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Management of dyslipidemia in HIV infected patients on combined antiretroviral therapy: effects of intervention

By

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- **Declaration**

I, **Mmabatho Ngoananoka Portia Dintwe**, hereby declare that this dissertation presented to the University of Pretoria for the degree Masters of Science in Clinical Epidemiology, is my own work and has not been presented previously by me for a degree at any other tertiary institution.

External examiners' suggestions and comments have been addressed in the dissertation.

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Dr Mmabatho Ngoananoka Portia Dintwe **Date**

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Management of dyslipidemia in HIV infected patients on combined antiretroviral therapy: effects of intervention

Mmabatho Ngoananoka Portia Dintwe, Piet Becker and Paul Rheeder

Abstract

Background: Clinical management of dyslipidemia is challenging, particularly hypertriglyceridemia in patients with HIV-infection. Changing combined anti-retroviral therapy (CART) and the use of lipid-lowering drugs have proven useful in treating dyslipidemia in HIV infected patients

Objective: To assess the efficacy of lipid lowering drugs (LLDs) and/or CART switching, in the management of HIV-associated dyslipidemia

Design: A retrospective, longitudinal cohort study

Setting: Phidisa HIV research project, 6 sites in South Africa, period April 2008 and April 2011

Patients: HIV positive South African National Defence Force (SANDF) members and their dependents; who are on CART and are 18 years or older. Four hundred and forty eight participants with dyslipidemia had non-fasted, total serum cholesterol ≥ 8.0mmol/l, serum triglyceride levels ≥4.52 mmol/l and naïve to lipid lowering drugs at baseline.

Measurements: Mean change over time of total serum cholesterol and serum triglyceride in the following treatment strategies were used: exercise and dietary advice, lipid-lowering drugs (statins or fibrates or both), CART switches separately and combined lipid lowering drug with ART switch was measured using panel data with first–order autoregressive-response and xtabond.

Results: The mean age for a total of 448 participants was 39.9 years; males were 87%, females were only 13%. The participants contributed to 1861 follow-up visits. CD4 count was normally distributed with the baseline mean value of 402 cells/mm^3 (18.5%).Mean change over time for total serum cholesterol and triglycerides increased by 0.099 mmol/l (p=0.007) and 0.248 mmol/l (p=0.018) respectively, with an increase in body mass

index while an increase in CD4 cell percent decreased mean over time for total serum cholesterol by 0.045 mmol/l (p=0.002).Our hypothesis was confirmed when lipid lowering drugs and ART switch combined treatment strategy even more decrease in the mean total serum cholesterol and triglycerides levels over time by 0.754 mmol/l (p<0.001) and 2.073 mmol/l (p<0.001) respectively compared to the exercise and dietary advice treatment strategy. Our findings showed that combined treatment strategy maintained a decrease in both the mean total serum cholesterol and triglycerides levels over time of 0.283 mmol/l (p=0.038) and 0.941 mmol/l (p=0.016) respectively, when compared to lipid lowering drugs; the mean serum triglycerides over time were also reduced by 0.486 mmol/l (p=0.048) when the combined treatment strategy was compared to CART switch only. Furthermore combined treatment strategy of lipid lowering drugs with ART switch showed significant virological suppression by decreasing log of viral load, 0.486 (p<0.001) when compared to the exercise and dietary advice group.

Conclusions: Combining lipid lowering drugs and ART switching as a treatment strategy in the management of HIV-associated dyslipidemia is effective in lowering the mean over time of both total serum cholesterol and triglycerides when compared to exercise and dietary advice strategy, while maintaining virological suppression.

Key words: HIV infection, Combination antiretroviral therapy, Triglycerides, Cholesterol, Dyslipidemia, Lipid lowering drug, CART change

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List of abbreviations:

- 95% CI: 95% confidence interval
- <: is less than
- >: is greater than
- =: is equal to
- 3TC: Lamivudine
- AIDS: Acquired immunodeficiency syndrome
- ART: Antiretroviral therapy
- AZV: Atazanavir
- BMIcat: Body mass index category
- BARC: Bioanalytical Research Corporation
- CART: Combination antiretroviral therapy
- Coef: Coefficient
- CYP: Cytochrome P450
- D4T: Stavudine
- D:A:D: Data collection on Adverse events of Anti-HIV Drugs
- DDI: Didanosine
- DoH: Department of health
- EFV: Efavirenz
- FTC: Emtricitabine
- GCP: Good Clinical Practice

- HAART: Highly active antiretroviral therapy
- HDL: High-density lipoprotein cholesterol
- HMG-CoA: 3-hydroxy-3-methylglutaryl–coenzyme A reductase
- HIV: Human immunodeficiency virus
- HPoAGfAa: HHS Panel on Antiretroviral Guidelines for Adults and Adolescents
- IRB: InstitutionaI Review Board
- LDL: Low-density lipoprotein cholesterol
- LLD: Lipid lowering drug
- LPVr: Lopinavir and ritonavir
- NNRTI: Non-Nucleoside reverse transcriptase inhibitor
- NRTI: Nucleoside reverse transcriptase inhibitor
- NVP: Nevirapine
- PCR: Polymerase chain reaction
- PI: Protease Inhibitor
- RNA: Ribonucleic acid
- RoSA: Republic of South Africa
- RTV: Ritonavir
- SANDF: South African National Defence Force
- SD: Standard deviation

- STD: Sexually transmitted disease
- TB: Tuberculosis
- TC: Total serum cholesterol
- TG: Total serum triglycerides
- TNV: Tenofovir
- VL: Viral load
- WHO: World Health Organization
- ZDV: Zidovudine

CHAPTER 1

1.0 Introduction

Combination antiretroviral therapy (CART) has led to dramatic reductions in HIV-associated morbidity and mortality, [1]improving life expectancy. This was confirmed by the calculated projected life expectancy of 71.5 years for HIV-positive men who have sex with men, living in a developed country with good access to ART and healthcare.(2) The study had a low diagnosis rate; diagnosing only when symptomatic with median CD4 cell count 140 cells/mm^3.[2]

