THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

Serum levels of Lipoprotein(a) in HIV positive patients on HAART at Livingstone General Hospital, Livingstone, Southern Province, Zambia.

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A Dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the degree of Master of Science in Pathology (Chemical Pathology).

Supervisor: Dr. T. Kaile
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2015

DECLARATION

I Sepiso K. Masenga, declare that this dissertation represents my own work and that all the sources I have quoted have been indicated and acknowledged by means of complete references. I further declare that this dissertation has not previously been submitted for Degree, Diploma or other qualifications at this or other Universities. It has been prepared in accordance for dissertations for the University of Zambia.

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DEDICATION

This dissertation is dedicated to my beautiful wife Joreen Povia Masenga who has been my source of encouragement and to my beautiful daughters, Sereen Lilato Masenga and Tumelo Masenga.

ACKNOWLEDGEMENTS

I give all the credit to my God for helping me in all this academic work, for the Lord God, he is God.

I would also want to express appreciation to my supervisor Dr. T. Kaile, who has provided the insights I needed to get on and complete my dissertation. I thank him especially for the mentorship and discipline he accorded me in mastering my MSc Pathology (Chemical Pathology) programme.

I would also like to express my sincere thanks to my co-supervisor Mr. T. Kantenga, for his unreserved commitment and input in making this thesis a reality. I thank him also for the encouragement, mentorship and insights he made into my research.

My gratitude is extended to the entire management of the University of Zambia, in particular the school of Medicine for facilitating my learning.

I would also like to render my heartfelt gratitude to my wife Joreen Povia Masenga for the encouragements and all the support she has given me during this time. May the Lord always be gracious to her life as long as she lives.

May the Lord almighty bless everyone who has rendered any kind of assistance including Mr Dean Ntuntuma and Mr Alex Chaaba of Livingstone General Hospital, during my sample collection process of the research project. I thank Mr Mubita also, H.O.D at the University Teaching Hospital Biochemistry Laboratory who helped during my sample processing stage of the research project with Beckman coulter AU480 analyzer.

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LIST OF ACRONYMS

AIDS: Acquired immunodeficiency syndrome

ART: Antiretroviral therapy

Apo(a) : apolipoprotein(a)

Apo(B-100) : apolipoprotein(B-100)

CHD : Coronary Heart Disease

cART : combination Antiretroviral Therapy

CVD : Cardiovascular Disease

HAART : Highly Active Antiretroviral Therapy

HIV: Human Immunodeficiency Virus;

HDL-c : High Density Lipoprotein Cholesterol

IFCC : International Federation of Clinical Chemistry and Laboratory medicine.

LDL-c : Low-density Lipoprotein Cholesterol

Lp(a) : Lipoprotein (a)

NCCLS : National Committee for Clinical Laboratory Standards

NNRTI : Nonnucleoside Reverse Transcriptase Inhibitor. Includes:

- ATV atazanavir
- ATV/r atazanavir/ritonavir
- **EFV** efavirenz
- **ETV** Etravirine
- **NVP** nevirapine

NRTI : Nucleoside Reverse Transcriptase Inhibitor. Includes:

- TDF tenofovir disoproxil fumarate (Nucleotide reverse-transcriptase inhibitors (NtRTIs)
- 3TC lamivudine
- FTC emtricitabine
- AZT zidovudine (also known as ZDV)
- ABC abacavir
- d4T stavudine
- ddI didanosine

PI : Protease Inhibitor

- RTV ritonavir
- LPV/r lopinavir/ritonavir
- LPV lopinavir

PROCAM : Prospective Cardiovascular Münster Heart Study

SPSS: Statistical Package for Social Sciences;

UNZABREC: University of Zambia Biomedical Research Ethics Committee

WHO: World Health Organization

DEFINITION OF KEY TERMS

Lipoprotein(a) – Biochemical assembly consisting of apolipoprotein(a), apolipoprotein B100 attached to an LDL like cholesterol core.

LDL-c – A blood-plasma lipoprotein that is high in cholesterol and low in protein content and that carries cholesterol to cells and tissue; also called bad cholesterol.

High Lp(a) : a Lp(a) concentration of 30mg/dL (1.07μmol/L) and above.

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ABSTRACT

Title: Serum levels of Lipoprotein(a) in HIV positive patients on HAART at Livingstone General Hospital, Livingstone, Southern Province, Zambia.

Background: Lipoprotein(a) (Lp(a)) is a highly atherogenic independent risk marker for Cardiovascular disease (CVD) and current studies have shown that Highly Active Antiretroviral Therapy (HAART) raises Lp(a) levels in HIV patients, thereby increasing their risk for CVD. In this study, Lp(a) distribution in HIV patients on HAART were determined as well as their risk category for CVD based on their Lp(a) concentrations.

Methods: This cross sectional laboratory based study was conducted between December 2014 to February 2015 at Livingstone General Hospital where fasting serum samples were collected and sent to the University Teaching Hospital Biochemistry laboratory for analysis. The Demographic and clinical data from patient files used were age, gender, ART combination and duration on ART. For data analysis, STATA version 12.0 and SPSS version 17.0 were used.
The Study sample comprised of 57 (39.9%) males and 86 (60.1%) females who had been on ART from one to ten years.

Results: Lp(a) concentration mean was 0.86 μmol/L ± 0.45μmol/L. Prevalence of high Lp(a) was 31.5% of which 29.4% and 2.1% were in the high risk and very high risk for CVD categories respectively. There was a significant relationship between age ($p=0.009$), duration on HAART ($p<0.001$) and high Lp(a) levels.

Conclusion: Since Lp(a) tended to be raised with increasing age ($p=0.009$) and longer duration on HAART ($p <0.001$), this implies that age & duration on HAART were risk factors for CVD and contributed significantly to high prevalence levels of high Lp(a) in HIV patients on HAART.

CHAPTER ONE

1.0 INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of mortality worldwide and it was projected that annual global CVD mortality was to increase from 16.7 million in 2002 to 23.9 million by 2030 (Roger 2012, Mathers and Loncar 2006). The HIV pandemic over the last decades has been identified as an important contributor to CVD mortality rates. A recent meta-analysis confirmed a significantly increased risk of CVD in HIV-infected versus uninfected people, and virus type, treatment, and disease activity (CD4+ T-cell count) were associated with elevated risk for CVD (Islam et al. 2012). Although a number of traditional risk factors have been shown to contribute to an excess risk of CVD in HIV patients, the exact mechanisms underlying this increased risk are not well understood. This issue warrants further investigations, as CVD is likely to be one of the major conditions in the future to be confronted by people living with HIV. A large body of genetic and epidemiological evidence now suggests a direct association between an elevated plasma lipoprotein(a) (Lp(a)) level and an increased risk for CVD (Byambaa et al. 2012).

The plasma concentration of Lp(a) varies over a wide range among individuals. Furthermore, the inter-individual variation in Lp(a) level is 90% genetically determined by the LPA locus on chromosome 6, although plasma Lp(a) in a particular individual remains stable over a lifetime (Boerwinkle et al.1992).

