THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PUBLIC HEALTH

Risk of Cardiovascular Disease among HIV Seropositive Adults on Protease Inhibitors Containing Antiretroviral Therapy at Adult Infectious Disease Centre at the University Teaching Hospital.

BY

BRIAN CHILUBA

A DISSERTATION SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN EPIDEMIOLOGY.

SUPERVISORS

DR. GERSHOM CHONGWE; MRS. CHOLA .N. DAKA; MR. MUMBI CHOLA

DECLARATION

I declare that this dissertation *(Risk of Cardiovascular Disease among HIV Seropositive Adults on Protease Inhibitors Containing Antiretroviral Therapy at Adult Infectious Disease Centre at the University Teaching Hospital)* presented for the degree of Master of Science in Epidemiology is my own work. It has never been submitted for any degree or examination at any other university. All the sources herein quoted have been acknowledged by complete references.

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CERTIFICATE OF COMPLETION OF DISSERTATION

I, Brian Chiluba hereby certify that this dissertation is the product of my own work and, in submitting it for the Degree of Master of Science in Epidemiology programme, further attest that it has not been submitted to another University in part or whole for the award of any programme.

Signature ……

Date…….

I, Dr Gershom Chongwe having supervised and read this dissertation is satisfied that this is the original work of the author under whose name it is being presented.

I confirm that the work has been completed satisfactorily and is ready for presentation to the examiners.

Signature/Supervisor……..

Date……

Head of Department……

Date……

Department……

CERTIFICATE OF APPROVAL

The University of Zambia approves this Dissertation by BRIAN CHILUBA titled Risk of Cardiovascular Disease among HIV Seropositive Adults on Protease Inhibitors Containing Antiretroviral Therapy at Adult Infectious Disease Centre at the University Teaching Hospital in partial fulfillment of the requirements of a Degree of Master of Science in Epidemiology by the University of Zambia.

SIGNATURE: …… DATE……..

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DEDICATION

To the orphaned child and the oppressed, those vulnerable in society who struggle to get an education. To those who struggle with them to help look after them.

"*It is only the oppressed who, by freeing themselves, can free their oppressors*"

Paulo Freire- Pedagogy of the Oppressed

ACKNOWLEDGEMENT

Attainment of a Degree of Master of Science in Epidemiology is a milestone in one's life. For this reason I thank the almighty God for giving me the strength, good health and peace of mind during my study period and while undertaking this hard but rewarding task.

Special thanks to my all-time friend Theresa Lesa Chisoso, for the support and encouragement

I extend my gratitude to my employer Ministry of Health, Roan General Hospital for granting me the study leave to further my studies.

I sincerely thank my supervisors Mrs. Chola Nakazwe Daka, Dr. Gershom Chongwe and Mr. Mumbi Chola, for their guidance, encouragement, patience and support throughout this study.

Special thanks go to my family, Beatrice Chansa Chiluba, Abigail Chiluba, Bupe Chiluba, Nkumbu Chiluba and Natasha Kafula Chiluba for their unwavering support and constant encouragements to pursue my dreams.

I am indebted to the support rendered by Dr. Phoebe Bwembya, Prof. Kumar Baboo, Prof. Charles Michelo, Dr. Victoria Nankabirwa, Prof. Esther Munalula-Nkandu and Dr. Oliver Mweemba, may God continue to bless you all. I also wish to thank my class mates: Margaret Nzala Munakampe, Uchizi Grace Chirwa, Mulenga Mary Mukanu, Perfect Shankalala, Asha Ramadhani, Nathan Kamanga, Namwaka Mungandi, Ntazana Nana Sinyangwe and the entire class.

I extend my gratitude to all who assisted me in one way or the other.

"epa shili paku leka"

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LIST OF ACRONYMS

AIDC	Adult Infectious Disease Centre
AIDS	Acquired Immune Deficiency Syndrome
CVD	Cardiovascular Disease
HIV	Human Immunodeficiency Virus
MI	Myocardial Infarction
PI	Protease Inhibitors
UNZA	University of Zambia
UNZABREC	University of Zambia Biomedical Research Ethics Committee
UTH	University Teaching Hospital
WHO	World Health Organisation

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CHAPTER ONE

1.0 EXECUTIVE SUMMARY

Introduction: The burden of cardiovascular disease (CVD) in Sub-Saharan Africa is rising in the background of a high prevalence of infectious diseases including Human Immune Deficiency Virus (HIV). The use of certain Antiretroviral Therapy (ART) drugs has been shown to cause dyslipidaemia, with little known about the burden of dyslipidaemia in the presence of ART particularly Protease Inhibitors (PIs) in Sub-Saharan Africa. The objective of this study was to determine the risk associated with the use of PIs- containing ART in the development of CVD among HIV seropositive adult's at the Adult Infectious Disease Centre University Teaching Hospital (UTH), Zambia.

Methods: This was a retrospective cohort study that reviewed records of patients on PIs and non- PIs between 2008 and 2016. The primary end point of the study was CVD and consisted of a total sample of 281, with PI consisting of 112 and 169 for the non PI group. A log-binomial model was used to assess covariates, while Kaplan Meier method for probability of survival to CVD and time to CVD comorbidity were utilised.

Results: The incidence of CVD among PIs was 62% versus 38% for the non-PI ART group. The risk of CVD was found to be 2.3 times higher in the PIs ART group than non-PI ART group. Associated factors include; age, CD4 cell count, type of ART, years since HIV diagnosis and BMI. Kaplan-Meier survival estimates for CVD showed a statistical difference (log-rank; p=0.003) in the two groups (PI and non-PI), were survival was more in the non-PI group, hence a higher cumulative incidence of CVD was observed in the PI group.

Conclusion: Prolonged use of PIs especially older generation drugs containing ART for at least a period of over 2 years were significantly associated with a higher incidence of cardiovascular disease. This study underscores the importance of new screening strategies to be effectively incorporated in ART program among Human immune deficiency virus seropositive adults. Cardiovascular disease has emerged as an important cause of morbidity among seropositive adults. Human immune deficiency virus, antiretroviral therapy and host factors contribute to cardiovascular disease. However, many opportunities exist for developing interventions for optimal screening, treatment and prevention of cardiovascular in Zambia.

CHAPTER TWO

2.0 BACKGROUND

The use of Protease Inhibitors (PI) has been implicated with a high risk of lipodystrophy syndrome in HIV patients leading to a cardiovascular disease (CVD) (Röhrig, 2009). A number of factors have been identified as important causes for CVD in most cross sectional studies (Triant, 2007; Rotger, 2013). The proposed mechanism is that CVD may be caused by an interaction between the HIV infection, immune recovery and antiretroviral medication consequently as well as the sociodemographic and clinical characteristics of the patient (Cioe, (2012). This presents challenges in the management of HIV infections (Alves *et al.*, 2014). According to the 2014 WHO Progress Report, the number of people living with HIV globally was about 35 million by the end of year 2013 and currently, about 50% of all patients with HIV worldwide die from causes considered unrelated to HIV; 11.7 million were in low- and middle-income countries. The 11.7 million people on ART represent 36% of the 32.6 million people living with HIV in low- and middle-income countries. In the same year, 28 million people were eligible for ART and about 1.8 million died due to HIV related complications mostly CVD (Worm et al., 2012). CVD refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial diseases (Bavinger *et al.*, 2013).

The associated metabolic changes that come has a result of the HIV infection, ART and the general factors like those of the general population may threaten long-term survival (Chu, 2011). The management of risk factors for CVD is an increasingly important part of the management of HIV infection (Chu, 2011). Over the past decade or more, the prevalence of these traditional risk factors for CVDs have been increasing in most of the developing world, including sub-Saharan Africa, which has recorded increases in the rates of coronary and cerebrovascular events. It has also been postulated that by 2020, CVDs will be a major cause of morbidity and mortality in most developing nations and this may pose as a major factor for the HIV population on ART (D'Ascenzo 2014). However, factors that might cause this increase have not been explained, including population of people who will be affected more (Ntsekhe & Mayosi, 2009).

Antiretroviral usage has been in existence since 2004 in Zambia but PI ART use is relatively a new treatment concept in the health delivery system. As such information generated locally on the side effects of the medicines involved is based mainly on case reports. This has resulted in the reliance on the outcome of external research (Esposito, 2008).

Cardiovascular disease which includes coronary heart disease, stroke, congestive cardiac failure and hypertensive disease, is steadily gaining ground as the leading cause of death worldwide (World Health Organization, 2015; Cappuccio *et al.,* 2004). In Zambia, there has been paucity of documented prevalence rates of chronic disease such as CVD, but documentation has been done for hypertension and diabetes over the last twenty years (de-Graft Aikins, 2007).

According to Eron *et al.,* (2010), the most probable link between ART and CVD is associated with lipodystrophy or fat redistribution. Since widespread use of ART began, the numbers of HIV positive people with lipodystrophy have increased. Today, lipodystrophy occurs in 30% to 50% of HIV infected people who are on PIs (Lang, 2010). There is debate on which HIV drugs cause lipodystrophy. Studies link it to PIs while others link it to Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (MoH Uganda, 2003; MoH, 2006). It should be noted at the same time that HIV itself may interfere with the way the body processes fat increases CVD risk, at the same time increase in the length of time one has had HIV would also increase the risk for CVD, as well as severity of the virus (Ramussen *et al,* 2010).

Management of HIV comorbidity is very crucial especially in this era when there is a paradigm shift from focus on Communicable Diseases to NCD. Cardiovascular diseases are among the NCDs receiving attention globally as they are the leading cause of death (David *et al.,* 2012). In 2008, 30% of all global deaths were attributed to CVDs (Zhu, 2003. Numbers of deaths caused by CVDs are also higher in low- and middle-income countries, where 80% of all global deaths occurred (Toma, 2001). It is also estimated that by the year 2030, over 23 million people will die from CVDs each year (Drechsler, 2011). CVD management for people on ART, therefore, should be prioritized regardless of whether or not there is a link to PIs or any other factors. An inclusive management should be aimed at improving the life expectancy of seropositive HIV persons (Ding & Andraca-Carrera, 2012).