However, an ongoing concern for patients with HIV infection is the higher prevalence of age-related comorbid conditions, such as coronary disease, compared with general population rates. The prevalence of cardiometabolic disease or risk factors has been show in HIV infected persons on CART and those not on CART.[3-5]

Antiretroviral agents, particularly PIs, adversely affect lipid levels in patients with HIV infection, increasing the risk of myocardial infarction.[6] Treatment of dyslipidemia in patients with HIV infection is challenging and drug interactions complicates it even further.[7] Two pharmacological approaches, substitutions in ART and the addition of lipid-lowering drugs have proven useful in treating dyslipidemia in HIV infected patients separately.[8-15]

The effects of these approaches and the comparison thereof need investigation in limited resource settings.

1.1 Definitions

1.1.1 HIV infection

Human immunodeficiency virus (HIV) belongs to the genus Lentivirus, there is HIV type-1 (HIV-1) responsible for the global pandemic and the HIV type 2 (HIV-2), less pathogenic, less transmissible and largely restricted to Western Africa, with limited spread to other countries.[16]The HIV virions enter the host and primarily target the lymphocytes, particularly T lymphocytes known as CD4 cells.[16, 17]The virus establishes itself within the cells, where it replicates using host cell material, and new virions are produced.[17] The new virions are then released from the host cell by budding and are free to invade other uninfected cells. Viral replication goes unchecked, high level of viremia may lead to symptoms such as fever, rash, pharyngitis, and enlarged lymph nodes. At this stage it is a transient illness, lasting days to several weeks, and called acute HIV infection.[17]The immune system eventually responds to the viremia and suppresses viral replication.[17] The viral load diminishes, and the symptoms of the acute infection resolve. Low-grade viral production continues at a steady state, and the host and virus reach a balance of viral suppression and continued viral production. This chronic steady state may be maintained for years until the immune system is eventually overwhelmed. Infection with HIV is a progressive disease. Following years of viral replication, and eventual destruction of CD4 cells, the immune system begins to fail.[17]

When the CD4 count approaches 200 cells/mm^3; opportunistic infections such as Kaposi sarcoma and pulmonary tuberculosis etc. begin to appear. At this stage the infected person is said to have AIDS and ART is indicated.[17, 18]The detection of HIV-specific antibodies in serum or plasma establishes the diagnosis of HIV infection.[17] Close monitoring of the level of viremia and of the CD4 cell counts is crucial in the management of the HIV-infected patient.[17]PCR testing quantitatively measures the viral in copies per milliliter of plasma, the viral load.[17]

The South African National Department of Health Guidelines[19] (updated in 2013) recommends lifelong ART when CD4 count is ≤ 350cells/mm^3 irrespective of WHO clinical stage OR in all types of TB (in patients with TB drug resistance or sensitive, including extra pulmonary TB) irrespective of CD4 count and when WHO stage 3 or 4

irrespective of CD4 count.[18, 19]All woman breastfeeding and diagnosed as HIV positive during pregnancy are initiated immediately on CART even if CD4>350 cells/mm^4; CART is continued for duration of pregnancy and for one week after cessation of breastfeeding.[19]

1.1.2 Combination antiretroviral therapy

The advent of potent combination antiretroviral therapy (CART) changed the course of the HIV epidemic.[1, 17, 18, 20] Combination of three or more antiretroviral drugs has significantly improved life expectancy to decades rather than months.[1, 17, 18, 20] Combination of two NRTIs as the backbone regimen and one protease inhibitor (PI) or one NNRTI leads to better patient quality of life, increased survival, slowed disease progression, decreased opportunistic infections, decreased viral loads, and increased CD4 counts.[1, 17, 20]Recent recommendations include the combination of NRTI (FTC and TNV) with a NNRTI (EFV), a ritonavir-boosted PI (AZV/RTV) or the integrase inhibitor (raltegravir) with less adverse events.[16, 17]

In the resource limited setting a ritonavir-boosted PI such as LPVr and NRTI such as ZDV and D4T are still used.[19]

Table 1: Antiretroviral Agents in HIV-1-Infected Adults and Adolescents

Nucleoside Reverse Transcriptase Inhibitors	Non-Nucleoside Reverse Transcriptase Inhibitors	Protease Inhibitors	Integrase Inhibitors
3TC, ZDV, D4T, DDI FTC, TNV	EFV, NVP	LPVr, RTV, AZV, AZV/RTV	raltegravir

1.1.3 Triglycerides

A triglyceride is an ester derived from glycerol and three fatty acids that, a chief constituent of fats and oils.[21] Triglycerides are blood lipids that enable the bidirectional transference of adipose fat and blood glucose from the liver.[21]Triglycerides are synthesized from carbohydrates during the process of digestion and stored in the body's adipose tissues.[21, 22] High levels of triglycerides in the blood are associated with insulin resistance.[22]

1.1.4 Cholesterol

Cholesterol is a lipid molecule found in animal tissues and various foods, that is normally synthesized by the liver and is important as a constituent of cell membranes and a precursor to steroid hormones (such as the estrogens, testosterone, and cortisol), vitamin D_2, and bile acids.[23] Its level in the bloodstream can influence the pathogenesis of atherosclerosis.[23]The measurement of serum cholesterol encompasses all these different types low-density lipoprotein and high-density lipoprotein.[23, 24] A lipoprotein is a carrier molecule that constitute proteins and lipids.[23, 24]

1.1.5 Low-density lipoproteins (LDL)

LDL is a complex of lipids and proteins, with greater amounts of lipid than protein that transports cholesterol in the blood.[24] High levels are associated with an increased risk of atherosclerosis and coronary heart disease[24]