A study done by Helmhold et al.(1991), on distribution of plasma Lp(a) concentrations in four groups of adults yielded the following results: Germany (mean=0.67µmol/L (18.7 mg/dL)), median=0.30µmol/L (8.5mg/dL) , San (mean= 0.75µmol/L (21.1mg/dL)), median =0.54µmol/L (15.2mg/dL), Chinese (mean= 0.82µmol/L (22.9 mg/dL)), median= 0.55µmol/L (15.3mg/dL), Ghanaians (mean= 1.29µmol/L (36.2mg/dL)), median = 0.93µmol/L (26.0mg/dL). In contrast to the skewed distribution of the German, San, and Chinese groups, the Ghanaians had a much higher mean value and an almost Gaussian distribution. They concluded that High Lp(a) levels seem to be characteristic of all black populations so far studied (Helmhold et al.1991,

Guyton et al.1985, Parra et al.1987 and Sandholzer et al.1991). This may suggest that blacks are more susceptible to CVD than whites in relation to Lp(a) levels. The proportion of individuals in Negroid populations with elevated Lp(a) levels is substantially higher than that of Caucasians. The Ghanaians (45%) had a plasma Lp(a) concentrations of 1.07µmol/L (30 mg/dL) or greater as opposed to only 19.5% of Germans (Helmhold et al.1991). In another study done in Zimbabwe (Dube et al.2002) assessing the Lp(a) concentration distribution in a Zimbabwean population, the mean and median values for Lp(a) concentrations were 1.81µmol/L (50.6mg/dL) and 1.25µmol/L (35.0 mg/dL) for the black participants and 0.99µmol/L (27.8mg/dL) and 0.51µmol/L (14.2 mg/dL) for the white participants ($p < 0.005$). They concluded in their study that environmental and metabolic factors may be responsible for the elevated Lp(a) levels observed in blacks.

Changes in cholesterol and triglycerides have been extensively studied, but the effect of initiating ART on newer cardiovascular risk markers such as Lp(a) has been less well investigated. However, in an earlier study on HIV-positive individuals initiating ART with mainly unboosted HIV protease inhibitors (PI), an increase in Lp(a) was observed, in particular in patients with higher Lp(a) baseline levels (Mauss et al.2008). It is well known in literature that protease inhibitors increase total cholesterol, LDL and triglycerides (Andrew 2003, Magenta 2005, Lorenzo 2005, Young 2005 and carr 1998) and plasma Lp(a) levels (Mauss et al.2008) and also that there is a positive relationship between plasma Lp(a) levels and antiretroviral therapy particularly non-nucleoside reverse-transcriptase inhibitor (NNRTI) and nucleoside reverse-transcriptase inhibitor (NRTI) combinations which is the first line ART according to WHO (2013).

In this study, the serum Lp(a) levels, the prevalence of high Lp(a) in HIV patients on HAART at Livingstone General Hospital were determined and the Lp(a) levels with LDL cholesterol were compared and used to determine the patient’s risk category for CVD.

1.1 STATEMENT OF THE PROBLEM

There are about 34 million people living with HIV worldwide (Global Report on the Global AIDS Epidemic 2012) and over the last few decades, it has become clear that chronic cardiovascular diseases (CVDs) and associated risk factors are on the rise on the African continent especially in HIV positive individuals on ART (Kearney et al. 2005). A meta-analysis study involving 25 african countries showed that HIV-seropositive patients on combined antiretroviral therapy could achieve a normal life expectancy with the accompanying exposure to risk for developing chronic CVDs as more than one-third had some form of cardiovascular disorders. Use of first-line antiretroviral therapy regimens were associated with raised total cholesterol, LDL-cholesterol, triglycerides and an established atherogenic lipid profile. They concluded and recommended that all lipid profiles should be performed periodically in HIV positive patients on treatment to screen and monitor any rising trends, in the assessment and management of cardiovascular risk factors in HIV (Mills et al.2011, Tilahun et al.2012 and Agete et al. 2012).

High Lp(a) levels seem to be characteristic of all black populations so far studied (Helmhold et al.1991, Guyton et al.1985, Parra et al.1987, Sandholzer et al.1991, Virani et al. 2012 and Byambaa et al. 2013). This may suggest that blacks are more susceptible to CVD than whites in relation to Lp(a) levels. Mauss et al.(2008) in a one year follow-up study showed that in patients initiating antiretroviral therapy, marked increases in Lp(a) after initiation of ART were mainly restricted to patients with high baseline levels. However, HIV patients on HAART have higher Lp(a) levels compared to treatment naïve patients (Sawadogo et al.2013). Since Lp(a) levels among HIV patients treated with HAART in Zambia is unknown, it is a problem, as it is not known how many in the HIV positive patient population receiving treatment have high levels of Lp(a) and at great risk of having CVD especially after more than one year of treatment considering the effect of HAART on Lp(a) levels as shown from the above cited studies.

1.2 STUDY JUSTIFICATION

This study was to provide the Lp(a) and LDL-c distribution levels in HIV positive individuals on treatment that can be used for future studies and reference. Since Lp(a) is strongly artherogenic, for the first time in Zambia, the study would not only provide Lp(a) distribution but also prevalence information of Lp(a) among people living with HIV in Livingstone which can be used i.e for identification of high risk patients, estimation of the magnitude of health problem and for administrative and planning purposes.

Changes in cholesterol and triglycerides have been extensively studied, but the effect of initiating ART on newer cardiovascular risk markers such as Lp(a) has been less well investigated. In an earlier study on HIV-positive individuals initiating ART with mainly unboosted HIV protease inhibitors (PIs), an increase in Lp(a) was observed and also, results from another study showed that those treated with HAART for 12 months had significantly higher levels of Lp(a) in comparison with those treated for six months ($p=0.034$) (Mauss et al.2008 and Hafsatou et al.2013 respectively).

However, there was scarce data on studies that have been done specifically assessing the distribution of Lp(a) levels in HIV patients on HAART, in Africa and Zambia. Furthermore, the Lp(a) increase in patients on ART being remarkable, because little change has been observed in the general population attributable to diet (Marcovina et al.2003 and Sawadogo et al.2013), a study on Lp(a) conducted in Zambia would be critical and necessary in this regard, not only to add relevant information in the global perspective of HIV patient management but locally also, to determine the actual treatment effects or burden of HAART on Lp(a) levels among the HIV patients.

Although a few similar studies on Lp(a) in HIV Patients on ART had been conducted (in Germany and Burkina Faso), it was important to carry out this study due to the fact that Lp(a) varies a 1000 fold between individuals, is 90% genetically determined, varies by geographical and environmental factors and within ethnic groups.

A study done in Lusaka, Zambia, by Kiage et al.(2013) assessing the early effects of combination antiretroviral therapy (cART) on CVD risk markers showed a significant increase in LDL-c (P=0.02) in the group on D4T +3TC +NVP. High LDL-c with Lp(a) levels >1.07µmol/L (>30mg/dL) is associated with high risk for CVD but in their study, Lp(a) was not measured hence the need to assess it.

Furthermore, in Zambia, Lp(a) & LDL-c are not routinely measured and considering that Zambia was rolling out ARVs country wide, studies on disturbances in lipid metabolism of ARVs are cardinal in management of HIV seropositive patients on treatment.