Some of the studies investigating ART have found an association between cumulative use of ARVs and cardiovascular disease, and others found an association with recent exposure (Rotger *et al.,* 2013; Lang, 2010). A meta-analysis based on three observational studies indicated that recent PI use was associated with Myocardial Infarction (MI) with an odds ratio of 2.13 (Worm *et al*., 2010). There was a caution, however, that this combined estimate is based upon studies that did not meet important criteria for quality (Bavinger *et al.,* 2013). In contrast to these findings, Capilli *et al*.,(2011) conducted a meta-analysis of RCTs and found no association between nelfinavir and risk of MI (point estimate not reported) or between indinavir exposure and risk of MI (0.7, 95% CI: 0.1, 7.75).

Worm *et al.,* (2010) found a significant increase in the risk associated with cumulative PIs classes of lopinavir and indinavir. These results were based on only two studies and their quality was fair. Caution for this study was put forward against interpreting these findings as conclusive. However, other studies suggest that HIV appears to independently increase the risk of cardiovascular disease via elevated cytokine levels, chronic vascular inflammation, and endothelial dysfunction (Esser *et al.,* (2013).

With the aforementioned features and circumstances, virally mediated vascular effects may then be compounded by lipid or metabolic changes caused by traditional risk factors of CVD, HIV infection and antiretroviral use, (Tesoriero *et al.*, 2010). For example, abacavir (Ziagen) has been widely investigated for direct cardio toxicity. In addition, some experts suggest that extra consideration be paid to current and previous antiretroviral exposure (i.e., abacavir, protease inhibitors) when evaluating patients for CVD (Sankatsing *et al.,* 2009).

A study was done in Nigeria on CVD risk factors among HIV-infected Nigerians receiving highly active antiretroviral therapy by Muhammad (2013). This study found a significantly higher burden of some CVD risk factors (such as hypertension, hypercholesterolemia, obesity and metabolic syndrome) among highly active antiretroviral therapy (HAART)-treated HIV patients than their HAART-naïve counterparts. Trends in published research findings on this subject have shown a mixture of association and lack of association between ART use and CVD risks.

Recent studies suggest that some types of ART may be associated with increased risk of CVD (Bavinger *et al.,* 2013; Choi, 2011), a cause for concern given that people living with HIV may take ART for decades. The mechanisms causing an increased risk of cardiovascular disease are unclear, but according to a review by the Antiretroviral Therapy Cohort Collaboration of 2010, "may relate to dyslipidaemia, insulin resistance, diabetes mellitus, inflammation, impaired fibrinolysis, factors specific to antiretroviral medications, or combinations of these factors." The authors further speculate that both HIV and ART might be associated with many of these risk factors (Antiretroviral Therapy Cohort Collaboration, 2010). They also stressed that it is important to place their results within wider contexts and to understand potential confounders. "Despite being a risk factor for CVD, ART use has increased the quality and length of life of HIV people, it is possible that the use of ART increases life expectancy and hence increases the average age of those taking ART in comparison to the reference group, which may lead to confounding of results" (Pearce *et al.*, 2012).

The conclusion is that the reasons for the excess risk of CVD among HIV-infected people are not very well known and require considerable attention as CVD is likely to be one of the major conditions to be confronted in the future among seropositive people. However, in a study by Bavinger *et al.*, (2013), they noted some overlap with a study by Islam *et al.,* (2012). Bavinger study highlighted comparisons of the risks of MI among HIV-positive people exposed to different regimens of ART while the study by Islam *et al.,* (2012) had a different scope that also included comparisons between HIV-positive and HIV-negative subjects. Islam *et al*.,(2012) observed an increased risk of CVD events with exposure to PIs as a class, lopinavir, and abacavir, and Bavinger *et al*.,(2013) analysis additionally revealed an increased risk in myocardial infarction (MI) from exposure to indinavir. Bavinger findings were based on methodological choices regarding pooling or summarizing estimates that differed from those of Islam who specifically looked at one form of ART and in one study without comparing with others studies.

CHAPTER THREE

3.0 RESEARCH FOCUS

3.1 Problem Statement

HIV is known to be associated with increased risk factors for CVD among HIV infected individuals. Management of HIV patients on ART has been associated with exacerbation of cardio-metabolic complications (Bavinger, 2012). Therefore, there is an increased morbidity and mortality due to CVD among patients treated with ART (Islam *et al.,* 2012). The increased morbidity and mortality due to CVD among HIV/AIDS patients on ART poses a new challenge in HIV/AIDS management. Despite present awareness on the rising cardiovascular events among HIV patients on ART, little is known on the extent of cardiovascular risks among HIV patients and their contribution to the overall risk in development of a CVD in our setting. Cardiovascular risk factors attributed by HIV disease and its treatment are modifiable. Inadequate knowledge on the magnitude of cardiovascular risk factors among HIV patients in Zambia is among the limiting factors for intervention.

The scenario in Zambia is that about 1 million people are living with HIV/AIDS and 200,000 of them require ART. Zambia is experiencing a generalized HIV/AIDS epidemic, with a national HIV prevalence rate of 13.3%(CSO,2015) Considering the fact the number of those accessing protease inhibitors type ART keep on getting bigger, this could at the same time be compounded by the current trend of the Ministry of Health in Zambia current step of introducing a new PI drug in its guideline, called Atazenavir as a daily dosage. Since launching of ART treatment, the number of patients enrolled onto ART program is increasing in many developing countries including Zambia, but many studies done across have shown that ART have shown a morbidity of about 20% (Guaraldi, 2010). This loss could be attributed to mortality due to CVD.

3.2 Rationale

An increase in cardiovascular attributable morbidity and mortality among HIV patients justifies the compelling need to understand cardiovascular risks attached with HIV management options (Tesoriero *et al.*, 2010). This study was conducted because of paucity of information on the magnitude of cardiovascular disease risk factors among HIV patients in Zambia. The results of this study provided knowledge on the magnitude and distribution of the cardiovascular risks factors in relation to HIV disease and its management. This knowledge is expected to improve care of HIV infected patients, particularly those with increased risk of cardiovascular morbidity, bearing in mind that most of cardiovascular risk factors are modifiable. This study was also able to form a baseline for further intervention studies aimed at modifying cardiovascular risk factors among HIV patients.

Several studies on CVD risk factors among HIV/AIDS patients on PI have been reported in the western literatures but there is paucity of information on this subject in Zambia, which has the greatest HIV/AIDS burden and increasing access to ART. Identification of the risk factor and appropriate intervention were necessary would allow assessment of impact of PI on CVD risk (Pearce *et al.*, 2012).

In Zambia just like in many other countries, there is increasing evidence of a changing disease profile from infectious diseases to non-communicable chronic diseases, including CVD (Guaraldi, 2010). Even after incorporating recent estimates for the spread of HIV/AIDS, projections of mortality and burden of disease suggest that by year 2030, CVD will become the leading cause of death in low-income countries in Africa, contributing 13.4% of total deaths, compared with 13.2% from HIV/AIDS, (Di Angelantonio & Sarwar, 2009). This study contributes to existing knowledge in overall HIV/AIDS management. This study provides also a baseline data which necessitate the need for assessment and further research regarding CVD manifestation among HIV seropositive and if any other associated cause exist.

3.3 Hypothesis

Prolonged use of PI's regimens for at least 2 years increases the risk of CVDs for people aged between 15 and 49 who are on ART.

3.4 Objectives of the Study

3.4.1 Main Objective

To determine the associated risk of CVD among HIV seropositive persons who are on PIs at the AIDC, UTH.

3.4.2 Specific Objectives

1. To determine and compare the influence of social demographic factors and clinical characteristics in the development of CVD's between the PI and Non PI groups
2. To establish the incidence and predictors of CVD among those on PI and Non PI ART groups
3. To determine the proportion of HIV seropositive patients with an enhanced risk for CVD from the time of commencement of ART
4. To compare the rate of survival between the PI and Non PI groups to CVD and their CVD comorbidity

3.5 A Theoretical Frame work showing the Development of CVD

The Grunfeld conceptual framework in figure 1.6 shows the development of CVD of those exposed to ART. This frame work was adapted to suit this study. In this theoretical framework the rationale was that that HIV increases the risk for a CVD. This risk may actually be doubled if one is on specific type of ART because both HIV and a Specific type of ART have the same effect on the metabolic syndrome. The effects is via elevated cytokine levels, chronic vascular inflammation, and endothelial dysfunction causing dyslipidaemia, insulin resistance, glucose increase, inflammation, lipoatrophy, lipohypertrophy and body composition.

Considering that HIV and a particular type of ART may both lead to a CVD, HIV in this study was considered to be a confounding variable. This confounding was taken care of by collecting information on years since HIV diagnosis. The confounding was also taken care of in analysis by adjusting for years since one has been diagnosed with HIV. The other cardinal aspect of this theoretical framework is that there are already established risk factors for a CVD which are divided into underlying social economic factors, common modifiable risk factors and common non-modifiable risk factors. This is why one of this study's objectives was to try

to establish the effect of socio-demographic factors and clinical characteristics of the participants on the risk of CVD.

This framework is very important because it tries to explain that there are already established risk factors for the general population irrespective of HIV status .It also points out that CVD may be facilitated by how long one has been HIV positive and at that CVD may further be enhanced by specific type of ART. Cardiovascular disease in this framework will referred to Stroke, heart disease and arterial disease (hypertension, diabetes and others). Framework originally adapted from the study "Contribution of metabolic and anthropometric abnormalities to cardiovascular disease risk factors", (Grunfeld, *et al.,* 2008).