1.1.6 High-density lipoproteins (HDL)

HDL is a lipoprotein that transports cholesterol in the blood; it is composed of a high proportion of protein and relatively little cholesterol; high levels are thought to be associated with decreased risk of coronary heart disease and atherosclerosis[24]

1.1.7 Dyslipidemia

Dyslipidemia, defined according to the latest National Cholesterol Education Program guidelines, is hypercholesterolemia (total cholesterol≥ 6.2mmol/l), high LDL cholesterol (≥ 4.1 mmol/l), low HDL cholesterol (≤1.03 mmol/l) and high triacylglycerol (serum triacylglycerol ≥2.26 mmol/l).[25] A triacylglycerol is also called a triglyceride. Dyslipidemia in HIV-infected individuals has been described before the availability of antiretroviral therapy in men, the abnormalities included increased levels of triglycerides and decreased levels of total-Cholesterol, HDL and LDL with greater degrees of abnormalities associated with more advanced immune suppression.[26]Untreated HIV-positive women had lower high-density lipoprotein cholesterol and higher triglycerides but LDL was not different in the HIV-negative women compared with the untreated HIV-positive women.[27]

1.1.8 Lipid lowering drugs

Statins are inhibitors of HMG-CoA reductase, are widely used treatment and prevention of atherosclerotic disease.[28] The treatment of dyslipidemia in HIV infection is often complicated by potential drug interactions between antiretroviral drugs and lipid-lowering medications.[12, 29] Other medications used in the treatment of HIV-infection such as macrolide antibiotics, azole antifungals or antimycobacterials may also have significant interactions with lipid-lowering agents.[29] Fibrates have proved to be effective in the treatment of hypertriglyceridaemia and mixed hyperlipidaemia in large population-based studies, and did not present clinically significant drug interactions with antiretroviral agents.[29] Statins are usually effective in reducing total cholesterol and triglycerides in the general population. Muscle toxicity (myopathy) is a potential adverse effect of all statins and fibrates, but the most severe form of myotoxicity, rhabdomyolysis, is very rare with currently used statins.[29] Simvastatin, Lovastatin, and Atorvastatin are metabolized by CYP3A4 (Simvastatin acid is also metabolized by CYP2C8); their plasma concentrations and risk of myotoxicity are greatly increased by strong inhibitors of CYP3A4 (e.g. itraconazole and ritonavir).Pravastatin, Rosuvastatin, and Pitavastatin are excreted mainly unchanged, and their plasma concentrations are not significantly increased by pure CYP3A4 inhibitors significantly increased by pure CYP3A4 inhibitors.[29]

EFV is also a mixed inducer/inhibitor of CYP 3A4, and many statins are primarily metabolized via CYP3A4.[30] EFV when administered with Simvastatin, Atorvastatin or Pravastatin can result in significant induction of statin metabolism. The reduced inhibition of HMGCoA reductase activity during co-administration of EFV may result in diminished antilipid efficacy at usual doses of Simvastatin, Atorvastatin, and Pravastatin[30] In an open label trial conducted in 52 healthy adult HIV-seronegative subjects EFV reduced Simvastatin acid exposure by 58% and active HMG-CoA reductase inhibitory activity by 60%. EFV reduced Atorvastatin exposure by 43% and the total active Atorvastatin exposure by 34%. EFV administration resulted in a 40% decrease in Pravastatin exposure.[30]

The PIs are metabolized by the Cytochrome P450 isoenzyme CYP3A4.[8, 29, 31] The area under the curve (AUC) for simvastatin increases 32-fold when co-administered with the PI combination of saquinavir/ritonavir due to inhibition of CYP3A4 by the PI. Ritonavir is a potent inhibitor of the P450 isoenzyme CYP3A4.[8, 29]

In studies where Pravastatin and Atorvastatin, were no or less of Simvastatin was used, fibrates and statins were safe and associated with a favourable but moderate effect on lipid plasma levels.[8, 13, 31]

Fibrates (e.g. Gemfibrozil, Bezafibrate) decrease the secretion of VLDL and increase lipoprotein lipase activity and increase HDL by binding and activating the enzyme peroxisome proliferator-activated receptor-alpha.[32] Bile acid sequestrants such Colestipol and Cholestyramine are not used in HIV patients on CART as they would interfere with absorption of antiretroviral drugs.[32] Sterol absorption inhibitor ezetimibe has been used to manage HIV related dyslipidemia, not readily available in resource limited settings.[32] Niacin (Nicotinic Acid) or Vitamin B3 increases HDL and decreases triglycerides, LDL levels.[32]

Lipid lowering drugs that were used in this study are statins and fibrates.

Table 2: Lipid lowering medications

HMG-CoA reductase Inhibitors (Statins)	Bile Acid-Binding Resins	Fibric Acid Derivatives	Nicotinic acid	Sterol absorption inhibitor
Atorvastatin, Simvastatin, Pravastatin, Rosuvastatin, Pitavastatin	Cholestyramine Colestipol	Gemfibrozil Bezafibrate	Niacin	Ezetimibe

1.2 Literature review

Prevalence of dyslipidemia ranges from 25-82% in different cohorts of HIV infected patients, in both the developed and developing countries.[32-35] Management of dyslipidemia in HIV infected patient is more challenging compared to patients who are HIV negative, the use of lipid lowering drugs have to carefully considered in view of the type of antiretroviral therapy the patient is on.[7, 12]

The pathogenesis of CART-related dyslipidemia is complex and involves various drug-induced effects, in association with hormonal and immunological influences.[7, 29, 36]

The dyslipidemia that is labeled ‘‘HIV related’’ is complex. The lipid abnormalities may be caused by HIV itself, by antiretroviral therapy, or by host factors (genetic and lifestyle).[36, 37]Advanced HIV disease in the absence of combination antiretroviral therapy has been associated with hypertriglyceridemia, low levels of total cholesterol, low levels of LDL, and low levels of HDL.[33, 34, 38]