CHAPTER TWO

2.0 LITERATURE REVIEW

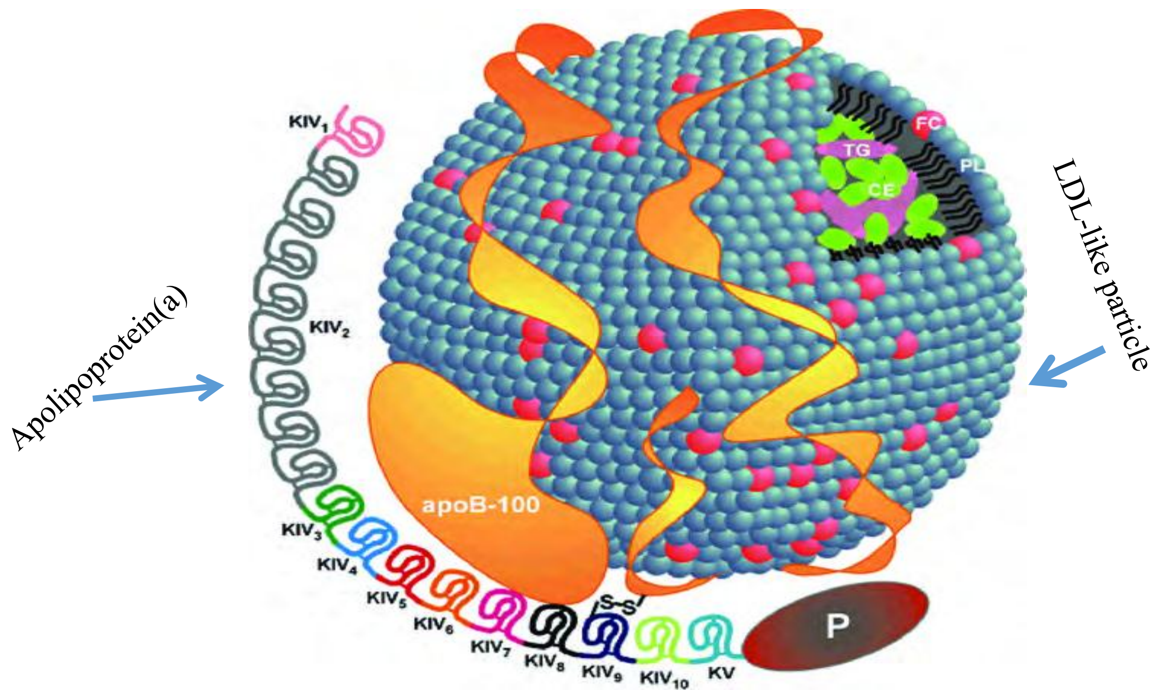
The number one cause of mortality worldwide has been cardiovascular disease with the HIV pandemic being an important contributor to CVD mortality rates (Global Report on the Global AIDS Epidemic 2012) especially in HIV positive individuals on HAART (Kearney et al. 2005. Roger 2012, Mathers & Loncar 2006 & Islam et al. 2012). Lp(a) has recently been implicated as being associated with CVD occurrence in HIV HAART treated patients (Byambaa et al. 2013).

2.1 Lp(a) synthesis and metabolism (based on Sawadogo et al.2013, Frank et al.1994, Siekmeier et al.2008, Lippi and Guidi 2000).

Lipoprotein(a) (Lp(a)) is synthesized and secreted by the liver and comprises a lipid core of LDL cholesterol and apolipoprotein B-100 (ApoB-100) surrounded by a unique glycoprotein apolipoprotein(a) (Apo(a)) that shares homology with plasminogen (Utermann 2001). The synthesis of Lp(a) is thus a two-step process. In a first step, which can be competitively inhibited by lysine analogues, the free sulfhydryl groups of Apo(a) and ApoB-100 are brought close together. The binding of apo(a) then occurs near the domain of ApoB-100 which binds to the LDL receptor, resulting in a reduced affinity of Lp(a) to the LDL receptor. Investigations into the metabolism have shown that the synthesis rate of Lp(a) is strongly related to its plasma concentration and therefore depends on the Apo(a) isoform that determines plasma concentration, while the catabolism rate is only of little significance. The metabolic path of the synthesized Lp(a) is to a large extent unknown. Kinetic investigations performed in man, demonstrated a longer plasma half-life for Lp(a) compared to LDL, allowing the conclusion that the LDL receptor practically has no function in the metabolism of Lp(a). The fractional catabolic rates for LDL and Lp(a) are 0.38 and 0.26, respectively, in normal persons and are practically the same as for homozygous patients with a defect of the LDL receptor (0.205 and 0.210, respectively). Apo(a) separates from apoB-100 in the blood and as a consequence it comes to a subsequent fragmentation into degradation products of

different molecular weight induced by metalloproteinases. The resulting degradation products are removed from circulation by different organs and tissues, and only a very small part of them (less than 1 % of Apo(a)) is excreted with the urine. Other, receptor-dependent metabolic paths are assumed for the Lp(a) metabolism. Among them are the LDL receptor related protein, megalin, the VLDL receptor and the galactose-specific asialoglycoprotein receptor.

Fig 1. Lipoprotein(a) particle picture



Utermann (2001).

2.2 Lp(a) Pathology

Lp(a) may be athero-thrombotic through its low-density lipoprotein moiety, but also through Apo(a). Apo(a) can be retained in the vessel wall and thereby mediate pro-inflammatory and pro-apoptotic effects including those potentiated by oxidized

phospholipids. Apo(a) may also exert anti-fibrinolytic effects (Sawadogo et al.2013 and Marcovina 2003).

Lp(a) belongs to the lipoproteins with the strongest atherogenic effect. Various atherosclerotic vasculopathies such as coronary heart disease, ischemic stroke, peripheral vasculopathy, abdominal aneurysm, peripheral arterial occlusive disease, cerebral stroke were associated with elevated Lp(a) plasma concentrations and conversely, persons with very low or no detectable Lp(a) plasma concentrations did not present a specific phenotype (Siekmeier et al. 2008, Berg 1963 and Kostner et al.2005).

The clinical interest in Lp(a) emanates from its association with cardiovascular disease (CVD) when present in high plasma concentrations. A meta-analysis of prospective studies demonstrated that elevated levels of Lp(a) was an independent risk factor for CVD. The pro-atherogenic influence of Lp(a) seemed to be particularly enhanced in subjects with elevated levels of LDL cholesterol (LDL-c) (Danesh et al.2000, Tsimikas et al.2005 and Suk et al.2006).
Plasma concentrations of Lp(a) vary over a 1000-fold-range, and the distribution of levels is highly skewed in most whites and Asians having low levels. The distribution of plasma levels in blacks is less skewed and black populations so far studied seem to have high Lp(a) levels (Helmhold et al.1991, Guyton et al.1985, Parra et al.1987 and Sandholzer et al.1991).

2.3 Influences on the Lp(a) plasma concentrations

Hormonal influence

Hypothyroidism is associated with an increase of Lp(a) concentrations, while hyperthyroidism is accompanied by a decrease of Lp(a). Thus triiodothyronine (T_3) or thyroxine (T_4) treatment results in a decrease of Lp(a) plasma concentration, while the administration of a thyroid hormone inhibitor is associated with an increase (Kostner et al.2005, Kostner 2002 and Pearce 2004).

Oestrogen treatment frequently leads to lower Lp(a) levels (International Task Force for Prevention of Coronary Heart Disease; Prospective Cardiovascular Münster Heart Study, 2010)

Nutrition and lifestyle influences

The chronic consumption of ethanol results in a marked decrease of the Lp(a) concentration which is dose-dependent (Kostner et al.2005, Catena et al.2003 and Fontana et al.1999) and the Lp(a) concentration rises again after termination or reduction of alcohol consumption (Delarue et al.1996, Paassilt et al.1998 and Rakic et al.1998). Trans-fatty acids such as elaidic acid, which is found in deep-fried food, lead to a 25 % - 50 % increase of Lp(a) concentration, which is particularly marked in individuals with initially elevated Lp(a) concentrations (Enas et al.2006, Katan et al.1995 and Sundram et al.1997). Several studies also discuss that mono- or poly-unsaturated fatty acids or fat-modified foods may have a lowering and protective effect on Lp(a) plasma concentrations (Enas et al.2006, Marcovina et al.1999, Pedersen et al.2005 and Shinozaki et al.1996).

Decreased Lp(a) plasma concentrations in smokers compared to non-smokers have been demonstrated (Kostner et al.2005 and Os et al.2003).

However, Serum levels of Lp(a) have little or no environmental impact. Diet, physical activity and body habitus are not known to affect Lp(a) levels to any significant extent. Further studies into this is warranted (Jayasinghe et al. 2014).

