1. INTRODUCTION AND LITERATURE REVIEW

1.1 HIV Epidemiology

Acquired Immunodeficiency Syndrome (AIDS) is a worldwide pandemic caused by the Human Immunodeficiency Virus (HIV). HIV infection results in suppression of the immune system exposing the patient to increased risk of morbidity and mortality from opportunistic infections. Both HIV type 1 (HIV-1) and HIV type 2 (HIV-2) are associated with development of acquired immunodeficiency syndrome (AIDS). HIV-1 is responsible for the majority of infections globally while HIV-2 is predominantly found in West Africa.

In 2009 it was estimated that 33.3 million people were living with HIV worldwide with 2.6 million newly infected people. AIDS deaths were estimated to be 1.8 million people. In 2009, in Sub-Saharan Africa alone, people living with HIV were estimated to be 22.5 million and 1.3 million died from AIDS.

1.2 Burden of HIV in Zimbabwe

Zimbabwe is experiencing a generalised HIV epidemic with an adult prevalence rate of 15.6% according to the 2007 National HIV/AIDS estimates. HIV prevalence in Zimbabwe is believed to be declining as a result of combined effects of behaviour change, the impact of...
mortality and out migration. An estimated 1,320,739 Zimbabweans were living with HIV and AIDS at the end of 2007. Of these, 1,085,671 were people aged between 15 and 49 years. Among these 60% were women. Currently it is estimated that 500,000 Zimbabweans need antiretroviral therapy (ART) largely due to the WHO (World Health Organisation) recommendations that anyone with a CD4 of less than 350 cells/mm$^3$ should be initiated on ART regardless of their WHO clinical stage.

The strategy of providing HIV drugs to developing countries is being implemented worldwide including in Zimbabwe. As the life expectancy of people adhering to antiretroviral drugs has dramatically improved, the chronic complications of HIV infection and antiretroviral therapy (ART) have become increasingly relevant. It is now recognised that ARV (antiretroviral) treatment is associated with certain metabolic complications.

1.3 Antiretroviral drugs used in Zimbabwe

Zimbabwe has adopted the WHO recommended treatment strategy for HIV. The drugs used belong to four classes. These include nucleoside reverse transcriptase inhibitors (NRTIs) which block the HIV reverse transcriptase enzyme and prevent the copying of the viral RNA into the DNA of the infected host cells. NRTIs imitate the building blocks of the DNA chain. The resulting DNA is incomplete and cannot be assembled into virion particles.

Another class are nucleotide reverse transcriptase inhibitors (NtRTIs) which act at the same stage of the viral life cycle as do NRTIs but have a better resistance profile. Non nucleoside reverse transcriptase inhibitors (NNRTIs) block the HIV reverse transcriptase enzyme so that
the virus cannot polymerise. Lastly there are protease inhibitors which block the enzyme protease and prevent the assembly and release of HIV particles from infected cells.⁹

The first line regimen recommended for adults in Zimbabwe adopted from WHO recommendations, usually contain two NRTIs in combination with one NNRTI. This combination is less expensive and has generic formulations. The NRTIs include stavudine (d4T), lamivudine (3TC), tenofovir, didanosine and zidovudine (AZT). The NNRTIs include nevirapine (NVP) and efavirenz. Indinavir, lopinavir, saquinavir and ritonavir belong to the PIs class.⁹

The first line currently recommended in Zimbabwe is stavudine, lamivudine and nevirapine (marketed as stalanev or triviro) or zidovudine, lamivudine (marketed as zidolum) and nevirapine. Ideally, patients who fail to respond to first line treatment should be treated with a different regimen that contains drugs not used in the first regimen. The second line regimen will still consist of two NRTIs but with a PI. This regimen is only used when there is evidence of treatment failure with first line regimen.⁹

1.4 Metabolic complications in HIV

The use of effective ART has resulted in tremendous reductions in morbidity and mortality in HIV positive patients. However, the widespread use of effective ART has coincided with increasing reports of metabolic abnormalities such as impaired glucose metabolism and insulin resistance, lactic acidosis, lipodystrophy, dyslipidemia, cardiovascular disease and pancreatitis.¹⁰,¹¹,¹²,¹³,¹⁴,¹⁵
Although the drugs are generally well tolerated several studies have reported that treatment of patients with protease inhibitors causes dyslipidemia with elevated total cholesterol, low density lipoprotein cholesterol (LDL cholesterol) and triglyceride\textsuperscript{6,8,13,16}. Clinical observations, the National Cholesterol Education Program (NECP) results of clinical trials and other studies have documented metabolic effects on lipid metabolism by PIs, NRTIs and NNRTIs\textsuperscript{6, 8, 13, 14}. The PIs effect is related to increased hepatic very low density lipoprotein (VLDL) secretion. The NNRTI effect may be related to increased hepatic synthesis of apo A-1 and increased hepatic lipoprotein secretion\textsuperscript{13}. Forty seven percent of protease inhibitors recipients at one clinic had lipid abnormalities according to the (NECP) guidelines for intervention\textsuperscript{17}. Thus dyslipidemia seems to be a common occurrence among HIV infected individuals receiving treatment\textsuperscript{17}. Impaired lipid metabolism in HIV infection can also lead to lipodystrophy a condition which can either be lipohypertrophic or lipoatrophic. Patients with HIV lipodystrophy syndrome are at increased risk for development of atherosclerosis and glucose intolerance. These are associated with increased risk of progression to cardiovascular disease (CVD)\textsuperscript{7}.

1.5 Metabolism of lipids involved in CVD

Abnormal lipid metabolism plays a central role in the pathogenesis of CVD. Lipid fractions such as elevated LDL cholesterol and reduced HDL cholesterol contribute to the development of CVD. Thus LDL cholesterol is a positive risk factor for developing CVD whereas HDL cholesterol is a negative risk factor. LDL particle transports cholesterol to peripheral tissues and HDL transports cholesterol from the tissues to the liver where it is metabolised and excreted. Apo B 100 allows LDL particles to bind to specific receptors on the surface of cells particularly in the liver. The receptors transport LDL cholesterol into the cell where they are
catabolised to release cholesterol. The cholesterol is then used by the cell, stored or excreted from the body\textsuperscript{18}.