The contribution of traditional risk factors has capital importance because they may occur with increased prevalence in HIV-infected patients (e.g. smoking). Human Immune Deficiency Virus and ART (PI's) independently affect many of the mediators of traditional cardiovascular risk factors. The bidirectional arrows indicate associations, but there is not yet adequate proof of causality. The orange arrow between body composition and cardiovascular disease indicates that body fat may have a direct effect, in addition its effects on other mediators of traditional cardiovascular risk factors. ART (PI's); FFA (Fatty free acids).

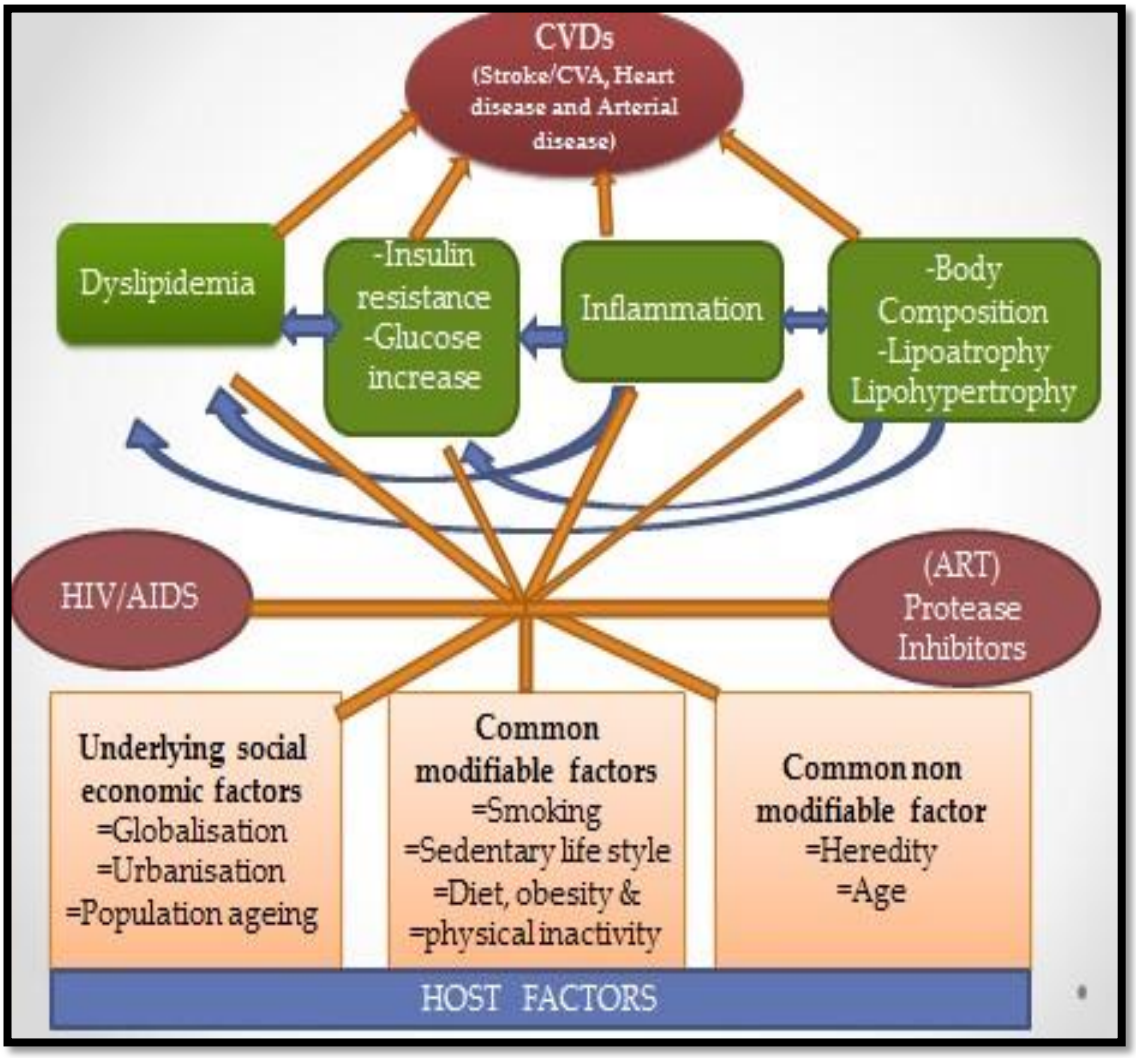

Figure 1 The (Grunfeld, *et al.*, 2008) adapted Theoretical framework

CHAPTER FOUR

4.0 METHODS

4.1 Study Design and Setting

This study was a retrospective cohort, utilizing a quantitative method. The cohorts of those who were exposed to PI's were selected from the HIV seropositive people using a systematic sampling procedure, with a sampling interval of 3. While the cohorts of those who were unexposed (non PI) group were chosen with a sampling interval of 2 using systematic sampling procedure. The sampling intervals were arrived at by dividing the respective sample size for PIs and non PIs into the study population sample estimates. These two groups were consistently followed up from 2008 to 2016, survival analysis was done and CVD was considered a failure in the analysis. The rate of CVD was compared between those exposed to PI's and unexposed group (non PI).

The study was carried out in Lusaka at the Adult Infectious Disease Centre, University Teaching Hospital (AIDC, UTH). AIDC is a research and treatment centre within UTH. AIDC has developed numerous HIV protocols in the study of HIV. The area was chosen partly because UTH is highest referral hospital and attends to a larger population of people in Zambia. The other reason is that Lusaka is the most urbanized city in Zambia. Further, there are a lot of people accessing ART in Lusaka due to the fact that it has one of the largest populations in Zambia and has a high HIV prevalence. It can be argued that the hospital to some extent can give a rough picture of the national composition of Zambian seropositive accessing ART. Furthermore, UTH had all subjects of interest that is, type of ART being accessed and different economic status of people accessing ART.

4.2 Study Population and Eligibility

The study population was comprised of HIV seropositive adults aged 15 and above. It was assumed that this group would provide accurate information on the risk of CVD due to PIs. The current population of HIV seropositive adults at AIDC who are on ART was 121,900 by the time of study. The age 15 was chosen because the WHO commencement of ART for adults is age15.All HIV seropositive adults aged 15 and above years who are or have been on PI's between 2008 and 2016 at AIDC, UTH were included. Seropositive HIV adults aged 15 and

above who have been on ART other than PI's between 2008 and 2012 at AIDC, UTH were also included for the unexposed group. Adults on ART who had already developed CVD prior to enrolment in the study were excluded from the study.

4.3 Sample Size Estimation and Assumption

The sample size was determined using the formula below.

$$n = \frac{(z_1 + z_2)^2 \times 2p(1-p)}{(p_2 - p_1)^2} \times \frac{c+1}{2c}$$

Table 1 Sample Size Estimation

Proportion who develop disease in unexposed group (p2)	0.01
Proportion who develop disease in exposed group (p1)	0.1
Average of p1 and p2 (p)	0.055
Prevalence of exposure	0.4
Ratio of unexposed to exposed (c)	1.5
z1 (95% confidence)	1.96
z2 (90% power), Alpha=0.05	1.28
Number in the exposed group (n)	112
Number in the unexposed group (cn)	169
Total sample size (n+cn)	281

The assumptions for Sample size in (Table 1) were arrived at by considering findings from Data collection on adverse events of Anti-HIV Drugs (DAD). DAD is a prospective multi cohort study that focuses on the early recognition of adverse events, amongst which are cardiovascular problems and liver and renal diseases that could result from HIV treatment with antiretroviral agents. Specifically the assumption was obtained from one of the 2012 DAD study on the Evaluation of HIV Protease Inhibitor Use and the Risk of Sudden Death or non-haemorrhagic Stroke, a study done by Worm *et al.,* (2012) in Australia. In this study it was found that the overall prevalence of diabetes mellitus in this DAD study was 1.1% in patients

not currently receiving antiretroviral therapy to 4.3% in patients receiving PI. This is consistent with other studies which have shown an association between impaired glucose tolerance, diabetes mellitus and use of PI. This study however acknowledged that other studies have reported the prevalence of diabetes in PI treated HIV patients to be in the range of 2–8%, though other few studies have reported a 10% increased prevalence of hypertension in PI treated patients or in conjunction with lipodystrophy (Hewitt *et al.*, 2001).

4.4 Sampling Procedure and Data Source

In this study, systematic sampling procedure was used to select both exposed (those on PI's) and unexposed (those not on PI's). Participants were assigned to each group. This was done after assigning numbers from 1 to the total number of those on PI's (112) and 1 to a total number of those not on PI's (162) giving each element in the population a chance to be chosen. Thereafter, an interval was calculated to pick numbers from 1 to 112 in the exposed and 1 to 168 in the unexposed group. The sampling interval in the exposed group was 281/112=3 and in the unexposed 281/168=2.

Data source was patients' files from AIDC, UTH who are the owners of data. This data is collected routinely. Data was collected using a checklist suited to answer the research objectives and variables for the study. The sources are important because they can give some empirical evidence for the topic under study. Data was drawn from a stored computer software records and the actual examination patients' files. Computer stored software data is collected as patients attend antiretroviral therapy clinic. Patients' records are for routine patient examination for any other conditions and particularly those relating to ART and CVD. Computer stored data was merged with patients' file data in order to have the variables well synchronized.

The information recorded in the file include some routine social demographic characteristics like age, gender, height and weight clinical characteristics of the patients like the type of ART one is taking, how long they have been on ART, CD4 count and development of any diseases including CVD. The centre captures a spectrum of data from patients, but this research only utilized some parts of that data.