Therapeutic modification of Lp(a)

The administration of niacin or nicotinic acid may produce a distinct decrease of Lp(a), with simultaneous favourable influence on other parameters of the lipid metabolism (Siekmeier et al.2008, Kostner 2005, Batiste et al.2002, Birjmohun et al.2002, McCormack et al.2005 and McKenney 2004).

Medical treatments such as carbamazepine or sodium valproate may induce an increase in lipoprotein(a); however, the mechanism for this increase is unknown (Voudris et al.2006 and Sonmez et al.2006).

Influence of disease

The plasma concentrations of Lp(a) are determined by genetic factors and are considered subject to only low variations during lifetime (Kostner 2002 and Puckey et al.1999). But a number of factors may lead to an increase or decrease of the plasma concentration. Cholestatic liver diseases are associated with reduced Lp(a) concentrations. In these cases it is assumed that a lack of normal LDL is the cause for this phenomenon, which leads to an impairment of the assembly of LDL and apo(a) (Kostner et al.2005).

Many studies have investigated the influence of chronic kidney diseases on the concentration and metabolism of plasma lipoproteins. Elevated Lp(a) plasma concentrations were found in patients with diabetic microscopic albuminuria (Enas et al.2006) nephrotic syndrome, nephropathy and kidney failure of different etiology and stages. Type 2 diabetes mellitus patients showed an increase in Lp(a) compared to healthy individuals. (Enas et al.2006, Hirata et al.1993 and Soulat et al.2000)

2.4 ART influence on Lp(a) plasma concentration and cardiovascular disease relations

Some studies have shown that the nucleoside reverse transcriptase inhibitor (NRTI) component of highly active antiretroviral therapy (HAART) contributed in an as yet undefined way to the lipid abnormalities and body fat distribution. The non-nucleoside reverse transcriptase inhibitor (NNRTI) component of HAART may also contribute to the dyslipidemia as shown by Mohsen et al. (2005), Clotet et al.(200)3 and Tashima et al.(2003).

A study by Gallant et al. (2004) showed that some antiretroviral drugs, such as stavudine (d4T) increased the blood levels of total cholesterol, LDL-cholesterol and triglycerides with variable effects on levels of HDL-cholesterol. Nevirapine (NVP) use was associated with increases in LDL-c (Van der Valk et al.2001, Mills et al.2011 and Aldeen 1999).

In a study assessing Lp(a) in patients initiating antiretroviral therapy (Mauss et al.2008) conducted in Germany, the study population was divided into individuals with lipoprotein(a) ≥ 1.07µmol/L (≥ 30 mg/dL), n=28 (29.5%) at baseline and those with < 1.07µmol/L (<30 mg/dL), n=67 (70.5%). [The 1.07µmol/L (30 mg/dL) threshold was chosen according to data from prospective studies showing a higher cardiovascular risk in patients with Lp(a) levels ≥1.07µmol/L (≥30 mg/dL) (Cantin et al.2002, Glader et al.2002, Stubbs et al.1999 and Shai et al.2005)]. Almost exclusively, patients with high Lp(a) at baseline (1.84 µmol/L (median 51.6 mg/dL)) showed a profound increase of median 0.95 µmol/L (26.7 mg/dL) (week 24). This effect was not associated with specific ARVs and was independent of changes in other lipids. In other words, a substantial increase in Lp(a) concentration was mainly observed in patients who already had high Lp(a) concentrations at baseline, independent of ARV regimen, ARV efficacy and baseline levels, or changes in, other lipids. The majority of patients in the Lp(a) <1.07µmol/L (<30 mg/dL) group showed only a small increase from baseline concentrations, from a median of 0.25µmol/L (7 mg/dL) at baseline (IQR 0.06-0.39 µmol/L (1.7–11 mg/dL)) to a median of 0.33µmol/L (9.3 mg/dL) at week 48 (IQR 0.14-0.54µmol/L (4–15 mg/dL)). Five (7.5%) of the 67 patients in the Lp(a) <1.07µmol/L (<30 mg/dL) group at baseline showed an increase in the serum Lp(a) concentration to >1.07µmol/L (>30 mg/dL). They concluded that marked increases in Lp(a) after initiation of ART were mainly restricted to patients with high baseline levels. This may have clinical implications as patients with high Lp(a) were at higher risk for myocardial infarction and stroke. Furthermore, there was no correlation between change in HIV RNA (viral load) or CD4 cell count and increase in Lp(a).

In another study conducted in Burkina Faso (Sawadogo et al.2013), the study population (136 subjects) comprised of 106 HIV-infected participants, and 30 HIV-infected participants that did not receive antiretroviral treatment; 36 HIV-infected participants on antiretroviral treatment for six months; and 43 HIV-infected participants on antiretroviral treatment for twelve months. All recruited patients had normal blood lipid values (total cholesterol < 5.2mmol/L, triglycerides < 2.26mmol/L, HDL-c > 0.9mmol/L). The Lp(a) level was significantly higher in HIV infected group 1.74 ±

11.0μmol/L (48.8 ± 30.8 mg/dL) compared to 1.13 ± 5.4μmol/L (31.6 ± 15.1mg/dL) in the HIV-negative control group (p=0.0036). Among the HIV infected participants, Lp(a) levels was higher in HAART treated group compared to the group that was not treated (p=0.004). Infected subjects on the antiretroviral treatment for 12 months had higher Lp(a) levels than those treated for six months (p=0.034). They concluded that in the adequate management of metabolic abnormalities and all HAART-treated HIV-infected patients, periodic measurement of Lp(a) levels must also be included. In another study, Shahmanesh et al. (2001) reported that Lp(a) level higher than 1.07μmol/L (30.0 mg/dL) was observed in 41% of subjects receiving non-nucleoside reverse transcriptase inhibitor (NNRTI) and 33% of retroviral-negative patients.

In a study by Niehues et al.(2005), a group of 300 HIV-positive adults that were followed at an HIV clinic in Stuttgart (Germany) [266 men, 34 women, mean age 40.4 ± 9.2 years, 245 treated with antiretroviral drugs (PI, NNRTI & NRTI)] found that median Lp(a) was 0.46μmol/L (13 mg/dL) (interquartile range 0.14-1.71μmol/L (4 – 48 mg/dL)). 59 (19.6%) patients had either Lp(a) > 2.50μmol/L (70 mg/dL) or >1.07μmol/L (30 mg/dL) plus an elevated LDL-c >4.14mmol/L (>160 mg/dL). 104 (34.6 %) patients had a Lp(a) > 1.07μmol/L (>30 mg/dL). They also reported the prevalence of Lp(a) > 1.07μmol/L (>30 mg/dL) in other smaller cohorts being 22 – 33 %.

A meta-analysis study done by Tilahun et al.(2012) involving 37 countries of which 25 were from Africa, revealed that more than one-third of people living with HIV attending health facilities receiving antiretroviral treatment (ART) had some form of cardiovascular disorders. They reported a prevalence of 42.3% with myocardial infarction in India, 30.0% prevalence of cardiovascular autonomic neuropathy in Mozambique, 17.7% prevalence of dilated cardiomyopathy in Rwanda, 37.4% prevalence of QTc interval prolongation in Nigeria. It is evident that the cases of CVD is a problem among HIV patients especially those on treatment as shown further below.