LDL is derived mainly from VLDL which is formed from triglycerides synthesised either de-novo or by esterification of free fatty acids. VLDL contains cholesterol, phospholipids apo B, apo C and apo E. VLDL is the principal transport form of endogenous triglyceride, and as triglycerides are hydrolysed by lipase, the VLDL becomes smaller and is converted to IDL (intermediate density lipoprotein). Some IDL particles are taken up by the liver via LDL receptors and some are converted to LDL. LDL is composed mainly of cholesterol and apo B 100.

\textbf{1.6 Dyslipidaemia and Cardiovascular Disease}

CVD is a collective term given to conditions such as atherosclerosis, coronary heart disease, stroke and acute myocardial infarction (AMI)\textsuperscript{19}. An association between dyslipidemia and CVD in general has long been recognised\textsuperscript{13}. Two general patterns of dyslipidemia are related to CVD. The first is increased concentration of LDL cholesterol, usually with a genetic predisposition, the second is elevated serum triglycerides and low HDL concentrations often observed in obese, diabetic and hypertensive patients\textsuperscript{13}. The Data Collection on Adverse Events of Anti HIV Drugs Study a prospective, multinational, observational study including cohorts from 21 countries has analysed over 150 000 person-years of follow up on ARV therapy. The initial objective was to determine the prevalence of CVD risk factors among HIV infected people and to investigate any association between risk factors, stage of HIV disease and use of ART. The study noted a high prevalence of CVD risk factors, particularly smoking, at baseline\textsuperscript{13}. Continued follow up for more than 7 years showed a progressive
increase in the risk for CVD particularly MI. CVD risk was related to drug class, with PI based therapy conferring an increased risk compared with NNRTI therapy\textsuperscript{13}.

Vascular disease is already the most common cause of death in the developed world and by 2020 may become the leading cause of death in the developing world as well\textsuperscript{17,20, 21, 22,23}. The link between dyslipidemia and CVD has been firmly established, first by epidemiologic studies and, more recently, by long-term outcome trials that demonstrated that lowering LDL-cholesterol levels significantly reduced the risk of CVD\textsuperscript{17,24}.

Many factors suggest that HIV-infected individuals are at greater risk for CVD as a result of the HIV infection itself or side effects of some antiretroviral agents\textsuperscript{8,10,13,20}. Reports suggest that premature vascular events may be related to protease inhibitors therapy and abnormal lipids such as elevated total cholesterol, LDL and triglycerides and low HDL\textsuperscript{13,16}.

Atherosclerosis is a complex series of biological responses to the trapping of an atherogenic particle within the arterial wall. Excess of apo B containing particles is a main trigger in the atherogenic process\textsuperscript{25}. High concentrations of LDL particles lead to competition at the apo B receptor. Since such a competition will prolong the residence time of LDL particles in the circulation, it may lead to greater opportunity for them to undergo oxidation or other chemical modification. Such modifications may lessen the particles’ ability to be cleared by LDL receptors and also alters the structure of apo B 100 making it a ligand for the scavenger receptors on macrophages. Macrophages laden with cholesterol become foam cells characteristic of fatty streaks. Fatty streaks typically consist of macrophages and T- cells embedded in a thin layer of lipids in the arterial wall. Oxidised LDL acquires new properties that are recognised as foreign by the immune system. Thus oxidised LDL produces several
abnormal biological responses, such as attracting leukocytes to the intima of the arterial wall, improving the ability of the leukocytes to ingest lipids which is a step in the formation of atherosclerotic plaque. Atherogenicity occurs when the resultant foam cells are cytotoxic to the arterial wall and stimulate inflammatory and thrombotic processes leading to CVD\textsuperscript{26,27,28}.

1.7 Apolipoprotein B 100 (apo B 100)

LDL cholesterol has been the cornerstone for diagnosis of lipid abnormalities and therapy\textsuperscript{29,30}. As the apolipoprotein constituents were recognised and characterised, awareness gradually developed that apo B 100, occurring as one molecule per LDL particle, was a more representative indicator of the concentration of LDL.

Apo B is a large glycoprotein with two isoforms; apo B 100 is synthesised in the hepatocytes, and apo B 48 is derived from the apo B 100 and is synthesised in the small intestines\textsuperscript{31}. Both apo B 48 and apo B 100 share a common N-terminal sequence, but apo B 48 lacks the C-terminal of apo B 100 which is responsible for LDL-receptor binding\textsuperscript{32}.

Apolipoproteins have three major functions which include modulating the activity of enzymes that act on lipoproteins, maintaining the structural integrity of enzymes that act on lipoproteins and facilitating the uptake of lipoproteins by acting as ligands for specific cell-surface receptors. Apo B 100 is a component of all atherogenic particles including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and lipoprotein (a). Therefore, apo B 100 provides a direct measure of the number of atherogenic lipoprotein in circulation\textsuperscript{33}. Because risk of atherosclerosis appears to be more directly related to the number of circulating atherogenic particles that enter the arterial wall
than to the concentration of cholesterol in these fractions, this would suggest that apo B 100 would be a better predictor of cardiovascular risk than the concentrations of cholesterol in the LDL fraction\textsuperscript{33,34}.

Over 90\% of the LDL particle is composed of apo B 100\textsuperscript{33}. Apo B 100 could therefore be a convenient marker for assessing the cholesterol depositing capacity of the blood, and studies have clearly indicated it is a better discriminator of coronary artery disease than LDL cholesterol\textsuperscript{22,30,32,34}. The Quebec Cardiovascular Study, the AMORIS study, the Thrombo study and the Northwick Park Heart Study all concluded that apo B is superior to total cholesterol or LDL cholesterol as an index of the risk of vascular disease\textsuperscript{30}.

Individuals with seemingly normal LDL cholesterol (<3.3 mmol/L) may in fact have high apo B 100 values and can still be at increased risk of cardiovascular events\textsuperscript{25,35}. This implies that current lipid guidelines for assessing CVD risk may miss these individuals\textsuperscript{36}. Among HIV-infected patients use of protease inhibitors is associated with an atherogenic lipid profile and increased apo B 100; thus evaluation of apo B 100 may prove useful to further define risk when LDL-cholesterol is normal and triglyceride levels are increased\textsuperscript{20}.

