART therapy, and what could be the effects of cART medication on the serum cardiac biochemical markers of these patients. NT-Pro-BNP is the marker of interest; it is a prime marker of heart disease diagnosis. Assessing the cardiac biochemical markers together with high sensitive CRP (hs-CRP) of cART-treated HIV-positive patients, will result into giving insights in cardiovascular disease risk, among the HIV population in Rwanda

Type of Research Project/Intervention:

This is a cross-sectional study, aimed at evaluating changes in biochemical markers of HAART-treated HIV-positive adults in Kigali. Under this cohort, stable HAART treated HIV-positive patients; shall be assessed for their heart function through cardiac NT-proBNP test levels, hs-CRP and metabolic changes , the lipid profile and glucose for those on NNRTIs, NRTIs and PIs based ARV therapy combination (cART) for a period of < 6months, 6 - 12 months and a much longer period of 2 years, against an HIV+ HAART treatment naive as the baseline population with matching age and gender. This study will involve sampling that will need a venipuncture to participants; which is a simple and harmless medical procedure that will be done typically to withdraw a 5 ml venous blood into plain/dry red stopper tubes. The blood collected will be used for

biochemical and inflammatory marker (hs-CRP) analysis. A questionnaire and data extraction sheet/ patient files; will also be used to obtain demographic data, other details of HIV infection, current HAART regimen and comedication. Measurements of blood pressure (BP), weight, height, and waist circumference (WC) along with the body mass index (BMI) will be taken by qualified nurses at the selected HIV/AIDS clinics.

Why have I been identified to Participate in this study?

This study is on WHO-recommended HAART-Treated HIV-positive adult patients, men and women of 18−45 years old. Participation to this study is subjective to HAART naives and stable HAART-Treated HIV-positive patients for a period of < 6 months, 6-12 months and 12-24 months. And eligible Participants should not have active cardiac conditions or posses significant symptoms of heart disease, diabetes and renal problems prior to enrollment. Participation is voluntary, provided you fulfill the eligibility criteria and consent.

How long will the study last?

You will be in this study for a period of 6 months, 4 months of data collection and 2 months for data analysis.

What will happen to me during the study?

A. Provide a brief introduction to the format of the research study.

We are asking you to help us learn more about the cardiac risk among HAART-Treated HIV-positive patients. If you accept to participate in this study, you will be asked to answer few designed questions in form of interview concerning demographic data, you will be asked also to answer questions on cardiovascular risk factors present before

enrollment including family history of hypertension, diabetes and about other habits like of smoking and drinking. Details of HIV infection, comorbidities and comedication will be obtained from your file. You will also be asked to allow withdraw of 5ml venous blood for biochemical markers and inflammatory marker, hs-CRP test analysis.

B. Explain the type of questions that the participants are likely to be asked in the focus group, the interviews, the survey or other relevant approach. If the research involves questions or discussion which may be sensitive or potentially cause embarrassment, inform the participant of this.

What side effects or risks I can expect from being in the study?

A little pain may occur while withdrawing venous blood and small swelling at the needle prick though this is less likely to happen.

Are there benefits to taking part in the study?

Yes. The benefit when participating in this study is that you will be helping in evaluating whether cardiac biochemical markers in HAART-Treated HIV-positive patients are accurate predictors of CVD risk, which may help in forming the basis for the initiation of new strategies for CVD diagnosis among patients in Rwanda. This could be also useful in early detection and monitoring of CVD in HAART-treated HIV-positive patients.

Reimbursements

There will be no reimbursement.

Who do I call if I have questions about the study?

Questions about the study: **Call +250783703222/ +250782726234 Marcus BUSHAKU**

Questions about your rights as a research subject: You may contact Institutional Review Ethics Committee (IREC) **Dr. Jean Baptiste MAZARATI, Telephone: +25078830980,**

Chairman of Rwanda National Medical Ethics Committee (RNEC). RNEC is a group of people that review studies for safety and to protect the rights of study subjects.

Will the information I provide be kept private?

All reasonable efforts will be made to keep your information protected (private and confidential). Protected Information is information that is, or has been, collected or maintained and can be linked back to you. Using or sharing ("disclosure") of such information must follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the uses and disclosures of your personal information. A decision to take part in this research means that you agree to let the research team use and share your Protected Information as described below.

As part of the study, **Marcus BUSHAKU** and his study team may share the results of your laboratory, echo and ECG/EKG information. These may be study or non-study related. They may also share portions of your medical record, with the groups named below:

- The National Bioethics. Committee,
- The Institutional Review and Ethics Committee,
- National institutes of health (NIH), representatives of Regional Alliance for Sustainable Development (RASD Rwanda)

National privacy regulations may not apply to these groups; however, they have their own policies and guidelines to assure that all reasonable efforts will be made to keep your personal information private and confidential.