A study done by Agete et al. (2012) on prevalence of dyslipidemia among HIV-infected

patients using first-line highly active antiretroviral therapy (2NNRTI + 1NRTI) in Southern Ethiopia revealed prevalence of total cholesterol ≥ 5.18mmol/L (200 mg/dL) occurred in 43.4% of HAART and 15.9% pre-HAART patients (p=<0.0001) [27.5% attributed to ART], whereas LDL-cholesterol ≥ 3.36mmol/L (130 mg/dL) occurred in 33.6% of HAART and 15% pre-HAART patients (p=0.001)[18.6% attributed to ART], while triglycerides ≥ 1.70mmol/L (150 mg/dL) occurred in 55.8% and 31.0% respectively, (p=0.001)[24.8% attributed to ART]. The adjusted odds ratio (95% CI) of HAART-treated vs. pre-HAART was 3.80 (1.34-6.55) for total cholesterol ≥ 5.18mmol/L; 2.64 (1.31-5.32) for LDL- cholesterol ≥ 3.36mmol/L and 2.50 (1.41-4.42) for triglycerides ≥1.70mmol/L. They concluded that use of first-line antiretroviral therapy regimens were associated with raised total cholesterol, LDL-cholesterol, triglycerides, an established atherogenic lipid profile and that lipid profiles should be performed at baseline before commencement of antiretroviral therapy and then periodically through treatment follow-up to monitor any rising trends.

Most studies with the aim of defining an absolute threshold of Lp(a) serum concentration have reported an approximately two fold increase in cardiovascular risk in patients with Lp(a) ≥ 1.07µmol/L (≥30 mg/dL) compared to patients with Lp(a) levels < 1.07µmol/L (<30 mg/dL). However, the increase in cardiovascular risk attributable to high Lp(a) is further modulated by other cardiovascular risk factors, in particular LDL-c (Cantin et al.2002, Kronenberg et al.1999 and von et al.2001).

In another study by Vandana et al.(2012), Lp(a) levels were significantly higher in coronary artery disease (CAD) patients as compared to controls showing a correlation between Lp(a) and cardiovascular disease.

A meta-analysis of 27 prospective studies, which included 5436 coronary heart disease cases, demonstrated a consistent, positive association between high plasma levels of Lp(a) and coronary atherosclerosis in whites. Comparison of individuals in the top third of Lp(a) measurements with those in the bottom third in each study yielded a combined risk ratio of 1.6 (95 % confidence interval 1.4,1.8) (Achim 2005).

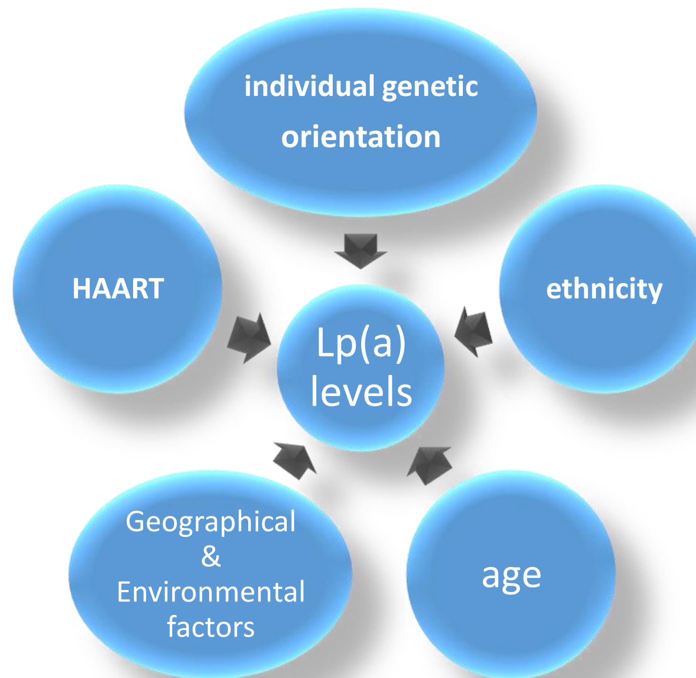
A case control study (Achim 2005 and Niehues et al.2005) in which 195 men who subsequently developed angina were compared to 195 men who remained free of cardiovascular disease for 5 years. In this study, a Lp(a) concentration measured by ELISA above the 95th percentile (~ 2.50µmol/L (70 mg/dL)) was associated with an almost fourfold risk. Using a commercially available test, a strong, nearly 12-fold increase in relative risk for angina was found at Lp(a) concentrations above 1.07µmol/L (30 mg/dL) with concomitantly elevated LDL-c above 4.14mmol/L (160 mg/dL).

In general, according to a report from the Prospective Cardiovascular Münster Heart Study 2010 (PROCAM) released by the International Task Force for Prevention of Coronary Heart Disease, both increased levels of LDL cholesterol and increased levels of Lp(a) are associated with increased risk of nonfatal myocardial infarction. LDL cholesterol and Lp(a) interact in increasing the risk of nonfatal myocardial infarction in men across all LDL cholesterol levels above 3.36mmol/L (130 mg/dL). The data indicated that Lp(a) does not increase risk at LDL cholesterol levels below 3.36mmol/L (130 mg/dL). This may suggest that in individuals with raised Lp(a), LDL cholesterol should be lowered to below 3.36mmol/L (130 mg/dL).

2.5 SUMMARY CONCEPTUAL FRAMEWORK:

The conceptual framework describes the various factors in the research on which the dependent variable, is influenced or rather, factors that seem to influence it's distribution.

Figure 2: conceptual framework of factors associated with Lp(a) levels



Geographical & environmental factors: as seen from literature, the distribution in normal population varies with geographical and environmental factors, hence those receiving treatment as well.

Ethnic groups: High prevalence levels are seen in blacks than in whites and varies by ethnic group

Individual variations: up to a 1000 fold, hence those having higher levels are more prone to CVD

HAART: raises Lp(a) levels

Age: Lp(a) is stable with age in HIV negative individuals but seems to be unstable in HIV positive individuals on HAART.

2.6 RESEARCH QUESTIONS

How is Lp(a) among HIV patients on HAART at Livingstone General Hospital distributed?
What is the prevalence of high Lp(a)?

2.7 General Objective

To assess the serum Lp(a) levels in HIV seropositive patients on HAART at Livingstone General Hospital, Livingstone, Southern Province (Zambia).

2.8 Specific Objectives

- **i.** To determine the Lp(a) and LDL-c levels in HIV Positive patients on HAART (NRTI & NNRTI based regimen) at Livingstone General Hospital.
- **ii.** To determine the prevalence of high Lp(a) levels in HIV positive patients on HAART (NRTI & NNRTI based regimen).
- **iii.** To compare the Lp(a) levels with LDL Cholesterol in patients on HAART (NRTI & NNRTI based regimen) and to determine their risk category for CVD.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design and site

This was a cross sectional laboratory based study that was conducted at Livingstone General Hospital adult ART Medical clinic and Laboratory between December, 2014 and February, 2015.

3.2 Study population

The estimated number of HIV positive people on ART in Livingstone District was 62,317. At Livingstone General Hospital alone, as of September, 2013, the estimated number of HIV positive adults enrolled at the Livingstone General Hospital ART Clinic was 6601 of which 3356 (Males = 1423, Females = 1933) were currently on HAART, which was the target population**.** The study population included all HIV positive adults enrolled at the Livingstone General Hospital ART Clinic above 18 years of age, who had been on HAART (NRTI AND NNRTI) for ≥ 1 year.

3.3 Sample size

Assuming 22% prevalence of high Lp(a) (Niehues et al.2005) in HIV seropositive individuals on treatment, a population size (N) of 3356, and using the Epi Info Statcalc formula for population surveys using random sampling below:

Sample size (n) = $Z^2*(P(100-P))/D^2$

Assuming 95 percent confidence level, Z=1.96, D=5%

A total sample size of 245 was arrived at. However, due to inadequate resources to procure enough Lp(a) reagents which were expensive at the time, we only managed to procure reagents for 200 samples but during the sample analysis process, we only managed to process 143 samples successfully. About 57 samples needed a repeat run (analysis) due to their concentration exceeding the assay range but there were no reagents to re-assay them. So, only a sample size of 143 were analyzed in this study.