\textbf{1.8 Risk factors for CVD}

Use of apo B 100 in association with established risk factor guidelines may serve as important tools in the management of CVD and thereby reducing CVD risk. The Framingham Risk Score is one of the models available for calculating global CVD risk. The objective of the Framingham Heart Study was to identify common factors or characteristics that contribute to CVD development by following up participants who had not yet developed
symptoms of CVD or suffered a stroke or MI over a long period of time. Over the years careful monitoring of the Framingham Study population has led to the identification of major CVD risk factors such as high blood pressure, high blood total cholesterol, low HDL cholesterol, advanced age, being a male, cigarette smoking, obesity, diabetes, and physical inactivity\textsuperscript{37}. Low, intermediate and high risk are defined as 10-year risk of CVD of \textless{}10\%, 10\% to 20\% and \textgreater{}20\%, respectively\textsuperscript{20, 37}. In this study the Framingham Risk Score was used to assess CVD risk. An online calculator was used to calculate 10-year risk. This calculator incorporates, age, sex, systolic blood pressure, total cholesterol, HDL cholesterol, diabetes and smoking to calculate the 10-year CVD risk\textsuperscript{38}.

1.9 Justification

Many factors suggest that HIV–infected individuals are at greater risk for development of CVD as a result of the infection itself and/or side effects of some antiretroviral drugs. This study seeks to assess the role of apo B \textsuperscript{100} in determining risk of CVD in HIV-infected individuals visiting AOIC at Harare Central Hospital. Apo B \textsuperscript{100} is reported to be a better predictor of CVD than LDL-cholesterol. Apo B \textsuperscript{100} is a component of all atherogenic particles including VLDL, IDL, LDL and lipoprotein (a). Therefore, apo B provides a direct measure of the number of atherogenic lipoprotein in circulation. Individuals at increased risk of CVD must be identified to receive preventive therapy and this must be one of the nation's highest priorities. In Zimbabwe CVDs have not been addressed under specific control programmes such as those that exist for infectious diseases. The treatment of HIV infection now should focus on long-term management of diseases such as CVD and better markers to assess risk must be identified and evaluated.
Of note the NECP guidelines now include a category termed coronary heart disease “risk equivalents” which include diabetes mellitus, other atherosclerotic disease and multiple risk factors that confer a 10 year risk of CVD of >20%. Because of the high risk of CVD in HIV individuals, they should perhaps be treated as those with established CVD\textsuperscript{17}. Risk of CVD in the HIV-infected population appears to be relatively higher than in the general population and thus appropriate screening strategies for CVD in this population are needed\textsuperscript{20}. Approaches to screening and assessment of CVD in HIV infected individuals were discussed at a State of the Science Conference in America\textsuperscript{20}. There is growing support that addition of apolipoprotein B100 measurement to the routine lipid panel would enhance patient management\textsuperscript{31}.

1.10 Research Question

Should apoB100 measurement be included to the routine lipid panel in risk profiling people at risk of developing CVD?

1.11 OBJECTIVES

1.11.1 Main Objective

The main objective of the study is to assess the potential usefulness of measuring apo B 100 in evaluation of CVD in HIV positive patients.
1.11.2 Specific Objectives

To evaluate performance of apo B method.

To assess the proportion at risk of CVD defined by each lipid parameter.

To determine the correlation between apo B and Framingham risk scores.

To determine the correlation between LDL-c and Framingham risk scores.

To determine the correlation between apo B and LDL-c.

To assess the effect of CVD risk factors on lipid parameters.
CHAPTER TWO

2 Methodology and Materials

2.1 Study design

In this cross sectional study 186 participants were enrolled at Harare Hospital adult opportunistic infectious clinic. These included ART naive and those on ART for varying durations. An HIV negative group was used as a control. A questionnaire eliciting socio-demographic data was administered prior to collection of a blood sample from each consenting participant for evaluation of lipids status.

2.2 Participants and Setting

HIV infected adult males and females between the ages of 18 to 65 years visiting Harare Hospital adult OIC who consented to participate in the study were enrolled. Harare Hospital adult OIC caters for patients in the western high density suburbs of the capital city Harare. These suburbs include Kambuzuma, Glen Norah, Glen View, Budiriro, Highfields, Mufakose and any suburb within its 30km radius. Most complicated cases from other cities, like treatment failure are also referred to Harare Hospital.

Although understaffed with 2 sisters in charge, 10 general nurses, 2 permanent senior doctors, 1 clinic head and 4 junior doctors who rotate on monthly basis, the clinic attends to about 500 patients per week. Adherence to medication is ensured by use of pill charts. Reviews are systematic after every 4 months whereby patients are resupplied with drugs and a review
register is available. A defaulter’s book is also in place and community sisters follow up these patients.

Participants were interviewed as they came for resupply of drugs or review. The enrolled participants included 60 ART naive patients and 35 of these were females. Sixty two HIV patients on treatment were also enrolled and 47 of these were females. A control group of 64 HIV negative participants were also enrolled and 52 of these were females. These were recruited from staff at Harare Hospital.

Participants completed a health and lifestyle questionnaire which also elicited drug regimen and treatment duration information. Participants who smoked regularly during the previous one month were classified as smokers. Blood pressure was measured and recorded. Hypertension was defined as either a systolic blood pressure ≥ 140 mmHg, and/or a diastolic blood pressure ≥ 90 mmHg, or on drugs for treatment for hypertension. Blood pressure was measured with the patient in a relaxed sitting position using an upper arm blood pressure monitor (Omrom M2 Basic manufactured by Omrom Healthcare, Japan). Two readings were taken 5 minutes apart and an average was calculated. Diabetes was considered present if the participant was on treatment with insulin or oral hypoglycaemic agents or fasting blood glucose of ≥ 7.0 mmol/L or random blood glucose of ≥ 11.0 mmol/L.

2.3 Study Factors

Blood concentrations of apo B 100, total cholesterol, LDL cholesterol, HDL cholesterol and glucose were the study factors. The Framingham risk score was also another study factor.
2.4 Outcome factors

Risk scores of developing CVD was the outcome investigated.

2.5 Inclusion Criteria

All HIV positive and negative adults between the ages 18 to 65 years who consented to participate in the study.

2.6 Exclusion Criteria

All patients who were on anti-tuberculosis treatment (to rule out interference of lipid metabolism by TB drugs) were to be excluded in the study.

Other self reported conditions which cause secondary hyperlidaemias like hypothyroidism and liver disease were also to be excluded.