Lipodystrophy, insulin resistance and diabetes have been recognized as important metabolic problems in HIV patients.[7, 36] The D.A.D study has shown that patients on CART, with risk factors for cardiovascular disease develop my myocardial infarction.[39]

Most PIs, with the exception of atazanavir, are associated with an elevation in levels of TC, triglycerides, and LDL.[38] NNRTIs produce increases in levels of TC, LDL, and triglycerides; however, increases in HDL levels may occur, particularly with NVP, which can yield a net reduction in the ratio of TC level to HDL level.[38] Switching from a PI to NVP or ABC has generally resulted in an improvement in total cholesterol and triglyceride levels whereas switching to EFV has produced less consistent results.[9, 38] D4T is linked to increases in levels of TC, LDL, and triglycerides.[38, 40] Atherogenic lipid profiles are commonly found in association with body fat changes.[38, 40] TNV showed sustained decrease in cholesterol and triglyceride on replacing stavudine.[40] Calza et al[9] found that the pharmacological treatment of the HAART related dyslipidaemia with Pravastatin or Bezafibrate had an antihyperlipidemic efficacy greater than the switching from the PI to NVP or EFV.[9]

The association of increased serum lipid levels with certain antiretroviral therapies has led to switching the potentially offending component for another drug. However, because of the multifactorial nature of dyslipidemia in HIV infection, abnormalities may not resolve simply by switching drugs.[7, 41]

Lifestyle modifications, including dietary changes, increased physical exercise, reduced alcohol intake and smoking cessation, can result in modest improvements in lipid levels and should be the first step in treating patients who do not have severe lipid abnormalities or established cardiovascular disease.[7, 41, 42] As with HIV-negative patients, the first step should be dietary counselling and a recommendation to increase aerobic exercise and stop smoking; unfortunately, lifestyle changes alone are often insufficient to reverse lipid abnormalities in patients with HIV.[37, 41]

Substitutions in ART and the use of lipid-lowering drugs have proven useful in treating dyslipidemia in HIV infected patients.[9] In choosing between these two pharmacological approaches, treatment of underlying HIV infection should generally take precedence over the other potential benefits of switching ART.[37, 41] Clinicians will need to weigh the risks of new treatment-related toxicities and the possibility of virologic relapse when switching antiretroviral drugs to the risks of potential drug interactions and new treatment-related toxicities from lipid-lowering agents that are added to existing regimens.[7, 37, 38, 41, 42]

Statins are recommended as first-line treatment of hypercholesteremia, whereas fibrates are a cornerstone for hypertriglyceridemia and mixed hyperlipidemia.[43]Statins and fibrates have broad spectrum pleiotropic activities, whose overall benefits appear to be greater than those related to changes in lipid levels, by multiple concurrent favourable activities exerted on endothelial function, oxidative stress, inflammation and thrombogenic response.[43]In a study comparing HIV-infected patients with hypercholesterolemia treated with statins and those with hypertriglyceridemia who received a fibrate, and a matched control group on CART-treated dyslipidemic patients on a dietary and physical program, no substantial variations occurred in the mean CD4 cell count, and viral load was suppressed up to 18 consecutive months of follow-up.[43]

Due to absence of PI-drug interactions, Pravastatin is the statin of choice when treating PI-related dyslipidemia.[37, 41, 42] If lipid targets are not met with Pravastatin, Atorvastatin is an acceptable alternative, provided the starting dose is 10 mg daily and there is close monitoring for evidence of hepatic or muscle toxicity.[37, 41, 42]

Standard laboratory practices regard non-fasting serum triglyceride level greater than 4.52 mmol/l (400 mg/dl); as an indication for a need for a fasting direct LDL cholesterol level.[12, 26] BARC used the same cut-off level for the same indication while the serum cholesterol level ≥ 8.0mmol/l was used as a cut-off to alert the need for a fasted lipogram.

Two pharmacological approaches, substitutions in ART and the addition of lipid-lowering drugs have proven useful in treating dyslipidemia in HIV infected patients. In choosing between these two pharmacological approaches, treatment for HIV infection should generally take precedence over the other potential benefits of switching ART.[41] It is also important to consider that the addition of lipid-lowering agents to the already complex treatment regimen of HIV-infected patients adds another layer of treatment complexity and may also be associated with toxicities related to introduction of the lipid-lowering therapy and/or due to drug interactions.[41] Williams et al. [44] described the association of ART with changes in total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and TG over almost 6 years of follow-up and found that the improvement in serum lipids levels was associated with an increased use of lipid-lowering agents and an improved selection of ART drugs.

In our study we compare the different pharmacological approaches, assessing their effects on serum lipid levels, their immunological and virological outcomes.

CHAPTER 2

2.0 Methods

2.0.1 Research question, aims and objectives

Would combination of the two pharmacological approaches; lipid lowering drugs and ART switching make a significant difference compared to each approach, in the management of dyslipidemia in HIV infected patients on CART?

2.0.2 Hypothesis

A combined strategy with lipid lowering drugs and CART switching is superior to either lipid lowering drugs or CART switch on their own regarding lowering cholesterol levels.

2.0.3 Primary objective

To assess the efficacy of lipid lowering drugs and/or CART switching, in the management of HIV-associated dyslipidemia

2.0.4 Secondary objective

Evaluate virological and immunological response of each treatment approach

2.0.5 Study design

A retrospective, longitudinal cohort study, between April 2008 and April 2011. A substudy of the main Phidisa observational study “A prospective, observational cohort study of HIV infection (treated and untreated) and risk related co-infections in the SANDF”.

2.0.6 Study Setting

Project Phidisa is a clinical research project focused on the management and treatment of HIV infection in the uniformed members of the SANDF and their dependents.