4.5 Data Collection

A structured checklist was used to gather information on factors that influence and/or contribute to patients' development of CVD. The checklist was adapted from a study by Cioe (2012). The tool was specifically chosen from this study as the features were related the objectives of this study were. The Cioe's study looked at CVD risk factors and tried to estimate the actual risk in HIV-Infected patients. This feature did not differ significantly from this study. However, the checklist was modified to suit the characteristics of this study. The tool contained information on sociodemographic characteristics captured routinely from AIDC. Certain information however was collected as AIDC does not capture information on certain socio-demographic data like occupation, smoking, alcohol and education. The tool captured information on clinical characteristics; information on both sociodemographic and clinical characteristics was collected as both may have had an effect in defining development of CVD. In order to preserve the reliability of the data collection instrument, it was tested prior to the study. Data collected was entered and stored in STATA software version 14. The checklists (hard copies) were kept in lockable drawers both before and after using them. The completed checklists were immediately checked for completeness and a single entry system was used for data entry. To ensure data quality, only the researcher and well-trained research assistants administered the questionnaire.

In order to ensure quality control, research assistants were chosen based on their ability to perform the required tasks. Before data collection, research assistants were trained on the requirements of data collection. This included procedures for monitoring how data is documented. During data collection, was ensured that self-checks were performed by checking for completeness and correctness of data recorded. Screening for data abnormalities check was done on daily basis after checklists collection.

4.6 Study Variables

4.6.1 Dependent Variable and Explanatory Variable

Dependent variable

The primary outcome was CVD, which was measured as a binary outcome variable. The basis was just to establish if one had a certain form of CVD as illustrated in Table 3.11 or not and not necessary trying to explain the different forms of CVD.

Explanatory Variables

Socio-demographic factors: these included age, gender, area of residence (certain variables in this category are not captured in routine collection of data from AIDC, like education level alcohol, smoking occupation and others). Therefore the research being a review of medical records failed to collect information on those variables, but this did not affect the scope of the research profoundly.

Clinical characteristic- these helped to explain the quality of life of study participants. When assessing development of CVD which is related to the quality of life of individuals with HIV/AIDS and its association with ART, one needs to identify the individual's clinical characteristics and which phase of the infection the individual is experiencing as well as the type of ART one is undertaking.

It is understood also from various literature and as demonstrated in theoretical framework that CVD being a developmental disease, there are many confounding variables at play as shown in the table below (Table 2), HIV is known to cause CVD but the effect may be doubled by specific ART regimens (hence Duration of HIV being a confounding variable as demonstrated in the theoretical framework as well, hence there was a need to collect information on such variables to avoid over estimation or underestimation of the risk of CVD from PI's.

The basis for measurement is that variables were left in their natural settings, independent continuous variables were not categorized to avoid type 1 error, loss of power, residual confounding and bias except for CD4 cell count which was categorized for determination sake (as it is usually categorized for clinical reasons and disease status).

Table 2: Operational Variable Framework

Variable	Operational definition	Indicator	Variable Measurement
Dependent Variable			
CVD	Presence of a CVD in patients file as indicated by the medica officer	Absent=0 Present=1	Binary
Independent Variables			
Age	Adults ranging from 15 years and above and as defined by WHO for commencement of ART for adults (15 years and above considered adults)	Age from 15 years and	Continuous
Residence	where participant stays (urban or rural)	Urban rural	Binary
Gender	State of being male or female	Female Male	Binary
ART	Being on Class PIs or not	Non PIs PIs	Binary
CD4 cell Count	Having a Low or High CD4 count	CD4 ≥350 CD4 <350	Binary
Years since HIVdiagnosis	How long has someone bee sick wit HIV	years	Continuous
Entry date	Date of entry into the study	Year	Continuous
Origin	Date started ART	Year	Continuous
Duration of infection	How long has been infected sincepositive diagnosis	Years	Continuous
Weight	Latest body weight of an individual on ART	Kg	Continuous
Height	Height of individual	M	Continuous
diastolic blood pressur	Average diastolic pressure	mmhg	Continuous
systolic blood Pressur	Average systolic blood pressure	mmhg	Continuous

4.7 Data Management

Each participant was assigned a unique research Identity (ID) number. A master sheet with participant names and matched study ID numbers was kept in a locked cabinet. All study data sheets were kept in a locked cabinet, and only the Principal Investigator (PI) had access. The PI entered the de-identified data into a database. A research assistant was trained and entered all de-identified data into a second identical database. Double entry was performed to ensure the accuracy of the data to be analysed. The two data sets were merged, and correlations were done on each variable to identify errors in data entry. Any variable that had a correlation of one (1) was identified. The data sets were then reviewed line by line, and errors in entry were corrected. Correlations were rerun between the two data sets to ensure that all errors were corrected. A clean data set file was established, and this data set was used for the remainder of the data analyses for the study.

4.8 Statistical Analysis

After quality control procedures were done, data was entered in Stata version 14 for analysis. Descriptive statistics were first done to observe the basic characteristics of the variables. Normality tests was done to assess how well data approximated by the normal distribution using histograms and Shapiro-Wilk W-test. For normally distributed continuous variables means and standard deviation were reported. If not median and interquartile range was reported. T-tests were done for mean difference for the two ART groups, otherwise Wilcoxon rank sum (Mann-Whitney) test was used to establish if there were significant median differences between the two ART groups on some continuous measure if assumption were satisfied.

For categorical variables, frequencies and percentages were calculated. Proportions were compared on both absolute and relative scales between the PI and non PI groups. The outcome which was a CVD was captured in binary and a time-to-event variable. Continuous variables that were right skewed were log-transformed to obtain more symmetrical distributions. The Pearson's Chi-square test was used to examine independent categorical variables with the outcome variables where the assumptions of the chi square were met, and if not Fisher's exact test was used.

Predictive risk equations were developed based on log binomial models and cox regression. Underlying time scale was the retrospective follow-up from baseline, until time of the event, the time of CVD, time of last follow-up visit in the study or 1 January 2016, whichever occurred first.

General linear model (GLM) of the log binomial family with a log link (obtaining direct risk ratios or Relative Risks (RRs) was used. This was so to avoid overestimating the risks. Utilizing log binomial models is also useful for the estimation of absolute risk reduction and the other analogue such as numbers needed to prevent as well as their 95% CIs interactions analysis was primarily conducted on a multiplicative scale. Univariate Relative Risk analysis was done to assess the relative contribution of each explanatory variable to the outcome variable. The multivariable model included parameters significantly and not significantly associated with CVD, at a level of P= 0.05 in the Univariate model.

The risk of CVD between PI exposure group and unexposed group in a cohort of HIV-infected patients was estimated using a time-dependent Cox proportional hazards model adjusting for the major CVD risk factors. Survival analysis was used for both comparison between those exposed to PI's and unexposed group and comparison was done within the two groups. Censoring was done for participants that could have dropped out due to a number of factors such as death, transfer or any loss to follow up and also for participants that reached the end of the study without developing CVD, but for those that developed a CVD before the end of the study were detected as failures. A corresponding Kaplan Meier curve was done to compare CVD of the exposed and the unexposed group. The Main goal was to estimate and compare survival experiences of different groups (exposed to PI's and not exposed to PI's). It also described the cumulative survival function: the hazard function was applied also to ascertain the probability of those that may have survived to CVD.

To establish if there is an exponential hazard or Weibull hazard between the PI's exposed group and non-exposed PI's group, a Cox Regression was modelled to establish the effect of predictor variables and time to CVD. In order to remedy attrition (selection bias) which is a major problem in a cohort study, in data analysis, sensitivity analysis was used for a loss to follow up and missing information. Selection bias is not remedied by analytic procedures such as restriction or survival analysis. Though sensitivity analysis does not

remedy selection bias from differential attrition, it may help in gauging its potential magnitude either towards the null or away from the null (overestimation and underestimation) (Röhrig *et al.,* 2009).

Sensitivity Analysis is a technique used to determine how different values of an independent variable will impact a particular dependent valuable under a given set of assumptions (dealing with bias). This is to deal with overestimation or underestimation of the problem that is if the loss to follow up will be more than 10%. This took into the best and worst scenarios. (Röhrig *et al.,* 2009).

4.9 Validity and Reliability

Two research assistants were trained to ensure efficiency. The instrument was pre- tested in a pilot study to determine if the desired information was achieved. Contents of the checklist were reviewed by experts and the research supervisor. An extensive editing and review of literature was conducted before compiling the checklist. Administering the same checklist throughout the study was done to eliminate biases and minimize study errors. A pilot study was conducted to measure reliability of the instrument.

A pilot study was conducted at Roan General Hospital in Luanshya. The pilot study was conducted on 10% of the total sample (10% of exposed= 12 and unexposed= 18). This was to test the tools for validity, reliability and to avoid bias. It assisted in obtaining clarity and guided the main study's direction. A pilot study also enabled adjustments to be made to the checklist before the major study was carried out.

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4.10 Ethical Considerations

As the research methodology required direct contact with human participants' records, there were ethical issues to be considered. The study involved itself with reviewing files of adult ART patients and this is sensitive as it may lead to knowledge of one's HIV status and other confidential information. Information obtained on participants' records remained confidential and was kept in a safe place under lock and key. The checklist will be kept for a period of five years before disposal.

Anonymity of participants' files was maintained by not using names but codes on the data collection form.

Privacy was maintained by allowing only research assistants to review the files, after they were received appropriate training on the importance of confidentiality and keeping the reviewed information to themselves.

Autonomy: a fair selection of participant's records was ensured.

Beneficence: The study added to the body of knowledge in helping the development of research towards improving the quality of health care and instituting holistic management for those on ART in the prevention of CVD. The study helped in coming up with effective strategies of addressing CVDs among those on ART.