2.7 Sample Size and Sampling Procedures

2.7.1 Sampling Procedure

Patients were interviewed as they came in for review and resupply of drugs. Participants were recruited consecutively until the sample size was reached.
2.7.2 Sample Size

A sensitivity analysis was used to calculate the sample size for each factor (apoB and LDL-C). Sensitivity analysis was based on an odds 1.68 and 2.64 of CVD in HIV negative patients (based on comparison of upper versus lower quartile of LDL-C and ApoB respectively) and assuming a 1.5-2 fold greater risk in HAART naïve and patients on HAART\textsuperscript{13}. The highest sample size was considered to come up with the final sample size that caters for all factors. Sample size of 204 was calculated based on the study factor LDL-C. Sample size was generated by computer using Power and Sample Size Calculations Version 3.0.13. A sample size of 186 was then used due to financial constraints.

2.8 Laboratory Methods

2.8.1 Sample collection

5mls of blood was collected by venepuncture into plain tubes and allowed to clot. Samples were centrifuged in a Hettich Rotanta 96 centrifuge (manufactured in Germany) for 3 minutes at 3000 rpm to harvest serum. Serum samples were stored at -80\textdegree C until time of analysis.

2.8.2 Principles of methods

LDL, HDL, total cholesterol and glucose were measured by enzymatic methods using reagents from the manufacturer on the Dimension, Dade Behring analyser (Siemens Healthcare Diagnostics). After calibration, controls were assayed. Controls and samples were
analysed according to standard operating procedures. Apo B was also measured by immunoturbidimetric method on the same analyser.

2.8.2.1 Apo B

The determination was based on the turbidimetric specific reaction which occurred between the anti – apolipoprotein B polyclonal antiserum and its corresponding antigen in optimal pH conditions and in the presence of polyethylene glycol polymer (PEG). The turbidity of the immunocomplex was proportional to the concentration of the analyte in the sample and the wavelength used was 340nm\textsuperscript{39}.

2.8.2.2 Total Cholesterol

Cholesterol esterase catalyses the hydrolysis of cholesterol esters to produce free cholesterol which was oxidised in a reaction catalysed by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase, the hydrogen peroxide oxidised a chromogen to produce a chromophore that absorbed at 540 nm. The absorbance was directly proportional to the total cholesterol concentration\textsuperscript{40}.

2.8.2.3 HDL Cholesterol

HDL cholesterol was measured in a two step reaction. In the first reaction chylomicrons, VLDL and LDL formed water soluble complexes with dextran sulphate in the presence of magnesium sulphate. These complexes were resistant to polyethylene glycol-modified
cholesterol esterase and cholesterol oxidase that reacted with HDL cholesterol. In the presence of oxygen the HDL cholesterol was oxidised to cholest-4-ene-3-one and hydrogen peroxide. The generated hydrogen peroxide reacted with 4-aminoantipyrine in the presence of peroxidase to form a coloured dye that was measured using a bichromatic (600 / 700 nm) endpoint technique. The colour intensity was directly proportional to the serum HDL cholesterol concentration.

2.8.2.4 LDL Cholesterol

The method was in a two reagent format and depended on the properties of one detergent which solubilised only non-LDL particles and the cholesterol released was consumed by cholesterol esterase and cholesterol oxidase in a non colour forming reaction.

Detergent two solubilised the remaining LDL particles. The soluble LDL cholesterol was then oxidised by the action of cholesterol esterase and cholesterol oxidase forming cholest-4-ene-3-one and hydrogen peroxide. The enzymatic action of peroxidase on hydrogen peroxide produced colour in the presence of toluidine, disodium salt and 4-aminoantipyrine that was measured using a bichromatic (540,700nm) endpoint technique. The colour produced was directly proportional to the amount of LDL cholesterol present in the sample.

2.8.2.5 Glucose

Hexokinase catalysed the phosphorylation of glucose in the presence of adenosine-5-triphosphate(ATP) and magnesium to form glucose- 6- phosphate (G-6-P) and adenosinediphosphate(ADP). G-6-P was then oxidised by glucose-6-phosphate
dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) to produce 6-phosphogluconate and NADH. One mole of NAD was reduced to one mole of NADH to each mole of glucose present. The absorbance due to NADH (and thus the glucose concentration) was determined using a bichromatic (340 and 383 nm) endpoint technique\textsuperscript{40}.

2.8.2.6 Evaluation of apo B method

Since the method had never been used at Harare Hospital Laboratory it was necessary to assess the performance of the method. To evaluate a method inter assays and intra assays must be carried out and the mean, SD and % CV calculated. Another way is to compare results with a gold standard method. Only intra assay was performed due to limited reagents.

2.9 Preferred Cut-off Values

Hypercholesterolemia was defined as total cholesterol concentration of ≥ 6, 2 mmol/L, low HDL cholesterol defined as ≤ 1,1 mmol/L, high LDL cholesterol defined ≥ 3,3 mmol/L and high apo B100 of ≥ 1,2 g/L\textsuperscript{41}.

2.10 Statistical Analysis

Demographic and clinical factors were described using percentage for categorical data. Mean and standard deviation was used to describe normally distributed continuous data. Median was used describe non-normally distributed data. Categorical data was compared between
groups using Chi-squared test. Continuous normally distributed data was compared between groups using ANOVA and the Kruskal Wallis test for non-normal continuous data. Multivariate linear regression analysis was used to determine factors independently associated with lipid parameters. Factors were included into the multivariate analysis if they were at least weakly associated (p<0.30) with any of the lipid parameters. A p-value <0.05 was considered as statistically significant. All data was analysed using STATA 10 (College Station, Texas).

2.11 Ethical Consideration

Ethical approval was sought from the Clinical Director of Harare Central Hospital and the Joint Parirenyatwa Hospital and Health Sciences Research Ethics Committee. Each participant gave written informed consent before enrolment into the study.
CHAPTER 3: RESULTS

Performance of Apo B method on Dimension Xpand analyser

Repeatability was determined on 18 replicates of one level of controls manufactured by Thermo Scientific in Germany. Intra assay results were as follows.