3.4 Sampling methods

Convenience sampling was employed when selecting HIV seropositive individuals as they reported for routine medical tests at the Laboratory. Convenience sampling was used due to the fact that there were only a few routine fasting lipid profile samples that were usually requested for routine analysis among HIV positive patients at Livingstone General Hospital hence making this type of sampling, the best under these circumstances. All samples collected were from HIV positive patients that were requesting to know their lipid profile and from HIV positive patients whose attending medical personnel requested for all routine chemistry tests including lipid profile. These were selected for lipoprotein(a) and LDL-c assessment using inclusion and exclusion criteria as outlined below.

3.5 Inclusion criterion

Those whose age was equal to or greater than 18 years.

Also, all HIV positive adult patients on NNRTI and NRTI based regimen (first line according to WHO: consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. 2013) reporting at Livingstone General Hospital Laboratory outpatient department and medical clinic for routine tests who were on ART for at least one year.

3.6 Exclusion criterion

Patients with any record of withdrawal from combination ART (NNRTI and NRTI), those on protease inhibitors and those whose age was less than 18 years were excluded from the study.

3.7 data collection

3.7.1 Clinical & demographical data

Clinical and demographic data was collected from patient files and laboratory forms using a data collection form (Refer to the appendix for the data collection form that was used). Clinical and demographic data information was collected from patient files using an ART number and laboratory serial numbers.

Beckman coulter AU480 analyzer was used to obtain Lipoprotein(a) and LDL cholesterol levels.

3.7.2 Specimen collection

Type of specimen:

Freshly drawn serum from a fasting individual is preferred (Beckman Coulter, Inc. 2010) and was obtained from the participants.

Serum, free from hemolysis, which is the recommended specimen was obtained after it was separated from red blood cells within 2 hours according to the National Committee for Clinical Laboratory Standards (1990) and laboratory standards used in Zambia.

Collection method for the samples that were selected and used for the research:

Blood samples which had been collected for routine testing in accordance with the standard procedure for collecting lipid profile samples were selected. Note the brief procedure used by the laboratory below:

When collecting samples for lipid profile, the patients had been advised by the requesting health personnel or phlebotomist at the laboratory to have their last meal at least by 22:00hrs on the day they were sent to the laboratory and to report the next morning for blood collection. Five milliliter of fasting blood samples was then collected from the patient's cubital vein the next morning for lipid profile and other chemistry related tests. After obtaining serum for the requested routine tests, the remaining serum was obtained by the principal investigator (PI) and put in small sample storage collection tubes from which lipoprotein(a) and LDL cholesterol concentrations would be determined. In all cases, blood from the patients was drawn from the cubital vein into plain collecting tubes.

Sample collection and storage started late December 2014 and by the end of February, 2015 all samples had been collected.

3.7.3 Specimen preparation & storage

In order to obtain serum, the blood was allowed to stand for 45 minutes at room temperature to allow complete clotting and clot retraction because a shorter period would

result in incomplete clotting and secondary clots may form later. During the clotting period, the collection tube was sealed. After approximately 45 minutes, specimens were centrifuged at 3000 cycles/ minute for three minutes, and then serum was separated from the clot, aliqouated and frozen at -20^{0}c for storage in the laboratory. Specimens are stable for up to 3 months when stored at -20^{0}C (Beckman Coulter, Inc. 2010). After all 200 samples were collected, they were transported on dry ice to the University Teaching Hospital (U.T.H), Pathology and Microbiology department in Lusaka and stored in another refrigerator still frozen, in readiness for processing.

3.8 Quality Control, Calibration and Reagents

The QC, Calibrator and Reagents for Lp(a) were procured from Randox Laboratories in Northern Ireland, UK and shipped to Zambia under cold chain (details given below under Lp(a) assay). The LDL-c reagents, calibrator and reagents were procured locally through JAP medical suppliers based in Kitwe, Zambia.

To ensure good quality of samples, only fasting samples were used (though non fasting samples could also be used for Lp(a)) to avoid chylomicron interference which appears in the circulation after a fat-containing meal. Chylomicrons are normally cleared within 9-12 hours, and no chylomicrons should be present after a 12 hour period of fasting. With chylomicrons interference, transient decreases in HDL-cholesterol and LDL-cholesterol occur, the magnitude of which depends on the fat content of the meal.

During operation of the Beckman Coulter AU analyzer, two levels of an appropriate quality control material were used. In addition, controls were performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide (Beckman Coulter, Inc. 2010). Quality control testing was performed in accordance with regulatory requirements and the standard operating procedures laid down by the U.T.H Biochemistry laboratory and all quality assurance protocols were adhered to as specified by the manufacturer (refer to appendix G & H for protocol).

Lipoprotein(a) section

Quality control (QC): To monitor accuracy and precision, the appropriate two levels of Lipoprotein(a) QC namely, LPD Control 1 (LE2668) which is a low level control and Lp(a) control 3 (LP2406) a high level control were assayed and the results were within the ± 1SD of the assigned range indicating that the analysis for QC and all the research samples were very accurate and precise.

1. LE2668 assigned mean & range were 0.43µmol/L (12.1mg/dL) and 0.35-0.52 µmol/L (9.68-14.5mg/dL) (1 SD = 1.2) and the results of the assayed QC were 0.44µmol/L (12.25mg/dL).
2. LP2406 assigned mean & range were 1.64µmol/L (46.0mg/dL) and 1.31-1.97µmol/L (36.8-55.2mg/dL) (1 SD= 4.6) and the results of the assayed QC were 1.60µmol/L (44.93mg/dL).

Lipoprotein(a) Assay :

For Lp(a) test, the Randox dedicated lipoprotein(a) assay for Beckman Coulter AU480 was used, which is proven to be the best methodology on the market. The reagents (two Lp(a) reagents LP3403, Controls LE2668 & LP3406 and Calibrator LP3404) were shipped by air from North Ireland under cold chain on 22 January 2015 and received five days later, cleared at customs and transported to U.T.H, Department of Pathology and Microbiology for storage at 2-8^{0}c. They were purchased from a company bearing the following name, physical address and company registration; Randox Laboratories Limited, company number N.I 15738.VAT Registered number. GB 151 682 708. Address 55 Diamond Road, Crumlin, county Antrim, BT29 4QY, United Kingdom Tell +44 (0) 28 9442 2413 Fax +44 (0) 28 9445 2912 Email marketing@randox.com website www.randox.com

The Randox assay is an immunoturbidimetric assay for the quantitative in vitro determination of Lp(a) in human serum or plasma. It contains a very high density of isoform-insensitive antibodies and detection reagent – ensuring more Lp(a) bound antibodies are detected and more accurate measurement. It is liquid ready-to-use IT

assay, excellent stability (open vial stability 30 days on board) and no sample preparation required.

Lp(a) Calibrator (LP3404)

While other types of calibrators are single point, Randox produces a 5-point calibrator [0.32µmol/L (9.0 mg/dL), 0.54µmol/L (15.1mg/dL), 1.04µmol/L (29.2mg/dL), 1.98µmol/L (55.4mg/dL) and 2.94µmol/L (82.3mg/dL)] which takes into account the heterogeneity of the Lp(a) molecule for each of the levels, resulting in excellent commutability of the calibrator with patient samples (Randox Laboratories Limited 2014).

The calibration was done and passed, hence QC passed as well. In cases where calibration fails, the accompanying QC and patient specimens give erroneous results but in many cases the analyzers do not assay the failed test hence sample analysis cannot proceed.

LDL-c section:

Quality control: QC was assayed and passed. LDL-c uses two levels of controls namely P(pathological) which is a high level control and N(normal) which is a low level control.