Justice-Participants' records had an equal chance of being picked for the study. Records were not exposed to any person other than research assistants or to anything else that may bring about discomfort or harm to AIDC as an institution or later on to patients that may result in physical, psychological or exploitation.

To ensure a fair selection of participant's records systematic sampling was used to select respondents so that there is fair selection and every file is accorded a chance for participation.

Ethical clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) and approval to conduct the study at AIDC was sought from the UTH.

CHAPTER FIVE

5.0 RESULTS

5.1 Basic characteristics of study participants

A total sample consisting of 281 seropositive adults aged between 17 and 69 participants were recruited from AIDC at the University Teaching Hospital, Zambia from 2008 to 2016. This consisted of two cohorts, the PI and the non PI cohorts. There were 112 and 169 sample distributions respectively. The average follow-up time was 4.8 years (interquartile range 3.0–7.0), for a total of 106.821 person-years. Different types of a risk for CVD among the study cohorts were categorised as those on PI and those who were Non-PI.

5.2 Sociodemographic and clinical characteristics of study participants

The demographic characteristics of the study participants are shown in Table 3. Of the patients records reviewed overall female constituted 157 (55.87%) and male were 124 (44.13%) of the study participants. The overall mean age of the respondents in years was 43.12) with a standard deviation of (10.58). Most of the participants were urban based 230 (81.85%) compared to those from rural parts 51 (18.15%).There was no statistical difference (p=0.972) in the distribution of different standardised age groups among the PI and non PI ART groups. There was also no statistical difference (p=0.261) in the overall distribution of males and females. The proportion of residence was significantly different (p =0.002) in the two ART groups with more urban representation among the two ART groups than rural presentation. Table (3) also shows no statistical difference in the distribution of weight between the PIs and non PIs (p<0.001). CD4 cell count showed a statistical difference (p=0.006) with more participants having a CD4 cell count of greater than 350 in both ART groups. Years since HIV diagnosis, age, systolic blood pressure and diastolic blood pressure showed no mean difference among the PIs and non PIs groups.

Table 1: Characteristics of participants in the protease and non-protease inhibitors containing ART retrospective cohorts of HIV seropositive adults during follow-up from 2008 through to 2016 for CVD risk (n=281)

		Frequencies [n (%		
Characteristic Variable	Overall	PI ART group	non PI ART group	chi-squared p-value
Standardised age groups				
17-33	56 (19.93)	22 (19.64)	34 (20.12)	0.972|[a]
34-41	52 (18.52)	23 (20.54)	29 (17.16)	
42-46	59 (21.00)	23 (20.54)	36 (21.30)	
47-52	57 (20.28)	22 (19.64)	35 (20.71)	
53-69	57 (20.28)	22 (19.64)	35 (20.71)	
Sex				
Male	124 (44.13)	54 (48.21)	70 (41.42)	0.261|[a]
Female	157 (55.87)	58 (51.79)	99 (58.58)	
Residence				
Urban	230 (81.85)	82 (73.21)	148 (87.57)	0.002|[a]
Rural	51 (18.15)	30 (26.79)	21 (12.43)	
Body Mass Index(kg/m2				
<18.5 (underweight)	32 (11.39)	6 (5.36)	26 (15.36)	<0.001|[a]
18.5-24.9(normal weight)	128 (45.55)	41 (36.61)	87 (51.48)	
25-29.9(overweight)	72 (25.62)	33 (29.46)	39 (23.08)	
>30(obese)	49 (17.44)	32 (28.57)	17 (10.10)	
CD4 cell count				
<350	75 (26.69)	29 (25.89)	46 (27.22)	0.006 |[a]
>350	206 (73.31)	83 (74.11)	123 (72.78)	
HIV Diagnosis (years)				
mean(SD)	9.66 (1.88)	9.54 (1.70)	9.73 (1.99)	0.801|[b]
Age				
mean(SD)	43.12 (10.58)	42.66 (10.52)	43.43 (10.64)	0.726 |[b]
SBP				
mean(SD)	126.63 (14.54)	127.7 (15.59)	125.92 (13.80)	0.156 |[b]
DBP				
mean(SD)	86.44 (22.96)	85.40 (16.22)	7.14 (26.53)	0.732 |[b]

a=Chi-squared P-value b= T-test p-value; SBP- Systolic blood pressure; DBP- Diastolic blood pressure

5.3 Influence of sociodemographic and clinical characteristics of CVD development

We studied the association between various variables (Table 4) and the development CVD in the two ART groups. The data shows that the distribution of standardized age was different in the two ART groups (p=0.003). There were significantly (0.002) higher proportion (70%) of CVD cases in PI group with CD4 <350 cells/ul compared to non-PI group. There is also an indication of an association of BMI categories of normal weight, overweight and obese. Being above the normal weight increases the risk for CVD for both ART groups, but more so in the PI group (p=0.004). There was no association of HIV since diagnosis and development of CVD, whether one was HIV for less than 7 years or HIV for longer than 7 years. Belonging to either category did not seem to influence CVD development (p=0.734). Systolic blood pressure (p=0.459) and diastolic blood pressure (p=0.232) does not seem to influence ones chance of developing CVD.

Table 4: Association of the demographic and clinical characteristics with CVD development

Characteristic Variable	Frequencies [n (%)] PI group(n=11: CVD PR (95% CI)	non PI group(n=169) CVD PR (95%CI)	p-value
Standardised age groups			
17-33	16 (10-25)	16 (02-21)	
34-41	15 (09-24)	11 (05-21)	
42-46	23 (15-32)	22 (13-33)	
47-52	23 (15-32)	23 (15-36)	
53-69	23 (15-32)	28 (21-55)	0.003|[a]
Gender			
Male	48 (39-58	39 (27-51)	
Female	52 (42-62)	61 (48-71)	0.078|[a]
Residence			
Urban	52 (42-67)	49 (41-56)	
Rural	48 (32-66)	51 (44-64)	0.082|[a]
Body Mass Index(kg/m2			
<18.5 (underweight)	04 (01-11)	03 (06-23)	
18.5-24.9(normal weight)	35 (26-45)	58 (45-69)	
25-29.9(overweight)	31 (22-41)	20 (12-32)	
>30(obese)	30 (22-40)	09 (04-20)	0.004|[a]
CD4 cell count			
<350	70 (60-78)	45 (33-58)	
>350	30 (22-40)	55 (42-67)	0.002|[a]
HIV Diagnosis (years)			
median	1.32	3.91	0.002|[b]
Age (years)			
Mean	47.66	53.43	0.003|[c]
Systolic blood pressure			
Mean	137.7	135.92	0.236|[c]
Diastolic blood pressure			
Mean	97.40	97.14	0.436|[c]

*Significant P value, [a]Chi-square test, [b]Wilkosum rank sum test, [c] t-test, PR: proportion

5.4 Risk of CVD among Seropositive ART Groups

The risk of CVD for those on PI was 86.6% while those not on PIs were 37.9%. Therefore, the relative Risk for PI ART group was 2.3 significant at 95% CI (p<0.001 95%CI 1.86, 2.81). The risk of CVD in the PI ART group was 2.3 times significantly higher (95%CI, 1.86,2.81;p<0.001) than in the group of non-PI not controlling for any other factors necessarily factors such as CD4 cell count, age, years since HIV diagnosis and the time to

CVD. This is an indication that just eliminating factors leading to CVD for those on PI would reduce the incidence of CVD by 56%.

Table 5: Two by two table of association between ART and CVD (n=281)

Frequencies [n (%)]	Cardiovascular Disease	No Cardiovascular disease	Total
Protease inhibitor ART group	97 (34.51)	15 (5.33)	112 (39.88)
non protease inhibitor ART group	64 (22.77)	105 (37.36)	169 (60.14)
Total	161 (57.29)	120 (42.70)	281 (100)
	Point	Estimates	95% CI
	Risk Difference	0.49	0.39-0.58
	Risk Ratio	2.28	1.86-2.81
	Attributable Risk	0.56	0.46-0.64
		P<0.001	

5.5 Effect of CD4 cell count, age and years since HIV diagnosis on protease inhibitors risk for CVD

Stratum specific risk of CVD among those with CD4 cell count above 350 compared with those with CD4 cell count below 350 were appreciably different ($p<0.001$), implying that a CD4 cell count below 350 had increased risk of 31% (RR 1.31; 95% CI 1.12 for CVD for PIs. Comparing the RRs of crude and the Mantel Hansel adjusted suggest no confounding suggesting that a CD4 cell count below 350 does not confound the effect of PI in the development of CVD. In test for homogeneity, the difference for stratum specific of CD4 cell count and the difference between crude and Mantel Hazel of CD4 cell count is statistically significant ($p<0.001$), implying that CD4 would confound the relationship between ART and CVD.

In order to test if years since HIV diagnosis have an effect in CVD development among PIs group, the variable since HIV diagnosis was categorised (that is 0 to 7 years and 8 to 16) years. After stratification of the variable the risk ratio for the two categories of years since HIV diagnosis were category 0 to 7 years had a relative risk of 2.22 while category 8 to 16 years had relative risk of 2.30 implying years since HIV diagnosis does not increase the effect of PIs in causing CVD. At the same time when comparing the crude RR and Mantel

Hazel adjusted RR for the two categories crude RR and Mantel Hazel are also not appreciably different, implying that years since HIV diagnosis is not a confounder for the association between PIs and CVD. The test for homogeneity showed no difference (P=0.908). Categorising age into 15 to 34 and 35 to 69, in the test for effect measure modification, this category of age modifies the association of PIs and CVD by 3.92 relative risk, comparing the crude and Mantel Hazel, age at the same time confounds the association of PI and CVD (test of homogeneity p<0.001).