Table 1: Performance of Apo B method on Dimension Xpand analyser

<table>
<thead>
<tr>
<th>Level</th>
<th>MEAN</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 2</td>
<td>0.48</td>
<td>0.015</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Ranges for level 2 controls was 0.35 – 0.52 g/L. This method was reproducible since it had a CV of <5%. Reproducibility by assaying for several days could not be achieved due to limited reagents.
Table 2: Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>HIV negative n=64</th>
<th>ART-naive n=60</th>
<th>On ART n=62</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>38.3 (10.4)</td>
<td>36.3 (11.2)</td>
<td>37.1 (10.1)</td>
<td>41.6 (9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>134 (72%)</td>
<td>52 (81.3%)</td>
<td>35 (58.3%)</td>
<td>47 (75.8%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Male</td>
<td>52 (28%)</td>
<td>12 (18.7%)</td>
<td>25 (41.7%)</td>
<td>15 (24.2%)</td>
<td></td>
</tr>
<tr>
<td>Smoking status n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>4 (2.1%)</td>
<td>2 (3.1%)</td>
<td>2 (3.3%)</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>Former</td>
<td>5 (2.7%)</td>
<td>0</td>
<td>3 (5%)</td>
<td>2 (3.2%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>177 (95.2%)</td>
<td>62 (96.8%)</td>
<td>55 (91.7%)</td>
<td>60 (96.8%)</td>
<td></td>
</tr>
<tr>
<td>Drinking status n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>13 (7%)</td>
<td>4 (6.3%)</td>
<td>7 (11.7%)</td>
<td>2 (3.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former</td>
<td>34 (18.3%)</td>
<td>2 (3.1%)</td>
<td>19 (31.7%)</td>
<td>13 (21%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>139 (74.7%)</td>
<td>58 (90.6%)</td>
<td>34 (56.6%)</td>
<td>47 (75.8%)</td>
<td></td>
</tr>
<tr>
<td>Family CVD history n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (7.5%)</td>
<td>2 (3.1%)</td>
<td>6 (10%)</td>
<td>6 (9.7%)</td>
<td>0.24</td>
</tr>
<tr>
<td>No</td>
<td>172 (92.5%)</td>
<td>62 (96.9%)</td>
<td>54 (90%)</td>
<td>56 (90.3%)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure ( mmHg )</td>
<td>130.1 (20.8)</td>
<td>131.7 (16.5)</td>
<td>123.8 (21.4)</td>
<td>134.5 (23.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure ( mmHg)</td>
<td>81.4 (14.0)</td>
<td>78.3 (13.0)</td>
<td>79.6 (14.7)</td>
<td>86.3 (13.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol level (mmol/L)</td>
<td>4.2 (1.4)</td>
<td>3.6 (1.1)</td>
<td>3.9 (1.3)</td>
<td>5.0 (1.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol level (mmol/L)</td>
<td>2.0 (0.8)</td>
<td>1.7 (0.7)</td>
<td>1.9 (0.7)</td>
<td>2.4 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol level (mmol/L)</td>
<td>1.4 (0.6)</td>
<td>1.6 (0.7)</td>
<td>1.4 (0.5)</td>
<td>1.1 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*Median triglycerides (mmol/L)</td>
<td>1.2 (0.9-1.8)</td>
<td>1.2 (0.9-1.6)</td>
<td>1.1 (0.9-1.7)</td>
<td>1.4 (0.9-2.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>Glucose levels (mmol/L)</td>
<td>4.4 (0.9)</td>
<td>4.4 (0.8)</td>
<td>4.5 (1.0)</td>
<td>4.5 (1.0)</td>
<td>0.99</td>
</tr>
<tr>
<td>Apolipoprotein B level (g/L)</td>
<td>0.6 (0.3)</td>
<td>0.5 (0.2)</td>
<td>0.5 (0.2)</td>
<td>0.6 (0.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: data are mean (sd) unless otherwise stated, *Kruskal Wallis p-value
One hundred and eighty six participants were enrolled into this study. Sixty four were HIV negative (34.4%), 60 (32.3%) were ART naïve and 62 (33.3%) were HIV patients on treatment. The mean age of all the participants was 38.3 (10.4) years. More of the enrolled participants were female (72%), there were statistically significant differences in gender distribution between the groups (p=0.014). More of the participants never smoked (95%), 5 (2.7%) were former smokers and 4 (2.1%) were current smokers. Thirteen participants (7%) were current drinkers, 34 (18.3%) were former drinkers and 139 (74.7%) never drank beer. Those with a family history of CVD were 14 (7.5%) and those without were 172 (92.5%). In this study, patients on ART were significantly older than patients not on ART and HIV negative participants (p<0.001). Drinking status, blood pressure, serum total cholesterol LDL-c, HDL-c and Apo B concentrations were all statistically significantly different between the three groups HIV negative, HIV ART naive and HIV on treatment (p<0.05).
Proportions of participants with a risk of developing CVD as defined by total cholesterol $\geq 6.2$ mmol/L was statistically significantly different between the three groups HIV negative, HIV ART naive and HIV on treatment ($p$ value $< 0.05$). About $3\%$ of HIV negative people, $5\%$ of ART naive patients and $18\%$ of HIV on ART patients had risk of developing CVD. Assessment of risk of CVD as defined by total cholesterol showed a strong dose response relationship between risk of CVD and exposure to ART (Chi-square test for trend; $p<0.001$).
Proportions of participants with risk of developing CVD as defined by LDL cholesterol $\geq 3.3$ mmol/L was statistically significant different between the three groups HIV negative, HIV ART naive and HIV on treatment ($p$ value $< 0.05$). Risk of having CVD as defined by serum LDL cholesterol concentration was also statistically significant different between the three groups HIV negative, HIV ART naive and HIV on treatment with a $p$ value of $< 0.05$. About 3% of HIV negative people, 5% of ART naive patients and 18% of HIV on ART patients had risk of developing CVD. Assessment of risk of CVD as defined by LDL cholesterol showed
a strong dose response relationship between risk of CVD and exposure to ART (Chi-square test for trend; p=0.002)

**Figure 3: Risk of CVD defined by HDL-c < 1.1 mmol/L**

Proportions of participants with risk of developing CVD as defined by HDL cholesterol <1.1 mmol/L was statistically significant different between the three groups HIV negative, HIV ART naive and HIV on treatment (p value < 0.05). About 18% of HIV negative people, 33% of ART naive patients and 63% of HIV on ART patients had risk of developing CVD
Assessment of risk of CVD as defined by HDL cholesterol showed a strong dose response relationship between risk of CVD and exposure to ART (Chi-square test for trend; p<0.001).