This study is a sub-study of Phidisa IA observational study at six PHIDISA investigational sites, 1 Military Hospital (Gauteng), 2 Military Hospital (Western Cape), Area Military Health Unit Free State Poli-clinic (Free State), Phalaborwa Sickbay (Limpopo), Mtubatuba Sickbay (Kwazulu-Natal) and Umtata Sickbay (Eastern Cape).

No protocol limits will be applied to the number of volunteers to be recruited at each site. Numbers of volunteers will be dictated by site capacity

2.0.7 Study population

All HIV infected uniformed SANDF personnel and their registered family members who are older than 18 years, and are on CART with laboratory evidence of dyslipidemia between April 2008 and April 2011, irrespective of, ethnicity, gender, beliefs, financial status, occupation, sexual orientation, or any aspect of lifestyle. All volunteers gave written informed consent in Phidisa 1a study, an observational study

2.0.8 Inclusion Criteria

Dyslipidemic levels for inclusion were:

1) Elevated total cholesterol ≥ 8.0mmol/l

2) Elevated triglyceride levels ≥4.52 mmol/l [12, 26]

2.0.9 Exclusion Criteria

- Previously received lipid-lowering therapy
- Pregnant women

2.0.10 Ethical and legal considerations

This study was conducted in accordance with South African Good Clinical Practice (GCP) and all applicable national and international regulations and guidelines as required through the PHIDISA project.

The SANDF Intelligence of South African Military Health Service, the Executive Committee member for the Republic of South Africa, Project Phidisa, the Phidisa project/SANDF Institutional Review Board (IRB); and the faculty of Health Sciences Research Ethics Committee of the University of Pretoria to evaluated this protocol, permission granted for the study to be conducted as set out in the protocol

2.0.11 Participant selection

All Phidisa 1a participants on combination ART were included in the study; they had to have total cholesterol ≥ 8.0mmol/l and /or triglyceride levels ≥4.52 mmol/l from the laboratory results (BARC Lancet), between April 2008 and April 2011. Participants with previous history of dyslipidemia or on lipid-lowering therapy were excluded. Women who were pregnant between April 2008 and April 2011 were also excluded.

2.0.12 Outcome Variables

Outcome variables are total serum cholesterol at every 6-month visit (continuous normal), serum triglycerides at every 6-month visit (continuous, skew).

2.0.13 Explanatory variables

Explanatory variables collected were age (continuous, normal), sex (categorical, male/female), weight (recorded at every 6-month visit) (continuous, normal), height (continuous, normal), body mass index (continuous, normal). Categorical variables: diabetes and lipodystrophy (all yes/no), lipid lowering drugs (categorical, yes/no). Continuous variables collected at every 6-month visit were: CD4+ T-lymphocyte cell count and percent (normal), Plasma HIV-RNA load (skew)

2.1 Treatment strategies

The 4 treatment strategies used are summarized in the next sub-sections.

2.1.1 Exercise and dietary advice only

No other treatment was given except dietary and exercise advice as the first treatment strategy to all participants

2.1.2 Lipid-lowering drugs only

Participants who only had lipid lowering drugs (statins or fibrates or both) as a treatment intervention, were assumed to have exercise and dietary advice as a standard practice. These participants had lipid lowering drug added to their treatment. (Binomial, yes/no)

Lipid lowering drugs used in the cohort were statins and fibrates. Pravastatin, Atorvastatin and Simvastatin were used. Doses for the statins may vary in this cohort. Participant were on statin or fibrate only, or both statin and fibrate to be in this group. Fibrate used was Bezafibrate CR 400mg at night. Participants were assumed to be on the lipid lowering drug from the time it was started throughout the rest of the study period.

2.1.3 CART switch only

Participants who had CART change but no lipid lowering drugs, were assumed to have exercise and dietary advice as a standard practice.

CART change/switch was done by replacing an offending ARV drug due to side-effects with a more tolerable one or switching CART due to treatment failure to a more effective CART regimen

The CART use in this cohort includes a combination of 2 NRTI with a boosted PI or an NNRTI as according to South African and WHO antiretroviral therapy guidelines.[45-47] Antiretroviral drugs used were: EFV, LPVr, 3TC, ZDV, D4T, DDI, FTC, TNV, AZV, RTV, NVP.[45-47]

2.1.4 Combined lipid lowering drug and CART switch

Participants on lipid lowering drugs and CART change concurrently, were assumed to have exercise and dietary advice as a standard practice

2.2 Data manipulation

All data were collected from the first high serum cholesterol or serum triglycerides (total cholesterol ≥ 8.0mmol/l and/or triglyceride levels ≥4.52 mmol/l) between April 2008 and April 2011 on 3 monthly bases. Baseline values are values on the first visit when patient had first serum triglycerides and/or cholesterol levels high enough to be included in the study. Different treatment interventions were implemented based on the previous visit's total serum cholesterol and triglycerides levels. All other data collection were done on a 6 monthly bases.

Female participant who were pregnant between April 2008 and April 2011 were excluded. All participants who were on lipid lowering drugs on the first high serum cholesterol or serum triglycerides were also excluded. Antiretroviral drug changes were noted according to the date the respective drug was changed and recoded.

Variable body mass index was calculated using the given weight and height (weight/height^2).

Participants were recorded as being on lipid lowering drug according to the date on which the lipid lowering drug was started.

Participants were seen at the clinics every 3 months. Lipid levels (total serum cholesterol and serum triglycerides) and all other data collection were done every 6 months for each participant. Visits on which lipid levels were not done as according to the Phidisa IA protocol, were therefore dropped and not used in the analysis of data.

Participant's Phidisa and laboratory (BARC) identification numbers were used to collate data. Participants' visits were coded and arranged sequentially.

2.3 Data Analysis

Time-series data analysis on longitudinal or panel data was performed using Stata version 12. Participant Phidisa identity number was the panel variable and visit code (0-

6) was the time variable. All participants had a baseline visit (visit 0). The panel data was unbalanced as not all participants had all subsequent 6 visits.