Table 6: stratification of CD4 and years since HIV diagnosis for CVD development

VARIABLE	Stratum RR (95% CI)	Crude RR (95% CI)	M-H RR (95% CI)	Test of homogeneity (p-value)
CD4 cell count				
350 above CD4	0.47 (0.49-0.85)	2.29 (1.89-2.81)	2.32 (1.89-2.83)	<0.001
Below 350 CD4	1.31 (1.12-1.55)			
Years since HIV diagnosis				
0-7 years	2.22 (1.37-3.61)	2.28 (1.86-2.81)	2.29 (1.86-2.81)	0.908
8-16 years	2.30 (1.83-2.88)			
Age (years)				
15-34	2.12 (1.99-2.21)	3.21 (2.14-4.31)	4.82 (3.12-5.67)	<0.001
35-69	3.92 (3.84-4.10)			

*Mantel Haze *RR Relative Risk

5.6 Age as a risk factor for CVD

Over all the proportion of ART adult clients that were on PI and developed CVD were 61.5 % (95%CI 52.9 to 69.4) and on the other hand the proportion of 38.5% (95%CI 30.6 to 47.1) who are not on PI developed CVD not adjusting for other important factors. Figure 1 shows the pattern of CVD development for standardised age groups adjusting for the effect of ART and traditional risk factors and HIV itself in the development of CVD. The trend is that in the lowest category of 17 to 33 years, incidence of CVD was not markedly different as about 11% seropositive adults on PI developed CVD compared to 9% in the Non PI group. The trend increased in the age group 34 to 41 years PI (14%) versus Non PI (12%) with an increased risk=RR 1.79 (95%CI .87-3.69; P=0.002), in the age group 42 to 52 years the gap between PI and Non PI seem to be appreciably different PI (25%) versus Non PI 21% with a risk of RR=3.70 (95%CI 1.94, 7.05; P=0.004). The proportion of CVD among the PI group further increases with a proportion of (35%) versus Non PI (25%) and a RR=3.89 (95%CI 2.02, 7.45). There is a marked highest difference for CVD development in the age category 53 to 69 years, with a higher CVD development in the PI

(50%) versus Non PI (29%) with a RR= 4.26 (95%CI 2.23, 8.12; P=0.020), indicating that the difference is not defined by chance.

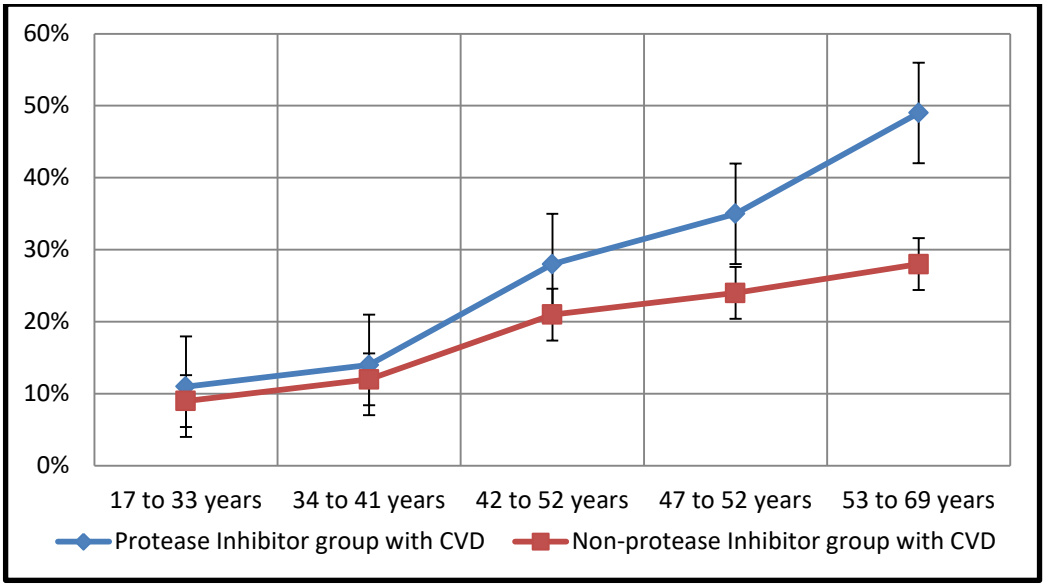

Figure 2: Distribution of CVD according to standardised age groups

Note: standardisation for age was done for equal number distribution in age categories

5.7 Rate of Survival between the PIs and Non PIs groups to CVD and their CVD comorbidity (Kaplan-Meir (K-M) Survival Estimates for CVD)

Preforming a proportional hazards regression analysis of CVD of HIV seropositive adults by Type of ART, the hazard ratio for PI type of ART relative to non PI type of ART is Hazard Ratio (HR)=1.8 (P=0.001 95%CI 1.3, 2.5). This suggests that the risk of CVD between the PIs and non-PIs groups differed significantly over a period of time with a higher risk for PI group.

The overall survival, number at risk and events experienced at each time interval in years during the study period is shown in figure 3Cumulative CVD morbidity is much higher in the PI type of ART even when the numbers at risk are much less compared to their counterparts.

In figure 3, the morbidity curves continually widen, which indicates that the PI ART group remain at greater risk than non-PI ART group regardless of the number of years since recruitment.

The median year of survival for PIs is six years and that of the non PIs is seven years, implying this the time it takes for half of the participants to develop CVD. The probability of survival in the non PI ART group was reduced by 0.7(70%), translating into a probability of about 30% of having CVD. The probability of having CVD is 20% higher in the PI group compared to the non PI group for a 7 years follow-up. Comparing the survival function of the Protease group to that of the non-Protease group (Log-rank p value=0.003).This indicates that the marked difference in survivorship between the Protease ART group to those of the Non Protease group is significant and is not likely to be due to chance.

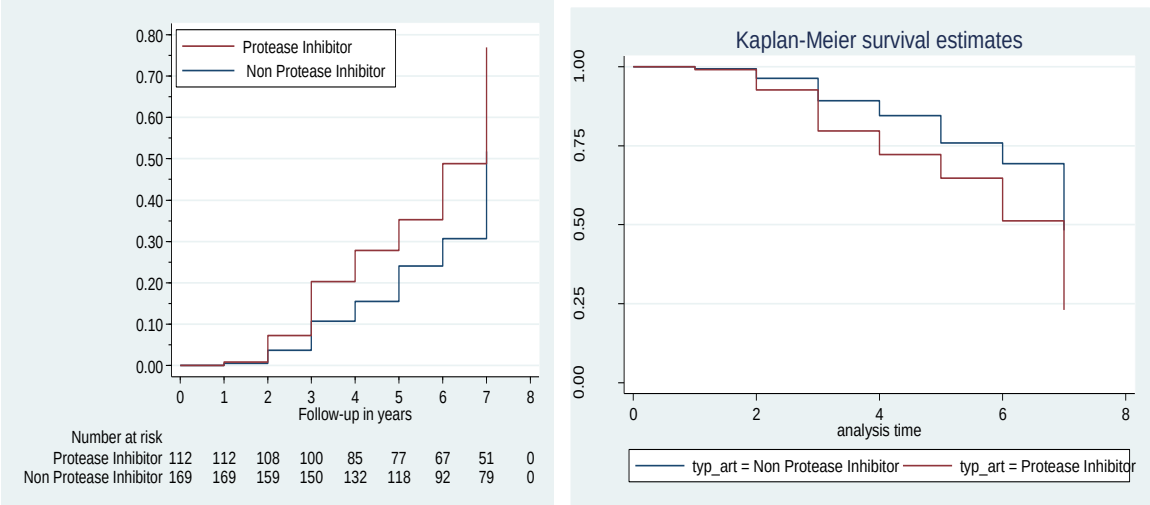

Figure 3: CVD cumulative morbidity curve and Kaplan-Meier survival estimates for PIs and Non PIs type of ART

Notes

In Figure 1 above, time (*t*) is constant over two years when no CVD are observed and drops abruptly after 2 years for both groups when CVD occur. The time interval is short enough that there is rarely more than one CVD per interval and then the height of the drop at each CVD year indicates the size of the cohort remaining on that year. The accuracy of the survival curve gets less as we move towards the right, as it is based on fewer patients.

5.8 Predictors of CVD among ART patients

Univariate and multivariate analysis in Table 7 shows the following; In the age group the age categories that were significant both at univariate and multivariate were 47-52 with a crude risk ratio of 2.13 (95%CI0.91-3.12 P=0.003) while at multivariate there was an adjusted risk ratio of 1.56 (95%CI1.34-1.72; P=0.043) and age category 53-69 with crude

risk ratio of 3.32 (95%CI0.12-4.13,p= 0.002) while at multivariate the crude risk ratio was 4.56(95%CI3.24-6.21, P=0.021) that male seropositive adult ART patients compared to female had same risk for CVD (RR 1.02; 95% CI 0.88, 1.68), although the findings were not statistically significant (p=0.23). When males were compared with females with respect to CVD, there was no statistical difference at both univariate (RR 1.02; 95%CI 0.88, 1.68; p=0.232 and multivariate (RR 1.29; 95%CI 0.85, 1.72; p=0.312). While on residence a participant coming from rural seem to influence CVD development at univariate (p=0.461), but not so at multivariate. When it came to BMI, being underweight seemed not to influence CVD development among ART patients, however the rest of the BMI categories were significant at univariate and multivariate stage, The adjusted risk of developing CVD for patients with normal weight was 0.98 (95%CI0.14-2.21; P=0.034) while adjusted risk of 1.01(95%CI0.123.21; P=0.032) for overweight patients the risk was1.34 (0.03-1.78; P=0.032) with adjusted risk of 1.57(95%CI1.21-7.87; P=0.004) and for obese patients the risk was 1.89(1.12-2.67; P=0.002) with adjusted risk ratio of 1.65(1.12-9.32; P=0.003)

ART patients with a CD4 cell count of less than 350 cells/mL were 4.35 times more likely to develop CVD at univariate than those with a CD4 cell count of 350 and above cell/mL (p=0.002), while the risk increased after adjusting for age and years since HIV Diagnosis to 5.10 time likelihood of CVD for PI group compared to their counterparts (P=0.006). Those on protease inhibitors had crude risk ratio of 2.31 (95%CI1.21-3.42; P<0.001) in this the effect of other variables were not accounted for, the adjusted risk ratio that is accounting for the effect of other variables was 2.60 (2.12-3.30) P= <0.001

It was also found that having lived with HIV for more than 8 years compared to less than 7 years significantly increased the risk for a CVD. Those who had lived with HIV for more than 7 years were 1.78 (p=0.032) times more at risk of developing CVD independent of other variables, while at multivariate there was no statistical difference (RR 2.41; 95%CI 0.12-3.21; p=0.632). The participants' Systolic blood pressure did not seem to predict CVD both at univariate and multivariate. Diastolic blood pressure on the other hand was significant in predicting CVD at univariate p=0.028and multivariate (p=0.026), but diastolic does not seem to either increase or decrease the development of CVD among ART.