Figure 4: Risk of CVD defined by Apo B 100 ≥ 1.2g/L

Risk of CVD defined by apo B was not statistically significantly different ( p= 0.15) between the three groups. About 2% of both participants of HIV negative and ART naive had risk of developing CVD. HIV positive on treatment had the largest proportion of about 5% of people at risk of developing CVD.
There was a positive weak significant correlation between Framingham risk score and apo B

\( r = 0.26, p < 0.001 \).
There was a weak positive correlation between Framingham risk score and LDL cholesterol ($r = 0.144$, $p=0.11$).
Figure 7: Correlation between apo B and LDL cholesterol

There was a moderate positive significant correlation between apo B and LDL cholesterol 
($r=0.44, p<0.001$).
Risk factors associated with lipid parameter in HIV negative patients, ART-naïve HIV patients and HIV patients on ART

Risk factors associated with lipid parameters were assessed by a multivariate analysis. Factors included in the multivariate analysis included those that were significantly associated or weakly associated (p<0.30) with the lipid parameter in the univariate analysis.

Table 3: Multivariate linear regression analysis for variables associated with lipid parameters in HIV patients on ART

<table>
<thead>
<tr>
<th></th>
<th>n (%) or median (iqr)</th>
<th>TC effect (se)</th>
<th>P</th>
<th>LDL-C effect (se)</th>
<th>P</th>
<th>HDL-C effect (se)</th>
<th>P</th>
<th>Apo-B effect (se)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>-0.25 (0.18)</td>
<td>0.18</td>
<td>+0.10 (0.14)</td>
<td>0.46</td>
<td>+0.09 (0.1)</td>
<td>0.36</td>
<td>+0.077 (0.08)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Age per 10 year</td>
<td>-0.008 (0.07)</td>
<td>0.91</td>
<td>+0.013 (0.05)</td>
<td>0.81</td>
<td>+0.001 (0.04)</td>
<td>0.98</td>
<td>-0.02 (0.03)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>+0.11 (0.33)</td>
<td>0.74</td>
<td>-0.059 (0.25)</td>
<td>0.81</td>
<td>-0.072 (0.17)</td>
<td>0.68</td>
<td>-0.16 (0.15)</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>+0.28 (0.17)</td>
<td>0.11</td>
<td>-0.40 (0.35)</td>
<td>0.26</td>
<td>-0.11 (0.25)</td>
<td>0.68</td>
<td>+0.035 (0.21)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>+0.50 (0.47)</td>
<td>0.29</td>
<td>-0.12 (0.13)</td>
<td>0.37</td>
<td>-0.11 (0.09)</td>
<td>0.23</td>
<td>-0.11 (0.08)</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>ART years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>+0.38 (0.17)</td>
<td>0.03</td>
<td>-0.25 (0.13)</td>
<td>0.07</td>
<td>-0.19 (0.09)</td>
<td>0.04</td>
<td>+0.019 (0.08)</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>+0.078 (0.15)</td>
<td>0.61</td>
<td>-0.038 (0.12)</td>
<td>0.74</td>
<td>-0.05 (0.08)</td>
<td>0.53</td>
<td>-0.016 (0.07)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>+0.01 (0.17)</td>
<td>0.95</td>
<td>+0.028 (0.13)</td>
<td>0.82</td>
<td>-0.03 (0.09)</td>
<td>0.75</td>
<td>-0.056(0.07)</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.001 (0.003)</td>
<td>0.76</td>
<td>+0.005 (0.002)</td>
<td>0.83</td>
<td>+0.004 (0.001)</td>
<td>0.79</td>
<td>+0.001 (0.001)</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.073 (0.06)</td>
<td>0.24</td>
<td>+0.051 (0.05)</td>
<td>0.28</td>
<td>+0.07 (0.03)</td>
<td>0.04</td>
<td>+0.033 (0.03)</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>
Every 10 year increase in age had a positive effect on levels of LDL-c and HDL-c although not statistically significantly different. Being a former smoker increased total cholesterol levels by 0.11 mmol/L, this increase was however not statistically significantly different (p=0.74). Higher systolic blood pressure had the effects of increasing all the lipid parameters though not statistically significantly different. Higher glucose levels significantly increased HDL-c levels by 0.07 mmol/L (p=0.04), similar effect were observed for LDL-c and apo B though not statistically significantly different. In HIV-infected patients on ART for the parameter total cholesterol, only duration of ART remained significantly associated with total cholesterol. Compared to those on ART for less than 1 year, those on ART for 1-2 years had significantly +0.38mmol/L higher total cholesterol (p=0.03). None of the covariates were independently associated with LDL-c, however duration of ART was weakly associated (p=0.07) and had the effects of lowering the LDL-c levels by 0.25mmol/L for an individual on ART for 1-2 years.
Table 4: Multivariate linear regression analysis for variables independently associated with lipid parameters in HIV infected ART naive patients

<table>
<thead>
<tr>
<th></th>
<th>n (%) or median (iqr)</th>
<th>TC effect (se)</th>
<th>P</th>
<th>LDL-C effect (se)</th>
<th>P</th>
<th>HDL-C effect (se)</th>
<th>P</th>
<th>Apo-B effect (se)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>-0.04 (0.21)</td>
<td>0.86</td>
<td>0.96</td>
<td>+0.03 (0.16)</td>
<td>0.84</td>
<td>-0.02 (0.08)</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per 10 year</td>
<td>-0.015 (0.07)</td>
<td>0.83</td>
<td>0.42</td>
<td>-0.012 (0.05)</td>
<td>0.83</td>
<td>-0.007 (0.03)</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>-0.09 (0.42)</td>
<td>0.84</td>
<td>0.75</td>
<td>-0.03 (0.33)</td>
<td>0.93</td>
<td>-0.06 (0.17)</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>+0.49 (0.29)</td>
<td>0.10</td>
<td>0.09</td>
<td>-0.38 (0.23)</td>
<td>0.10</td>
<td>+0.07 (0.11)</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>+0.23 (0.26)</td>
<td>0.37</td>
<td>0.56</td>
<td>+0.03 (0.20)</td>
<td>0.88</td>
<td>+0.04 (0.1)</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>-0.18 (0.21)</td>
<td>0.41</td>
<td>0.24</td>
<td>+0.03 (0.17)</td>
<td>0.84</td>
<td>-0.05 (0.08)</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pressure</td>
<td>-0.007 (0.003)</td>
<td>0.05</td>
<td>0.07</td>
<td>+0.01 (0.002)</td>
<td>0.001</td>
<td>-0.002 (0.001)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+0.14 (0.08)</td>
<td>0.07</td>
<td>0.15</td>
<td>-0.19 (0.05)</td>
<td>0.001</td>
<td>+0.05 (0.03)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Every 10 year increase in age had a positive effect on LDL- c levels which was not statistically significantly different (p value 0.42). In the former smoker it was observed that there was an increase in total cholesterol by 0.49 mmol/L and apo B by 0.07 g/L although not statistically significantly different. For a current drinker it was observed that there was an increase in total cholesterol by 0.23 mmol/L, HDL- c by 0.03 mmol/L and apo B by 0.04g/L although not statistically significantly different. Systolic blood pressure had a positive effect on LDL-c (p=0.07) and there was also a positive effect on HDL-c which was statistically significantly different (p = 0.001). Glucose had a positive effect on total cholesterol which
was weakly associated (p=0.07) and apo B but not statistically significantly different (p
value=0.13)