Outcome variable serum triglyceride was log transformed as it was not normally distributed.

Lag-1 response variable was generated for outcome variables total serum cholesterol, serum triglycerides, CD4 count percentage and log of viral load.[48] First–order autoregressive-response model was specified by including the previous response of the outcome variable as a covariate in a linear regression model.[48] Each observation is not independent of previous observation of the same participant in the panel data and each variable has an equation explaining its evolution based on its own previous values of the same participant.[49]

For descriptive data, mean values at baseline, standard deviations and ranges of values for serum cholesterol and log of triglycerides were used. Body mass index was categorized while CD4 count and log of viral load were categorized according the WHO categories.[50]Health status variable was generated according to CD4 count level below or above 350cells/mm^3 (CD4 level recommended for ART initiation as according to 2010 WHO guidelines for adults and adolescents including pregnant women regardless of WHO clinical stage)[47]and viral load count below or above1000 copies/ml (plasma viral load level above which virological failure would be suspected).[47]

Interaction variables were not generated for simplicity.

Variables without collinearity and were plausibly explained biologically to influence levels of serum cholesterol and triglycerides were included in the regression models.

The Arellano–Bond estimator called xtabond is an extension of a generalized method of moments (GMM) problem; it sets up the model as a system of equations, one per time period, where the instruments applicable to each equation differ; equations in later time periods include additional lagged values of the instruments.[51]

Xtabond estimator is used in the analysis of this dynamic panel data as it is designed for situations with:[49, 51]

- ‘small T, large N’ panels: few time periods and many individual units
- a linear functional relationship
- one left-hand variable that is dynamic, depending on its own past realizations, each observation is not independent of previous observation of the same entity
- right-hand variables that are not strictly exogenous: correlated with past and possibly current realizations of the error
- fixed individual effects, implying unobserved heterogeneity
- heteroskedasticity and autocorrelation within individual units’ errors, but not across them

- **CHAPTER 3**

3.0 Results

In the period between April 2008 and April 2011, 449 participants met the inclusion criteria and 1 participant was dropped from the study due to high CD4 count secondary to non-Hodgkin's lymphoma. The participants contributed to 1861 follow-up visits, the follow-up visits ranged from 0 to 6 per participant, 92.5% of the visits were completed by end of visit 4.

Table 3: Descriptive table of the study participants at baseline

Variables	Values
	Mean (SD)
N	448
Age(years)	39.9 (4.6)
Weight (kg)	76.5 (13.1)
Height (m)	1.68 (0.2)
BMI (m/kg^2)	26.8 (4.4)
CD4 cell count(cells/mm^3)	402 (190.4)
CD4 percentage	18.48 (6.7)
Log of Viral Load (Median(IQR))	2.06 (1.7 to 2.8)

The mean age for a total of 448 participants were 39.9 years with a standard deviation of 4.6; males were 87%, females were only 13% as shown in Table 3 and 4 respectively. The mean body mass index at baseline was 26.8kg/m^2. Contrary to the usual, CD4 cell count was normally distributed, with the mean value at baseline of 402 cells/mm^3 (18.5%).

Diabetic participants were 56 (12.5%) and participants with lipodystrophy 94 (21.0%) compared to those without the respective conditions. Most participants, 47.6% were in the overweight category and at better health status, 45.1% at baseline; see table 4.

Table 4: Baseline summary statistics of total cholesterol and log of serum triglycerides by sex, diabetes, lipodystrophy, body mass index and health status

	Total serum cholesterol (mmol/l)			Log of serum triglycerides	
	n (%)	Mean (SD)	Range	Mean (SD)	Range
Sex					
Male	390(87.05)	5.77(1.64)	2.23 to 12.54	1.75(0.39)	0.25 to 3.90
Female	58(12.95)	6.49(1.79)	2.92 to 10.61	1.52(0.63)	-0.49 to 3.12
Diabetes					
No	392(87.5)	5.82(1.68)	2.23 to 12.54	1.70(0.45)	-0.49 to 3.9
Yes	56(12.5)	6.18(1.59)	3.55 to 11.18	1.82(0.36)	1.19 to 3.05
Lipodystrophy					
No	354 (79.02)	5.87(1.67)	2.23 to 12.54	1.71(0.44)	-0.49 to 3.9
Yes	94 (20.98)	5.83 (1.70)	3.09 to 12.21	1.76(0.42)	0.42 to 3.17
BMIcat	442(100)				
<18.5(underweight)	10(2.26)	5.79(2.38)	2.82 to 9.27	1.39(0.45)	0.58 to 1.98
>18.5 to 24.9(Normal)	138(31.15)	5.76(1.69)	2.82 to 12.54	1.70(0.42)	-0.07 to 3.90
>24.9 to 29.9(0verweight)	211(47.63)	5.84(1.58)	2.92 to 12.21	1.77(0.44)	0.25 to 3.68
>29.9(Obese)	83(18.78)	5.98(1.72)	3.65 to 12.54	1.68(0.40)	-0.49 to 2.80
Health Status					
CD4>350, VL<1000	202(45.09)	5.91(1.70)	2.23 to 12.54	1.78(0.42)	-0.07 to 3.67
CD4<350, VL<1000	163(36.38)	5.93(1.69)	2.92 to 12.54	1.65(0.47)	-0.49 to 3.90
CD4>350, VL>1000	42(9.38)	5.69(1.31)	3.65 to 8.54	1.71(0.41)	0.31 to 3.00
CD4<350, VL>1000	41(9.15)	5.49(1.80)	2.79 to 9.49	1.72(0.38)	0.58 to 2.77

Table 5: Adjusted and unadjusted total serum cholesterol levels.