Table 7: predictors of CVD among seropositive adults on PIs (univariate and Adjusted Analysis)

Variable	Univariate RR (95%CI)	p-value	Adjusted RR (95%CI)	p-value
Standardised age groups				
17-33	1.00	1.00	1.00	1.00
34-41	1.23 (0.25-3.34)	0.008	1.73 (1.87-9.98)	0.251
42-46	1.87 (0.12-2.13)	0.023	1.98 (2.01-3.21)	2.342
47-52	2.13 (0.91-3.12)	0.003*	1.56 (1.34-1.72)	0.043*
53-69	3.32 (0.12-4.13)	0.002*	4.56 (3.24-6.21)	0.021*
Gender				
Male	1.00	1.00	1.00	1.00
Female	1.02 (0.88-1.68)	0.232	1.29 (0.85-1.72)	0.312
Residence				
Urban	1.00	1.00	1.00	1.00
Rural	1.03 (0.91-3.12)	0.461*	1.12 (0.79-2.10)	0.631)
Body Mass Index(kg/m2				
<18.5 (underweight)	1.00	1.00	1.00	1.00
18.5-24.9(normal weight)	0.98 (0.14-2.21)	0.034*	1.01 (0.12-3.21)	0.032*
25-29.9(overweight)	1.34 (0.03-1.78)	0.032*	1.57 (1.21-7.87)	0.004*
>30(obese)	1.89 (1.12-2.67)	0.002*	1.65 (1.12-9.32)	0.003*
CD4 cell count				
<350	1.00	1.00	1.00	1.00
>350	4.35 (2.31-7.43)	0.002*	5.10 (3.43-8.43)	0.006*
Type of ART				
non Protease inhibitors	1.00	1.00	1.00	1.00
protease inhibitors	2.31 (1.21-3.42)	<0.001*	2.60 (2.12-3.30)	<0.001*
HIV Diagnosis (years)	1.78 (1.21-2.32)	0.021*	2.41 (0.12-3.21)	0.632
Age (years)	1.30 (1.02-1.05)	<0.001*	0.69 (0.91-1.02)	0.230*
Systolic blood pressure	1.00 (0.98-1.01)	0.883	1.01 (0.836)	0.836
Diastolic blood pressure	1.00 (0.99-1.02)	0.028*	1.00 (0.99-1.01)	0.026*

*significant p values; Abbreviation: RR= Relative Risk

After carrying out an investigator led stepwise regression, the best fit model predicting the risk of developing CVD among PI patients included age, sex, body mass index, systolic blood pressure, CD4 cell count, years since HIV diagnosis and the main exposure being exposed to either PI and non PI. Relative rates from this study log binomial regression model are illustrated in Table 8.

The following parameters were assessed and excluded based on non-significance: gender, diastolic blood pressure and Age independent of categories. Systolic Blood pressure was

retained in the model despite its marginal statistical significance for CVD because of its well-known association with CVD. The variables in the study included age, systolic blood pressure , diastolic blood pressure, CD4 cell count, type of ART, years since HIV diagnosis and BMI. The best predictor model after doing investigator led stepwise regression and likelihood ratio test, only remained with four variables (Age, CD4 cell count, Type of ART, systolic blood pressure and BMI). The model was able to correctly classify by 80.4%, which actually entails that the model is not unreliable. The ROC curve (0.8525) indicated that the results were not due to chance (output results not shown for model classification and ROC curve).

Table 8: Adjusted predictors of Cardiovascular Disease (relative risk) from the best model

Predictors	RR(95%CI)	p-value
Age (years)		
17-33	1.00	1.00
34-41	1.73(1.87-9.98)	0.009
42-46	1.98(2.01-3.21)	0.003
47-52	1.56(1.34-1.72)	0.003
53-69	4.56(3.24-6.21)	0.006
CD4 cell count(cells/ml)		
≥350	1.00	1.00
<350	5.10(3.43-8.43)	0.006
BMI (Kg/m2) categories		
<18.5(underweight)	1.00	1.00
18.5-24.9(normal weight)	1.01(0.12-3.21)	0.032
25-29.9(over weight)	1.57(1.21-7.87)	0.004
≥30(obese)	1.65(1.12-9.32)	0.043
years since HIV diagnosis		
1 to 7	1.00	1.00
8 to 16	2.41(0.12-3.21)	0.003
Type of ART		
Non-PI	1.00	1.00
PI	3.51(2.31-4.10	<0.001
Systolic blood pressure	1.32(0.45-1.92)	0.008

RR= relative risk

CHAPTER SIX

6.0 DISCUSSION

This study has revealed that adult patients accessing PI face the threat of developing CVD. The study determined the influence of sociodemographic factors and clinical characteristics in the development of CVD between PIs and non-PIs based on retrospectively reviewing records of HIV seropositive patients at UTH, AIDC. The study also determined the incidence and predictors of CVD among the two ART group. Furthermore the study determined the proportion of PIs HIV seropositive adults with an enhanced risk for CVD and compared the rate of survival between the PIs and non-PIs ART groups to CVD.

This study found that the associated risk of CVD was 2.3 times higher in the PI ART group than the non-PI ART group not controlling for any other necessary indicators such as CD4 cell count, Age, Years since HIV diagnosis and the time to CVD. At the same time after adjusting for other variables the risk of CVD was 3.51 times higher for those on PI compared to the non-PIs. It is worth mentioning that this study could not manage to collect information on smoking and alcohol consumption as these have been found to be important risk factors for CVD among the HIV population (Pearce *et al.*, 2012). Therefore, the predicted risk in our study differs with most studies based on the fact that other studies collected information on smoking and alcohol consumption, which this study could not as no information is routinely collected from the study setting (Islam *et al.*, 2012). The other reasons for differences may be that other studies compared the HIV population against the general population while this study was comparing the effect of ART type within the HIV population. Based on this, in earlier studies (including the Framingham study), higher rates of CVD in HIV-infected individuals compared with the general population have been reported than those in our finding (Triant *et al.*, 2007; Asztalos et *al.*, 2006).

Going with reviews and observations from other studies, reasons have been advanced for this CVD changing paradigm. Some of the reasons are the effect of metabolic complications including dyslipidaemia, altered fat distribution and insulin resistance. This phenomenon has been advanced by certain agencies that collect data on people that take ART drugs (Collection on Adverse Events of Anti-HIV Drugs (DAD) Study Group, 2003).

This study reported a time to developing CVD for PI patients was lower than the non-PI ART group. This finding suggests a higher CVD cumulative incidence for the PI group. This would mean that for a period of 7 years under, those on PI were more likely to have a higher proportion of CVD than their counterparts. This is consistent with earlier studies which showed an association of prolonged use of ART being linked to CVD (Lang et al., 2010). Other studies have shown the contrary and these have been attributed to the relatively short duration (six months) of exposure of the participants to ART (Trian, 2007; Bavinger, 2012).

These results are in keeping with the major trial, the DAD study (Fontas *et al.,* 2005) which had reported earlier the high incidence of CVD among HIV patients on PI. Ritonavir, a PI common in use has been found to be the most notorious cause of CVD and obesity. Considering this it is also worth noting that ritonavir boosted Lopinavir is the only PI used in the UTH and AIDC as the second line treatment according to Zambia National HIV treatment guideline (Wood et al., 2003). This study confirms this that even after adjusting for other factors PI use remained to be independent predictors of CVD among patients on ART.

The incidence of CVD (60.9%) for the PI ART group found in this study was, however lower than a previous study among HIV patients in Dar es salaam in which the incidence of CVD was found to be 76% (Constans *et al.,* 1994) .The difference in incidence with the current study could be due to the fact that the previous study studied only the ART naive patients while the current study studied both ART experienced patients. It is imperative to note that even though the incidence differed with Dar es Salaam, these results are similar to those which were reported from Latin American HIV cohort of patients on ART in which the prevalence of CVD was reported to be 65% (Leite *et al.,* 2010). One of the reasons for looking at ART experience patients for comparisons is that is postulated that CVD is a result of the metabolic effects of the HIV virus itself and the metabolic effects of ART. Considering this fact this increases the risk of future cardiovascular events in HIV patients (Fitch et al., 2013).