Table 5: Multivariate linear regression analysis for variables associated with lipid
parameters in HIV negative patients

<table>
<thead>
<tr>
<th>n (%) or median (iqr)</th>
<th>TC effect (se)</th>
<th>P</th>
<th>LDL-C effect (se)</th>
<th>P</th>
<th>HDL-C effect (se)</th>
<th>P</th>
<th>Apo-B effect (se)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>-0.22 (0.18)</td>
<td>0.23</td>
<td>+0.15 (0.11)</td>
<td>0.19</td>
<td>+0.36 (0.24)</td>
<td>0.14</td>
<td>-0.03 (0.14)</td>
<td>0.80</td>
</tr>
<tr>
<td>Age per 10 year</td>
<td>+0.01 (0.05)</td>
<td>0.80</td>
<td>-0.02 (0.03)</td>
<td>0.62</td>
<td>+0.05 (0.07)</td>
<td>0.46</td>
<td>+0.004 (0.04)</td>
<td>0.91</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>-0.09 (0.40)</td>
<td>0.82</td>
<td>+0.15 (0.25)</td>
<td>0.55</td>
<td>-0.08 (0.54)</td>
<td>0.88</td>
<td>+0.12 (0.3)</td>
<td>0.70</td>
</tr>
<tr>
<td>Former</td>
<td>+0.21 (0.39)</td>
<td>0.59</td>
<td>-0.23 (0.25)</td>
<td>0.34</td>
<td>-0.49 (0.52)</td>
<td>0.35</td>
<td>-0.14 (0.29)</td>
<td>0.60</td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>-0.04 (0.35)</td>
<td>0.92</td>
<td>-0.05 (0.22)</td>
<td>0.80</td>
<td>-0.25 (0.46)</td>
<td>0.59</td>
<td>-0.14 (0.25)</td>
<td>0.60</td>
</tr>
<tr>
<td>Former</td>
<td>+0.01 (0.03)</td>
<td>0.70</td>
<td>+0.002 (0.002)</td>
<td>0.27</td>
<td>-0.003 (0.004)</td>
<td>0.47</td>
<td>+0.001 (0.002)</td>
<td>0.65</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.001 (0.003)</td>
<td>0.57</td>
<td>-0.03 (0.05)</td>
<td>0.49</td>
<td>-0.06 (0.10)</td>
<td>0.57</td>
<td>+0.16 (0.05)</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>+0.04 (0.08)</td>
<td>0.57</td>
<td>-0.03 (0.05)</td>
<td>0.49</td>
<td>-0.06 (0.10)</td>
<td>0.57</td>
<td>+0.16 (0.05)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

An increase of 10 years in age in the HIV negative group had a positive increase on total
cholesterol by 0.01 mmol/L, HDL-c by 0.05mmol/L and apo B by 0.004g/L which were not
statistically significantly different. For a current smoker it was observed it had a positive
effect on LDL-c and apo B although not statistically significantly different. Higher systolic
blood pressure had a positive effect also on LDL-c and apo B although not statistically
significantly different. Glucose only affected total cholesterol although not statistically
significantly different but increase in apo B of 0.16g/L was statistically significantly different (p value <0.05).
CHAPTER 4

4.0 DISCUSSION

Mean age, drinking status, blood pressure, serum total cholesterol concentration, LDL-c, HDL-c and apo B concentrations were statistically significantly different (p<0.001) between the three groups HIV negative, HIV positive ART naive and HIV positive on treatment. The mean total cholesterol concentration for those on ART was 5.0 (±1.4) mmol/l whereas for the ART naïve it was 3.9(±1.3) mmol/L and HIV negative it was 3.6 (±1.1) mmol/L. The mean total cholesterol concentration for those on ART was higher than the other groups. According to the Framingham Heart Study, people on ART have a greater risk of developing CVD since high total cholesterol has been implicated in atherogenesis.

The mean LDL-c concentration for those on ART was significantly higher (2.4 mmol/L ± 0.9) than that of ART naïve patients (1.9mmol/L±0.7). The mean LDL-c for the HIV negative was the lowest (1.7mmol/L±0.7). The mean for HDL was higher in the HIV negative group (1.6mmol/L±0.7) than in the ART naïve (1.4mmol/L±0.5). In the ART adherent group the HDL-c concentration was lowest (1.1mmol/L±0.5). A high HDL-c concentration is considered to be protective against heart disease and therefore HIV negative group had a low risk of developing CVD. HIV positive on ART had the highest mean in apo B levels, 0.6 (0.2) g/L as compared to the other groups who both had the same mean 0.5(0.2) g/L. The findings showed that ART seems to increase lipid parameters. However the mean systolic blood pressure for the HIV negative group was higher than the ART naïve group, 131.7± 16.5 and 123±21.4 respectively. This could have been to the fact that the group was
not healthy even though they were HIV negative or it could have been due to the small sample size used. Hypertension is also a risk factor for CVD.

There could have been a bias on the drinking status and smoking status since these were self reported. In this study it was observed that 95.2% of patients enrolled never smoked. This was commendable since tobacco smoking is one of the clearest CVD risk factors. There were 74.7% of participants who never drank beer, 18.3% former drinkers and 7% drank beer.

Risk of having CVD as defined by total cholesterol was statistically significantly different between the three groups HIV negative, HIV ART naive and HIV on treatment (p<0.05). This implies that that ART has an impact on the lipid parameters as there is an increase in proportion at risk of CVD from HIV negative to HIV ART naive and finally to HIV on treatment patients.