Variables	Total Serum Cholesterol							
	Unadjusted				Adjusted			
	Coef	95% Confidence Interval		P-value	Coef	95% Confidence Interval		P-value
Age	-0.224	-0.321	-0.127	<0.001	-0.064	-0.186	0.056	0.292
BMI	0.070	-0.006	-0.146	0.069	0.099	0.027	0.17	0.007
CD4 Percent	-0.064	-0.092	-0.037	<0.01	-0.045	-0.074	-0.016	0.002
Log viral load	0.162	0.047	0.276	0.006	0.075	-0.04	0.19	0.201
Treatment Strategies:								
Exercise and dietary advice only	Reference group				Reference group			
Lipid-lowering drugs only	-0.396	-0.658	-0.135	0.003	-0.352	-0.611	-0.093	0.008
CART switch only	-0.674	-0.934	-0.414	<0.001	-0.561	-0.835	-0.288	<0.001
Combined lipid lowering drug and ART switch	-0.8	-1.121	-0.478	<0.001	-0.754	-1.078	-0.43	<0.001

Table 6: Adjusted and unadjusted serum triglycerides levels.

Variables	Serum Triglycerides						
	Univariate				Multivariate		
	Coef	95% Confidence Interval		P-value	Coef	95% Confidence Interval		P-value
Age	-0.343	-0.633	-0.052	0.021	-0.073	-0.425	0.288	0.684
BMI	0.185	-0.028	0.398	0.088	0.248	0.042	0.454	0.018
CD4 Percent	-0.087	-0.168	-0.005	0.037	-0.02	-0.125	0.055	0.447
Treatment Strategies: Exercise and dietary advice only	Reference group				Reference group			
Lipid-lowering drugs only	-1.049	-1.795	-0.304	0.006	-1.090	-1.846	-0.335	0.005
CART switch only	-1.659	-2.412	-0.906	<0.001	-1.779	-2.548	-1.01	<0.001
Combined lipid lowering drug and ART switch	-1.928	-2.848	-1.008	<0.001	-2.073	-3.008	-1.138	<0.001

Table 7: Adjusted total serum cholesterol and serum triglycerides levels, comparison between the treatment strategies.

Treatment Strategies:	Total Serum Cholesterol				Serum Triglycerides			
	Coef	95% Confidence Interval		P-value	Coef	95% Confidence Interval		P-value
Lipid-lowering drugs only	Reference group							
Exercise and dietary advice only	0.429	0.11	0.689	0.001	1.238	0.486	1.990	0.001
CART switch only	-0.116	-0.526	0.294	0.579	0.062	-1.141	1.265	0.920
Combined lipid lowering drug and ART switch	-0.283	-0.551	-0.016	0.038	-0.941	-1.71	-0.176	0.016
CART switch only	Reference group							
Exercise and dietary advice only	0.539	0.203	0.875	0.002	1.091	0.122	2.061	0.027
Lipid-lowering drugs only	0.109	-0.283	0.502	0.585	-0.199	-1.348	0.95	0.734
Combined lipid lowering drug and ART switch	-0.179	-0.55	0.191	0.342	-0.486	-2.183	-0.011	0.048

Table 8: Unadjusted CD4 cell percentage and log of viral load levels according to the treatment strategies.

	CD4 percentage				Log viral load			
	Coef	95% CI		P-value	Coef	95% CI		P-value
Treatment Strategies:								
Exercise and dietary advice only		Reference group				Reference group		
Lipid-lowering drugs only	0.084	-0.68	0.849	0.829	-0.113	-0.297	0.07	0.225
CART switch only	1.291	0.453	2.129	0.003	-0.36	-0.544	-0.176	<0.001
Combined lipid lowering drug and ART switch	0.576	-0.383	1.536	0.239	-0.486	-0.71	-0.261	<0.001

3.1. Total serum cholesterol

In the final adjusted linear regression model, mean change over time for total serum cholesterol increased by 0.099 mmol/l with an increase in body mass index (p=0.007), while an increase in CD4 cell percent decreased mean over time for total serum cholesterol by 0.045 mmol/l (p=0.002). Age and log viral load failed to maintain statistical significance in the adjusted model. When both lipid lowering drugs and CART switch treatment strategies were compared to exercise and dietary advice treatment strategy separately, the mean total serum cholesterol levels over time decreased by 0.352mmol/l and 0.561mmol/l with p-values of 0.008 and <0.001 respectively. Our hypothesis was confirmed when lipid lowering drugs and ART switch combined treatment strategy showed even more decrease in the mean total serum cholesterol levels over time by 0.754 mmol/l compared to the exercise and dietary advice treatment strategy (p<0.001). This effect was maintained both in the adjusted and unadjusted models, see Table 5. The same effect was only maintained when lipid lowering drugs and ART switch combined treatment strategy was compared to lipid lowering drugs only with a decrease in total serum cholesterol of 0.283 mmol/l (p=0.038) but failed to show statistical significant decrease in total serum cholesterol 0.179 mmol/l (p=0.342) when compared to CART switch only, see Table 7.

In final adjusted linear regression model, mean change over time for total serum cholesterol increased by 0.099 mmol/l with an increase in body mass index (p=0.007), while an increase in CD4 cell percent decreased mean over time for total serum cholesterol by 0.045 mmol/l (p=0.002). Age and log viral load failed to maintain statistical significance in the adjusted model. When both lipid lowering drugs and CART switch treatment strategies were compared to exercise and dietary advice treatment strategy separately, the mean total serum cholesterol levels over time decreased by 0.352mmol/l and 0.561mmol/l with p-values of 0.008 and <0.001 respectively. Our hypothesis was confirmed when lipid lowering drugs and ART switch combined treatment strategy showed even more decrease in the mean total serum cholesterol levels over time by 0.754 mmol/l compared to the exercise and dietary advice treatment strategy (p<0.001). This effect was maintained both in the adjusted and unadjusted

models, see Table 5. This effect was also maintained when lipid lowering drugs and ART switch combined treatment strategy was compared to lipid lowering drugs only with a decrease in the mean total serum cholesterol over time of 0.283 mmol/l (p=0.038) but failed to show statistical significant decrease in the mean total serum cholesterol over time of 0.179 mmol/l (p=0.342) when compared to CART switch only, see Table 7.