1. P assigned mean and range were 2.47mmol/L (2.40-2.52mmo/L) and the assayed results were 2.51mmol/L
2. N assigned mean and range were 1.44mmol/L (1.42-1.46) and the assayed results were 1.44mmol/L

From the above data, it is seen that the QC had a very narrow range for more accuracy and precision hence quality results were assured.

The Beckman Coulter AU System LDL-Cholesterol Reagent: is liquid, ready for use. No preparation is needed. See details in appendix G

For the LDL-C calibrator, 1.0 mL of deionized water was added to the calibrator vial. Mixed thoroughly to avoid foaming. Calibrator performance and storage were done and it passed as recommended by the manufacturer (Beckman Coulter, Inc. 2010). Calibrator

lot 6393, bottle no.2704 & 2696 concentration at 2.87mmol/L and optic density of 0.1685.

3.9.0 Specimen and Data Analysis

Specimens were all analyzed on Beckman Coulter AU480 on the 17th of March 2015. Below is a brief principle of the two tests (LDL-c & Lp(a))

LDL ANANLYSIS PRINCIPLE:

The Beckman Coulter AU System LDL-Cholesterol test is a two reagent homogenous system. The assay is comprised of two distinct phases. In phase one, a unique detergent solubilizes cholesterol from non-LDL-lipoprotein particles. This cholesterol is consumed by cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrine to generate a colorless end product.

In phase two, a second detergent in the R2 reagent releases cholesterol from the LDL-lipoproteins. This cholesterol reacts with cholesterol esterase, cholesterol oxidase, and a chromogen system to yield a blue color complex which can be measured bichromatically at 540 / 660nm. The resulting increase in absorbance is directly proportional to the LDL-C concentration in the sample.

Lp(a) ANALYSIS PRINCIPLE :

Immunoturbidimetry is the technique that Beckman coulter AU480 analyser uses for Lp(a) analysis. The Lp(a) test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

CHEMICAL REACTION SCHEME:

Lp(a)(sample) + particle bound anti-Lp(a)(antibody) → [Lp(a)(sample)-Antibody complex]

For a detailed LDL & Lp(a) specimen analysis, methodology, principle, handling, performance parameters, reference ranges, limitations, precision & accuracy refer to

appendix 5 and Nordestgaard et al. 2010 (European Atherosclerosis Society Consensus Panel. 2010)

Data analysis

Data was first inserted in excel and exported to STATA and SPSS for the purposes of defining variables, labels and also values. Then it was also coded to make analysis easier. Coding and transformation of continuous variables into categorical variables was done as briefly explained below.

The categorical variable 'sex' was coded as follows; 0= male, 1= female.

A categorical variable for the continuous variable 'age' was created, categorized into four groups and coded (1= 18-24 years; 2=25-31 years; 3= 32-38 years and 4= 39-45 years) and the new variable was named 'age category'.

The continuous variable 'Duration on ART' was transformed into a two level categorical variable and coded as follows; 0= on HAART for ≤ 5 years, 1= on HAART for > 5 years. The new categorical variable was named 'duration category' which was a five year duration. This five year cut-off was arrived at, using the few similar studies (sawadogo et al.2013, Mauss et al. 2008 and Niehues et al. 2005) conducted on Lp(a) in HIV patients on HAART, where patients were grouped in 2 comparable categories using a double time interval for the second group compared to the first.

The variable 'ART combination' was not transformed but coded in five categories as follows; 1=TDF/FTC/EFV; 2= TDF/FTC/NVP; 3= D4T/3TC/NVP; 4= AZT/3TC/NVP; 5= D4T/3TC/EFV

The dependent continuous variable 'Lp(a) concentration' was also transformed into another new categorical variable in question form and called 'high Lp(a)' which was the primary outcome variable and coded as follow; 0= NO. LP(a) <1.07 μmol/L (<30mg/dL); 1= YES.LP(a) ≥ 1.07μmol/L (≥30mg/dL). However, the continuous variable 'Lp(a) concentration' was not removed but maintained to be used in analysis as needed.

The other dependent continuous variable 'LDL-c concentration' was also transformed into another new categorical variable in question form and called 'high LDL-c' which was the secondary outcome variable and coded as follow; 0= NO (LDL-c < 3.36mmol/L); 1= YES (LDL-c = ≥ 3.36mmol/L. However, the continuous variable

'LDL-c concentration' was also not removed but maintained to be used in analysis as needed.

Other two categorical variables were created namely 'LPA CVD RISK' and 'LDL CVD RISK' purely for easier data analysis to help in categorizing the participants in their CVD risk according to the Lp(a) thresholds and LDL-c thresholds.

The codes for 'LPA CVD RISK' was as follows; 1=NORMAL < 0.50µmol/L (<14mg/dL); 2=LOW RISK 0.50-1.06µmol/L (14 - 29 mg/dL); 3=HIGH RISK 1.07 - 1.79µmol/L (30 - 49 mg/dL); 4=VERY HIGH RISK > 1.79µmol/L (>50 mg/dL)
The codes for 'LDL CVD RISK' was as follows; 1 = OPTIMAL < 100 mg/dL (<2.59mmol/L); 2= NEAR OPTIMAL/ABOVE OPTIMAL 100 – 129 mg/dL (2.59-3.35mmol/L); 3= BORDERLINE HIGH 131 – 159 mg/dL (3.36-4.12mmol/L); 4= HIGH 160 – 189 mg/dL (4.14-4.90mmol/L)

Descriptive analysis was used to obtain frequency distributions, means, median and standard deviation for Lp(a) and LDL concentrations across all dependent variables.

To test whether data in the study population was normally distributed, using SPSS, both graphical and descriptive statistics were used. Using histograms, Q-Q plots (for visually comparing quantiles of a sample against the population quantile of the normal), and box plots, the dependent variable, Lp(a) and LDL-c distributions were graphed against the independent variables i.e gender. Independent variables (continuous and categorical) were also graphed to visually check for normality.

Shapiro-wilk's test was also used to numerically determine further normality of data. In SPSS, a Shapiro-wilk's significant (p-value) value > 0.05 is an indication that data is approximately normally distributed (Shapiro and Wilk, 1965)

Kurtosis and skewness values with their accompanying standard errors (SE) were obtained and used to calculate normality by dividing e.g skewness value by its SE, a value within the critical value ± 1.96 is considered approximately normally distributed.

In STATA, two sample t test with equal variance was used to test for Lp(a) & LDL-c concentration means between males and females to obtain p-values and means for table 4.The values were reproduced using SPSS also.

Analysis of variance was used in both STATA and SPSS to compare the Lp(a) and LDL-c concentration means between groups of each independent categorical variable and p-values were obtained for statistical significance.

For correlations between the continuous variables age, duration and Lp(a) concentration, Pearson's correlation was used to obtain the correlation coefficients after visually inspecting the scatter plot for linearity and found that it was approximately linear (a straight line could be drawn following linear characteristic dots).

To test for association between the dichotomous outcome variable 'high Lp(a)' and the independent predicting variable or in other words association between having high levels of Lp(a) and sex, age category, duration category and ART combination, chi-square was used. The same test was employed to test for association between having high LDL-c levels and sex, age category, duration category and ART combination.

Univariate logistic regression was used to predict or measure the effect of a single independent variable in contributing to the outcome variable (high Lp(a) levels).

For combined effect, multivariate logistic regression was used to measure how much each independent variable (sex, age category, duration on ART category and ART combination) was contributing in predicting the outcome variable (high Lp(a) concentration levels). Odds ratios (OR), Adjusted Odds Ratio (AOR) and p-values were obtained from this analysis for statistical significance.

For associations between having high Lp(a) concentration levels and a low or high LDL-c, chi-square test of association was used.

For tabulation of participant's risk categories for CVD, descriptive statistics, in this case bar charts, were used.