This study found that having a CD4 cell count below 350 cell count modifies the effect of PIs on the risk of CVD. This is supported by another study which found a lower CD4 cell count to be associated with increased cardiovascular risk (Friis-Møller *et al.,* 2010). The

explanation could be that a low CD4 could be related to having higher viral load, signalling a higher HIV burden. This HIV infection may increase the metabolic events that would eventually lead to a CVD. However, these findings contrast what was found by the DAD study which observed that CVD was associated with a high CD4 cell count (DAD) Study Group, 2003). It has also previously been reported that a lower baseline CD4 count has shown to be strongly associated with CVD after initiating PI (Erlandson *et al.,* 2013), this could help explain the finding of this study that a lower CD4 cell count modifies the effect of PI in the risk of a CVD among seropositive people as earlier eluded to. This explains in part why CD4 count could be at the same time predictors of CVD.

The finding regarding the relationship of CD4 and CVD in this study was that a nadir CD4 cell count of less than 350 cells⁄L, which also reflect advanced immunodeficiency, was a predictor of CVD in PI ART group individuals both at univariate and multivariate analysis. One previous study has reported a nadir CD4 cell count less than 200 cells⁄L to be associated with CVD in univariate analysis, but this association did not persist after multivariate adjustment (Medina-Torne *et al.,* 2012). In contrast, a cross-sectional study failed to reveal a link between prevalent CVD and CD4 cell count (Arruda *et al.,* 2010). In this study, CD4 cell count was analysed either as a continuous variable or with the cut-off level at 100 or 200 cells⁄L which makes comparison with our study difficult.

It was found that the risk of CVD increased with older age and higher BMI for both ART groups but was significantly higher for the PI ART group. This finding is consistent with other studies (Danoff *et al.,* 2005; De Wit *et al.,* 2008; Ledergerber *et al.,* 2007; Justman *et al.,* 2003; Galli *et al.,* 2012) indicating that sociodemographic factors and clinical characteristics apply in the same way in HIV-infected individuals as in HIV naïve. The explanation is that on effective ART including PIs BMI tends to increase, due to improved nutritional and immunological status. This indicates that PIs would improve the immunological status further and making one have a higher BMI which would pose a CVD risk in the long run. This study also found that having a much higher BMI is a predictor of CVD for PIs ART group.

As an increasing prevalence of overweight and obesity is associated with an increasing risk of a large number of diseases including CVD, this could potentially explain the findings of

an increasing prevalence of CVD over time more on PI groups than their counter part. The understanding that supports BMI to be a predictor of CVD among the PI ART group is the fact that on top of the effect of PIs and the HIV virus itself; the dyslipidaemia leading to CVD is also necessitated by in part wrong eating habits among HIV seropositive patients (Arruda *et al.,* 2010). This can be explained by the fact that what is common in practice in the local population is to encourage HIV patients to 'over-feed' on rich foods so that they maintain their weight and improve immunity at the same time avoid stigma. Thus HIV patients strive to get nutritional support and use food supplements to avoid such stigma from society. This may lead to overweight and dyslipidaemia and more likely contribute to CVD.

This study found that age increase the effect of PIs in the development of CVD, it was also found that there is an association between age and CVD. This is explained by the fact the older you grow the higher the risk of CVD. The fact that age itself consistently increases the risk of CVD irrespective of ART and HIV, this was taken care of at design stage by randomly allocating age in the PI and non PI groups. Recently, it was shown that HIV-infected individuals with an age of 65 years or older had an almost 4 times higher risk of CVD relative to HIV-infected individuals younger than 50 years (Hasse *et al.,*2011). Similarly, a recent study by Petoumenos *et al.,* (2013) showed a significant interaction between HIV, age and CVD, suggesting that HIV potentially modifies the relationship of age and ART on CVD, thus emphasizing the need to critically consider age when discussing CVD in the presence of other risk factors. Our finding is not the same but much closer to the DAD study because the HIV-infected population in the DAD study is slightly younger, our predicted risk may not be as exact as the DAD study because of its diverse geographical distribution and predominantly European population, though the majority is ART exposed. Age is an important predictor of CVD.

It has been proposed that chronic infections, and in particular, the faulty immunological processes seen in HIV infection, may be associated with an accelerated aging process (Polsky *et al.,* 2011). However, at present, our findings do not suggest a larger-than expected effect of aging with respect to CVD risk. The pattern in our in our study is that the older one get the risk for CVD increases, what has been noted is that the age trend differed remarkable with the PI having a much higher risk with age increase than their

counter part CVD in younger HIV-infected individuals does not exceed predictions from the Framingham score (D'Ascenzo *et al.*, 2014).

Meanwhile it is also widely accepted that HIV-positive patients have increased risk for CVD as reported by Grunfeld *et al.* (2009) compared to the general population and this was after adjusting for demographic characteristics. The significance level was only slightly attenuated after adjusting for traditional CVD-risk factors. This study reviewed evidence across studies investigating the association between cumulative and exposure to specific ART classes, that is PI and Non PI groups and the risk of CVD. Our findings implicated recent cumulative exposure to PIs in general. There are several issues, however, that need to be considered when interpreting our findings. An issue that may be considered is that CVD development may also be contributed by traditional risk factors such as age, alcohol consumption and sedentary life styles. This should be considered especially that records that were reviewed in this study did not contain information on these parameters.

In addition, some facilities have it as a policy to switch therapy to a much more user friendly therapy with fewer side effects. This switch is usually from old generation PIs to safer newer generation PIs for a period of six months to minimize the effects of ART. If duration alone is considered, this could mean that the difference in the development of CVD could be more in the PI group. It is also important to note the retrospective reviewing of patients' records may have its own challenges such as a loss to follow up, also the follow-up period of the study was rather short and considerable changes were not to be expected owing to short study period. With the foregoing a longer prospective follow-up period would probably have resulted in more cases with new-onset CVD and increased the statistical power. At the same time it has been reported in this study that the Kaplan Meier curve began to show difference in rate of survival after a period of 2 years. This being consistent with similar with a study by Saves *et al.*, (2002) which reported a median time of 18 months from the use of protease inhibitors to the development of CVD.

While the fight to reduce traditional risk factors for CVD remains supreme, the extent to which PI contributes to CVD in HIV-infected population will need further evaluation. Ensuring good quality of life for HIV-infected individuals in the era of ART is also associated to preserving their corporal self-image, physical aspects, and self-esteem.

These findings highlight raises a need to educate first-line ART takers regarding drug compliance to reduce the need to introduce second-line drugs. This is so because PIs are second line drugs and can be avoided if patients adhered to their first line treatment. Despite the risk of long-term complications including CVD, ART still provides substantial survival benefits for HIV infected populations. In extension, substantial evidence indicates that although some antiretroviral drugs may increase the risk of CVD, continuous ART in general may indeed reduce the risk of not only AIDS associated conditions and death, but also non-AIDS associated conditions like CVD (Klein & Hurley, 2003). The reason may be associated with reductions in inflammation markers, viral replication and improved immune function. However, this still needs further exploration.

6.1 Study Limitations and Strengths

This study was limited by the fact that it was a single-Centre, hospital-based study and findings of this study may not be generalizable in the entire HIV population in Zambia. The study was also unable to collect information on important variables considered important predictors of CVD, such as smoking and alcohol consumption. Another limitation was not knowing or being able to detect prior history of CVD which is also an important limitation in our study as this group of patients are likely to be a confounder in the risk factor profile.

The strength of this study however was the fact that it used a log binomial model to obtain more accurate estimates of risk ratios, unlike most cohort studies that use logistic regression to estimate the risk ratio. This study also looked at a much wider length of ART follow up of 7 years retrospectively and this gives a much longer period of time for those who would have developed inflammatory markers for CVD to develop symptoms for the condition.

6.2 Conclusion

This study has found that PI ART therapy is associated with a higher risk for CVD compared to non PIs. These CVD risks may be accelerated by a low CD4 cell counts, weight gain and duration of HIV. Given this situation, Zambia and most developing countries, especially in Africa where the prevalence of HIV/AIDS is high, have also been reported an increase in chronic disease burden including CVD, which threatens to outpace morbidity and mortality from HIV/AIDS. The use of ART has revolutionised and

successfully prolonged the life expectancy of many HIV-infected patients. However, CVD attributable morbidity seems to be an emerging paradigm for ART clients. For this reason, the fight against HIV/AIDS has seen a sustained global campaign to reduce the cost of ART and make these medicines available to people who need them. Much as this idea is laudable, the indirect risk of CVD with the use of ART especially PIs, in a population where prevalence of HIV is high and with growing trend of CVD morbidities including type II diabetes and hypertension raises serious concerns. It is therefore imperative that the risk of CVD with PI ART is noted and accordingly addressed.

In these years the scarce resources have become a reality. Hence, prioritization of health resources is of outmost importance. As such, it seems prudent to attempt to reduce modifiable risk factors. Physicians should encourage therapeutic lifestyle interventions to avoid a sedentary lifestyle with risk of obesity. Furthermore, careful selection of antiretroviral drugs should be applied according to the underlying CVD risk profile.

6.4 Recommendations

This study showed that exposure to PIs containing ART whilst improving the immune status of patients, predispose them more to CVD. Thus, it is advisable to monitor for cardiovascular risk factors or assessment before initiation of ART followed by periodic monitoring while on the treatment. This will ensure prompt detection and management of cardiovascular risk factors and the prevention of CVD events, ultimately leading to screening of patients and whenever possible, choosing the antiretroviral medication least likely to worsen the dyslipidaemia or dysglycaemia such as newer generation PIs.

To that aim, follow-up and nutritional and physical activity intervention studies are needed to evaluate the efficacy of nutritional therapy for modifying risks of morphological and metabolic abnormalities associated with the use of antiretroviral therapy among HIV-infected patients. Once the efficacy of this type of intervention has been proven, it can be incorporated into the set of integrated health care actions available for people living with HIV/AIDS.

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APPENDICES

UNZABREC APPROVAL LETTER

APPROVAL LETTER FORM THE UNIVERSITY TEACHING HOSPITAL