3.2. Serum triglycerides

An increase in body mass index increased the mean over time of serum triglycerides levels by 0.248 mmol/l (p=0.018) while an increase in CD4 percentage and age failed to show statistically significant decrease serum triglycerides, p=0.447 and p=0.684 respectively. When compared to the exercise and dietary advice group, lipid lowering drugs and CART switch treatment strategies decreased the levels of serum triglycerides by 1.090 mmol/l and1.779 mmol/l (p=0.005 and p<0.001) respectively; the same effect maintained when both strategies were combined and the mean levels of serum triglycerides over time by 2.073mmol/l (p<0.001), see Table 6. Combined lipid lowering drug and ART switch treatment strategy was effective in reducing the mean serum triglycerides over time when compared with either strategy separately; more decrease was when compared with lipid lowering drugs only with a decrease of 0.941mmol/l (p=0.016) and a marginally statistically significant decrease of 0.486mmol/l (p=0.048) when compared to CART switch only, see Table 7.

These results confirmed our primary objective that lipid lowering drugs and/or CART switching are effective in improving the mean total serum cholesterol and serum triglycerides over time in the management of HIV-associated dyslipidemia

3.3. CD4 percentage and log of viral load

Immunological response to the different treatment strategies was assessed using CD4 percentage.CD4 percentage increased by 1.291% when CART switch treatment strategy was compared to the exercise and dietary advice treatment strategy (p=0.003) but failed to maintain the same statistically significant effect in the category where lipid lowering drugs and ART switch treatment strategies were combined (p=0.39).

CART switch treatment strategy separately, and combined treatment strategies of lipid lowering drugs with ART switch showed significant virological suppression by decreasing log of viral load, 0.36 and 0.486 respectively when compared to the exercise and dietary advice group; p-values <0.001 in both groups (see Table 8)

- **CHAPTER 4**

4.0 Discussion

Our study shows that the mean over time of total serum cholesterol as a cardiometabolic risk factor in HIV infected patients; increases as body mass index with increases. This confirms the positive association of total serum cholesterol with high BMI as previously showed by Knuiman.[52] The mean over time total serum cholesterol decreased with improving immune response evidenced by increasing CD4 percentage suggestive of the cardiometabolic protective effect of improving immunological response. This confirms the findings in Krishnan et al. [53] study that cardiometabolic risk factors worsen with high body mass index and low CD4 count levels. The mean over time of serum triglycerides increased with an increase in body mass index therefore implying worsening of the cardiometabolic risk factors and increasing the risk of sensory peripheral neuropathy[54] and mortality.[55] High triglyceride levels are associated with increased risk for HIV-sensory neuropathy in HIV-positive individuals independently of other risk factors.[54]

Higher CD4 lymphocyte levels have been associated with higher lipid levels in HIV infected patients on CART. This needs to be verified in other larger studies.[56] A study done by Fourie et al. [57] in South Africa shows that HIV-1 subtype C is associated with dyslipidemia, but not with a higher incidence of metabolic syndrome in never antiretroviral-treated HIV-infected Africans.

Combining lipid lowering drugs and ART switching as a treatment strategy in the management of dyslipidemia has shown more beneficial effects in lowering the mean over time of both total serum cholesterol and triglycerides when compared to exercise and dietary advice strategy. The effect is better than when each strategy is compared to exercise and dietary advice strategy separately. Studies have shown that combining statins and fibrates is effective and safe in the treatment of dyslipidemia in HIV infected patients on CART.[8, 9] CART switching was not as effective as combining statins and fibrates in lowering lipid in the treatment of dyslipidemia in HIV infected patients.[9] The use of lipid lowering drugs combined with CART switch to ARV drugs with less side

effects was shown in this study to effectively reduce total serum cholesterol and serum triglycerides as shown by Williams et.al. [44] The lack of consistent reduction in the mean over time of total serum cholesterol when comparing combined lipid lowering drugs and CART switch treatment strategy to CART switch only may suggest other unmeasured multifactorial factors that contribute to the regulation of serum total cholesterol. The mean levels of serum triglycerides over time were consistently reduced when the combined lipid lowering drugs and CART switch treatment strategy was compared to either treatment strategy separately.

Viral suppression was maintained in all the treatment strategies when compared the exercise and dietary advice strategy. Immunological response with statistical significant increase in CD4 percentage was seen when CART was switched. These findings partly correlate with Manfredi et al.'s [43] work which showed that lipid lowering drugs in CART treated dyslipidemic patients had no substantial variations in the mean CD4 cell count, and the viral load levels were suppressed.

Due to diverse regions of location for sites in Phidisa project, fasting blood result could not be obtained. The mean serum triglycerides level in this cohort may be higher compared to other studies.

Safety and tolerability of the different treatment strategies was not assessed in this study. Lifestyle modification such as exercise and dietary advice, is the first step in treating patients with lipid abnormalities, enforcement of this practice in this cohort was not closely monitored. Other risk factors such as smoking history, alcohol intake and physical exercise were not collected. Dyslipidemia is part of a more complex HIV-associated dyslipidemic lipodystrophy with underlying body morphological changes, metabolic syndrome and insulin resistance, and the incidence and prevalence of diabetes are increasing in this population.[7]

More studies with cardiovascular risk outcomes are needed to show that combining lipid lowering drug and CART switch as a treatment strategy is effective and well tolerated in the management of dyslipidemia in HIV infected patients on ART.

4.1 Conclusion

Our study has shown that combining lipid lowering drugs and ART switching as a treatment strategy in the management of HIV-associated dyslipidemia is effective in lowering the mean over time of both total serum cholesterol and triglycerides when compared to exercise and dietary advice strategy, while maintaining virological suppression.

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