The sponsor may give your personal health information, not containing your name, to others or use it for research purposes other than those listed in this form. In handling your

personal information, the sponsor, **Marcus BUSHAKU** and associated staff will keep your information in strict confidence, and shall comply with any and all applicable laws regarding the confidentiality of such information.

The study results will be retained in your research record for at least six years after the study is completed. At that time, the research information not already in your medical record will be stored for a period of five years under a password encrypted computer. Any research information entered into your medical record will be kept indefinitely.

Unless otherwise indicated, this permission to use or share your Personal Information does not have an expiration date. If you decide to withdraw your permission, we ask that you contact **BUSHAKU** in writing and let him know that you are withdrawing your permission.

The mailing address is Regional Alliance for Sustainable Development (RASD Rwanda), P.O. Box 1544 Kigali, KK 11 Avenue Kamashashi- Mulindi. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for the purposes of reporting and research quality. You have the right to see and copy your personal information related to the research study for as long as the study doctor or research institution holds this information. However, to ensure the scientific quality of the research study, you will not be able to review some of your research information until after the research study has been completed.

Your treatment, payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You will receive a copy of this form after it is signed.

Part II: Consent of Subject:

I have read or have had read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all of the questions I have at this time. I have been told of the potential risks, discomforts and side effects as well as the possible benefits (if any) of the study. I freely volunteer to take part in this study.

_____	_____	_____
Name of Participant (Witness to print if the subject is unable to write	Signature of subject/thumbprint	Date & Time

_____	_____
Name of Representative/Witness	Relationship to Subject

_____	_____	_____
Name of person Obtaining Consent	Signature of person Obtaining Consent	Date

_____	_____	_____
Printed name of Investigator	Signature of Investigator	Date

APPENDIX 4: URUPAPURO RUGARAGAZA KWEMERA KUGIRA URUHARE MU BUSHAKASHATSI KU BUSHAKE (INFORMED CONSENT FORM)

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES / MOI TEACHING AND REFERRAL HOSPITAL
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC) INFORMED CONSENT FORM (ICF)

INYITO Y'UBUSHAKASHATSI: EVALUATION OF BIOCHEMICAL MARKERS OF CARDIAC RISK IN HAART-TREATED HIV-POSITIVE PATIENTS IN KIGALI, RWANDA

Muraho, Nitwa **……..**, ndi Umuforomo/ Umuganga nkaba nfasha mubushakashatsi bw'umunyeshuri wiga muri kaminuza ya Moi University, mu ishami ry'ubuvuzi ho mu gihugu cya Kenya. Ubushakashatsi bugamije **" Gusuzuma ibyago byo kurwara indwara z'umutima mu bantu babana n'ubwandu bw'agakoko gatera SIDA; bafata imiti igabanya ubukana"**.

Ubu bushakashatsi bugakorwa hifashishijwe bimwe mubipimo by'amaraso (NT-proBNP, glucose, Insulin, hs-CRP & lipid profiles) mubemeye kubushake kugira uruhare muri ubu bushakashatsi mubantu bagipimwa amaraso bagasanganwa ubwandu bw'agakoko gatera SIDA bataratangira imiti, ababana n'ubwandu bari kumiti igabanya ubukana mugihe kingana n'amezi 6, hagati y'amezi 6 na 12 ndetse no mubantu bamaze kumiti igihe kigera ku myaka 2 ; bemeye kubushake gufasha muri ubu bushakashatsi. Urupapuro rwa bimwe mubibazo tuza kukubaza ruzatwara iminota hafi **30**.

Ibisubizo bizagirwa ibanga hagati y'ukora ubushakashatsi n'uwemeye kubushake kugira uruhare muri ubu bushakashatsi. Ibizava muri ubu bushakashatsi bizatuma hamenyekana zimwe mungaruka zishobora guterwa n'imwe mumiti igabanya ubukana ; ku byago byo

kurwara indwara z’umutima hashingiwe kubisubizo bizagaragazwa n’imwe mumihindagurikire ya bimwe mubipimo bizafatwa mumaraso (NT-proBNP, Glucose, Insulin, hs-CRP & lipid profile) hakoreshejwe uburyo bugezweho bwabigenewe. Ibi kandi bizafasha mu kurushaho kwita ku bantu babana n’ubwandu bw’aka gakoko gatera SIDA.