Risk of having CVD as defined by serum LDL cholesterol concentration was also statistically significantly different between the three groups HIV negative, HIV ART naive and HIV on treatment. This also implies that ART has an impact on the lipid parameters as there is an increase in LDL cholesterol concentration from HIV negative to HIV ART naive and finally to HIV on treatment patients. These findings in this study can be related to other studies which have documented that there is metabolic effects on lipid metabolism by PIs, NRTIs and NNRTIs. In Donald P. Kotler´s paper which reviewed the contribution of both HIV infection and the different components of highly active antiretroviral therapy to dyslipidemia, it was summarised that the average patient initiating ARV therapy is likely to experience a moderate rise in serum lipids. In Jean Michel Petit´s study, his results summarised that PIs could have a direct effect on lipid metabolism leading to an increase in the production of
VLDL-apo B and IDL-apo-B\textsuperscript{14}. Kate Buchacz and others also concluded in their study that rural Ugandans on nevirapine or efavirenz based HAART experienced elevations in total cholesterol, LDL-c and triglycerides\textsuperscript{42}.

The mean serum HDL cholesterol concentration was also statistically significantly different between the three groups HIV negative, HIV ART naive and HIV on treatment. In the study population data, it showed that HIV on treatment patients had the lowest HDL cholesterol concentrations with a mean of 1.1mmol/L compared to HIV on treatment patients who had a mean concentration of 1.4mmol/L and HIV negative who had a mean concentration of 1.6mmol/l. This also implies that ART has an effect on the lipid parameters. However Buchacz and others found out that HDL –c increases in HIV positive on ART patients\textsuperscript{42}.

Those on ART for 1-2 years (11 patients) had significantly +0.38mmol/L higher total cholesterol (p=0.03) than the 2 to 3 years (18 patients) and the greater than 3 years (18 patients) durations although after 2 to 3 years the increase was not significant. This finding is almost the same with the findings done in rural Uganda by Kate Buchaaz and others although the findings had increases in both total cholesterol and HDL cholesterol\textsuperscript{42}. In line with what has been discussed above this supports the fact that lipid parameters should be monitored routinely in HIV positive on ART patients.

The risk factors for CVD used in this study like age, smoking status, drinking status, diabetes and blood pressure had a positive effect on the lipid parameters analysed. This is consistent with the findings of the Framingham Heart Study. The study identified major CVD risk factors such as high blood pressure, high blood total cholesterol, smoking, diabetes, age, gender and physical inactivities \textsuperscript{37}. 

The Framingham risk score was developed from the Framingham Heart Study. This is a model for calculating global CVD risk. In this study an online calculator was used to calculate CVD risk\textsuperscript{38}. There was a weak positive significant correlation between Framingham risk score and apo B levels (r = 0.26, p<0.001) and there was no significant correlation between Framingham risk score and LDL cholesterol levels (r=0.144, p=0.11). In this aspect we can argue that apo B levels can predict CVD risk than LDL cholesterol.

There was a moderate positive significant correlation between apo B and LDL cholesterol (r =0.44, p<0.001). However this is against C. Serban et al finding’s who found a strong and significant correlation between apo B and LDL cholesterol (r\textsuperscript{2} = 0.78, p<0.001) in the case study they carried out\textsuperscript{43}.

4.1 CONCLUSION

In this study it was observed that ART seems to increase lipid parameters like total cholesterol, LDL cholesterol and apo B levels. HDL cholesterol levels were decreased. The current guidelines should recommend routine monitoring of lipid parameters to actively investigate these changes. Apo B should be included in lipid profiles since it correlates with the Framingham risk score in assessing risk of developing CVD and it reflects the atherogenic particles not only LDL but also VLDL and IDL. Apo B methods have been internationally standardised and reference materials are traceable to the WHO-IFCC. Furthermore fasting blood samples are not needed which is a practical advantage to patients\textsuperscript{44}.
4.2 LIMITATIONS

The data on drug regimen was poorly collected since it was self reported and not checked against medical records. This ended up with regimens like just combivir or coviro without stating the third drug. This limited the study in not analysing effect of drugs on lipid levels.

4.3 RECOMMENDATIONS

For future studies data on physical activities and diet habits should be collected. Diet is a modifiable risk factor for CVD. This would conclusively establish changes in lipid levels due to diet. Body mass index should have been measured as it can influence lipid levels. A bigger sample size is needed and the HIV negative group should be healthy and just not HIV negative since secondary causes of dyslipidemia were not fully investigated.
REFERENCES


2) www.cdc.gov/hiv/topics/basic/index.htm/ Aug3, 2011


10) Kathrine Samaras, MBBS, FRACP, PhD, Handan Wand, PhD, Matthew Law, PhD, Sean Emery, PHD, David Cooper, DSC, MD, FRACP, FRCPA and Andrew Carr, MBBS, MD, FRACP, FRCPA. Prevalence of Metabolic Syndrome in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy Using International Diabetes Foundation and Adult Treatment Panel 111 Criteria. Diabetes Care. January 2007 vol. 30 no 1 p 113-119


20) Priscilla Y. Hsue, MD, Co-Chair; Kathleen Squires, MD, Co-Chair; Ann F. Bolger, MD, FAHA; Bernadette Capilli, DNSc, APRN, NP-C; George A. Mensah, MD, FAHA; Zelalem Temesgen et al. Screening and Assessment of Coronary Heart Disease in HIV-infected Patients. *J of American Heart Association*, 2008;118:e41-e47.


29) Wim A, Van der Steeg, MD; S. Matthijs Boekholdt, MD, PhD; Evan A. Stein, MD, Karim El-Harchaoui, MD; Erik S. G. Stroes, MD, PhD; Manjinder S. Sandhu, PhD; etal. Role of the apolipoprotein B – Apolipoprotein A – 1 Ratio in Cardiovascular Risk Assessment. *Annals of Internal Medicine* 2007; 145: 640 – 648.


   (cited June 3, 2011)

37) [http://www.framinghamheartstudy.org/about/history.html](http://www.framinghamheartstudy.org/about/history.html). (cited July 25, 2011)


39) Package insert from Sentinel Diagnostic on apolipoprotein B REF11042D. p1/2

40) Package inserts from Dimension chemistry analyser reagent cartridges.

42) Kate Buchaaz, PhD, Paul J. Weidle, PharmD, MPH, David Moore, MD, Willy Were, MBChB, MSc, Jonathan Mermin, MD, MPH, Robert Downing, PhD et al. Changes in Lipid Profile Over 24 Months Among Adults on First-Line Highly Active Antiretroviral Therapy in the Home-Based AIDS Care Program in Rural Uganda. J Acquir Immune Defic Syndr. March 1, 2008 Volume 47, Number 3 p 304 - 311.