Uramutse wemeye kugira uruhare muri ubu bushakashatsi, turagufata amaraso yo mu mutsi (ml 5) mugacupa kabigenewe ndetse n’ibindi bipimo birimo ibiro byawe, uburebure ndetse nokubazwa bimwe mubibazo bireba ubuzima bwawe busanzwe ; birimo kukubaza niba unywa itabi ndetse n’inzoga Urupapuro rwibibazo ruzatwara iminota hafi 60. Hashobora kubaho ububabare buke cyane mu gihe amaraso aza kuba afatwa’ ariko ubwo bubabare buramara igihe gito cyane. Kugira uruhare muri ubu bushakashatsi ni ubushake kandi ushobora guhagarika uruhare rwawe mu bushakashatsi n’igihe waba waramaze gutangira. Nta ngaruka mbi zabaho ziturutse ku kwanga cyangwa guhagarika kugira uruhare muri ubu bushakashatsi. Wemerewe kumbaza ikibazo icyo ari cyo cyose igihe icyaricyo cyose kandi ni uburenganzira bwawe kubona ibisobanuro

Murakoze cyane

Amazina/Igikumwe…………..…..**Itariki :** ……..….

- Umukono cyangwa igikumwe cy’ugira uruhare mu bushakashatsi cyangwa umuhagarariye
- Mugize ikibazo mwambaza kuri **telefoni** ifite nomero: **+250783703222/ +250782726234**, cyangwa **E-Mail: marcusbushaku@gmail.com.**
- Mushobora no kubaza Urwego rw’igihugu rushinzwe iyubahirizwa ry’amategeko mu
- Bushakashatsi; mukabaza uwitwa:
- **Dr Jean Baptiste MAZARATI kuri Telephone: +250788309807**, Umuyobozi mukuru.

Appendix 5:IREC Approval

MU/MTRH-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2018/213
Approval Number: 0003163

6th December, 2018

Marcus Bushaku,
Moi University,
School of Medicine
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
06 DEC 2018
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Mr. Bushaku,

RE: FORMAL APPROVAL

The MU/MTRH- Institutional Research and Ethics Committee has reviewed your research proposal titled: -

"Evaluation of Biochemical Markers of Cardiac Risk in HAART-Treated HIV-Positive Patients in Kigali, Rwanda".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 3163*** on 6th December, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; hence will expire on 5th December, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat *two months prior to the expiry date. You will be required to submit progress report(s) on* application for continuation, at the end of the study and any other times as may be recommended by the Committee.

Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. You will also be required to seek further clearance from any other regulatory body/authority that may be appropriate and applicable to the conduct of this study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO	-	MTRH	Dean	-	SOP	Dean	-	SOM
	Principal	-	CHS	Dean	-	SON	Dean	-	SOD

Appendix 6:Republic of Rwanda National Ethics Committee

REPUBLIC OF RWANDA/REPUBLIQUE DU RWANDA

NATIONAL ETHICS COMMITTEE / COMITE NATIONAL D'ETHIQUE

Telephone: (250) 2 55 10 78 84
E-mail: info@rnecrwanda.org
Web site: www.rnecrwanda.org

Ministry of Health
P.O. Box. 84
Kigali, Rwanda.

FWA Assurance No. 00001973
IRB 00001497 of IORG0001100

June 24,2020
No.695/RNEC/2020

Marcus BUSHAKU
Moi University School of Medicine Medical Biochemistry Department, Eldoret-Kenya

Your research project: **ANNUAL RENEWAL for "Evaluation of Biochemical Markers of Cardiac Risk in HAART-Treated HIV-Positive Patients in Kigali, Rwanda."** has been evaluated by the Rwanda National Ethics committee.

		Involved in the decision		
			No (Reason)	
Name	Institute	Yes	Absent	Withdrawn from the proceeding
Dr. Jean-Baptiste MAZARATI	Biomedical Services (BIOS)	X		
Prof. Jean Paul RWABIHAMA	University of Rwanda		X	
Prof.Laetitia NYIRAZINYOYE	University of Rwanda	X		
Ass .Prof. Egide KAYITARE	University of Rwanda	X		
Mr. Spencer BUGINGO	Lawyer	X		
Ass. Prof. David K. TUMUSIIME	University of Rwanda	X		
Ass. Prof. Lisine TUYISENGE	Kigali Teaching Hospital	X		
Dr. Darius GISHOMA	University of Rwanda	X		
Sr.Epiphanie MUKABARANGA	Rwamagana Nursing and Midwife school		X	
Dr. Vedaste NDAHINDWA	University of Rwanda	X		
Prof. Claude MUVUNYI	Biomedical Services (BIOS)	X		

After review of the protocol and consent forms, during the RNEC meeting of 13 June 2020 where quorum was met, **we hereby provide approval for the above-mentioned protocol.**

Please note that approval of the protocol and consent form both English and Kinyarwanda version is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrollment of participants
3. All consent forms signed by subjects should be retained on file. The RNEC may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the RNEC in a timely fashion and before expiry of this approval.

5. Failure to submit a continuing review application will result in termination of the study.

6. Notify the Rwanda National Ethics committee once the study is finished.

Sincerely,

Date of Approval: June 13,2020
Expiration date: June 12,2021

Dr. Jean- Baptiste MAZARATI
Chairperson, Rwanda National Ethics Committee.

C.C.
- Hon. Minister of Health.
- The Permanent Secretary, Ministry of Health