3.9.1 Ethical considerations

The study was undertaken after ethical approval from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) Assurance No. FWA00000338 IRB00001131 of IORG0000774, REF No. 007-10-14 obtained on the 12th of December, 2014. For the participant's samples, there was no need for patient consent as only blood samples for routine testing were used. There was no direct contact with the patient. However, permission was sought and granted from Livingstone General Hospital Management and ART clinic before data collection was started. Permission was also sought and granted for the use of clinical samples from Livingstone General Hospital Management. Only ART numbers were used to obtain information from patient files using laboratory sample serial numbers. The information obtained in the data collection forms was treated with total confidentiality and was not disclosed to anyone. It was only used for research purposes. No patient identifier was extracted in the data collection forms used. The participant's names, address, and other personal information was not obtained and only a study number (laboratory serial number) was used to connect their ART number (or name where necessary) without identifying them. Data obtained from this study and the records was kept in a private locked cabinet.

3.9.2 VARIABLES AND THRESHOLDS

Table 1. INFORMATION ON THE VARIABLES USED

NO.	OUTCOME VARIABLE	DEFINITION	UNIT & SCALE OF MEASURE AND VARIABLE INFORMATION
1^0	High Lipoprotein (a) concentration	Serum concentration levels ≥ 1.07µmol/L (30mg/dL)	µmol/L or mg/dL. Scale of measure: categorical. This is the primary outcome variable in the study
2nd	High LDL-C concentration	Serum concentration levels ≥ 3.36mmol/L	mmol/L Scale of measure: categorical
	INDEPENDENT VARIABLES	**DEFINITION**	**UNIT & SCALE OF MEASURE AND VARIABLE INFORMATION**
1	Age category	Number of years from date of birth of the participant, to the time when sample was collected from the participant.	Measured in years. Scale of measure: categorical variable ranging from 18-45 years. Categorized in four groups ; 18-24,25-31,32-38,39-45
2	Sex	Defined as either male or female	Scale of measure: categorical variable
3	Duration on HAART	In this study, this variable was defined as the period that the HIV positive participant was initiated on ART to the time a sample was taken for lipoprotein (a) and LDL cholesterol determination.	- Unit used is Years. - Scale of measure: categorical variable - Categories were used in this study - Categorized in 2 groups namely; 1-5 years and 6-10 years

4	ART combination	Was the current ART combination the participant was taking	Name of drugs combined Only NNRTI/NRTI based regimen. Categorized in 5 groups TDF/FTC/EFV; TDF/FTC/NVP; D4T/3TC/NVP; AZT/3TC/NVP; D4T/3TC/EFV

TABLE 2: Lp(a) THRESHOLDS

Lp(a)	Normal serum level of Lp(a) : < 0.50 µmol/L (<14mg/dL) Low risk for CVD : 0.50-1.06µmol/L (14 - 29 mg/dL) High risk for CVD : 1.07 -1.79µmol/L (30 - 49 mg/dL) Very high risk for CVD : ≥ 1.79µmol/L (≥50 mg/dL)

(Ryan, GM & Julius, T 2005)

TABLE 3: LDL-c THRESHOLDS (National Cholesterol Education Program (NCEP) 2001)

LDL-c	1. < 100 mg/dL (<2.59mmol/L) : Optimal 2. 100 – 129 mg/dL (2.59-3.34mmol/L) : Near optimal/Above optimal 3. 130 – 159 mg/dL (3.36-4.12mmol/L) : Borderline high 4. 160 – 189 mg/dL (4.14-4.90mmol/L) : High 5. ≥ 190 mg/dL (≥ 4.92mmol/L) : Very high

CHAPTER FOUR

4. RESULTS

4.1 Characteristics of participants

The total number of participants in the study was 143 and comprised more females, 86 (60.1%) than males, 57 (39.9%) with a male to female ratio of 2:3. The males had a mean Lp(a) concentrations of 0.88 µmol/L ranging from 0.01 µmol/L – 1.83 µmol/L with a standard deviation (SD) of 0.45 µmol/L and the females had mean Lp(a) concentrations of 0.85 µmol/L ranging from 0.02-1.85 µmol/L (SD ± 0.44 µmol/L) . The LDL-c concentration means for the males and females was 2.23 mmol/L ranging from 0.94 -4.14 mmol/L and 2.43 mmol/L ranging from 1.10-3.79 mmol/L respectively.

The participant's mean age was 34 years, ranging from 18 to 45 years. The ages of the participants were categorized in four groups namely; 18-24 years comprising of 21(14.7%) participants, 25-31 years comprising of 31(21.7%) participants, 32-38 years comprising of 48 (33.6%) participants and 39-45 years comprising of 43 (30.1%) participants.

Duration on ART was categorized in two groups of five year interval (1-5 years & 6-10 years) with the majority of participants (88 (61.5%)) in the 1-5 year category. The 6-10 year category had 55 (38.5%) participants.

There were 5 groups of different HAART combinations into which the study participants were grouped, with the majority in group 1 , as follows; group 1 had 101 (70.6%) participants who were on tenofovir disoproxil fumarate + emtricitabine + efavirenz (TDF+FTC+EFV), group 2 with 18 (12.6%) participants on tenofovir disoproxil fumarate + emtricitabine + nevirapine (TDF+FTC+NVP), group 3 with 10 (7.0%) participants on stavudine + lamivudine + nevirapine (D4T+3TC+NVP), group 4 with 9 (6.3%) participants on zidovudine + lamivudine + nevirapine (AZT+3TC+NVP) and group 5 with 5 (3.5%) participants on stavudine + lamivudine + efavirenz (D4T+3TC+EFV)

Table 4: Demographic and clinical characteristics of the study population in comparison with mean Lp(a) and LDL-c concentrations .

characteristic		n (%)	Lp(a) µmol/L (range)	p-value	LDL-c mmol/L(range)	p-value
[tt]Sex						
	Male	57 (39.9)	0.88 (0.01,1.83)	0.719	2.23 (0.94,4.14)	0.078
	Female	86 (60.1)	0.85 (0.02,1.85)		2.43 (1.10,3.79)	
Total		143(100)				
[a]Age category(years)						
	18-24	21 (14.7)	0.57(0.02,1.48)	0.001	2.26(1.10,3.54)	0.129
	25-31	31 (21.7)	0.84(0.02,1.73)		2.19(0.94,3.73)	
	32-38	48 (33.6)	0.85(0.01,1.75)		2.34(1.16,3.79)	
	39-45	43 (30.)	1.04(0.17,1.85)		2.53(1.41,4.14)	
[a]Duration category (years)						
	1-5	88 (61.5)	0.75(0.01,1.83)	<0.001	2.32(0.94,4.14)	0.420
	6-10	55 (38.5)	1.04(0.10,1.85)		2.41(1.54,3.79)	
[a]ART combination						
	TDF/FTC/EFV	101 (70.6)	0.83(0.01,0.85)	0.136	2.34(0.94,4.14)	0.971
	TDF/FTC/NVP	18 (12.6)	1.01(0.10,1.75)		2.44(1.41,3.76)	
	D4T/3TC/NVP	10 (7.0)	0.80(0.45,1.42)		2.37(1.68,3.54)	
	AZT/3TC/NVP	9 (6.3)	1.12(0.40,1.65)		2.25(1.61,3.40)	
	D4T/3TC/EFV	5 (3.5)	0.65(0.25,1.07)		2.37(1.54,2.81)	

tt= two sample t test with equal variance was used to obtain p value; a= analysis of variance test was used; TDF= tenofovir disoproxil fumarate; FTC= emtricitabine; EFV=efavirenze; NVP=nevirapine; D4T= stavudine; 3TC= lamivudine; AZT= zidovudine. Note: read percentages by column.

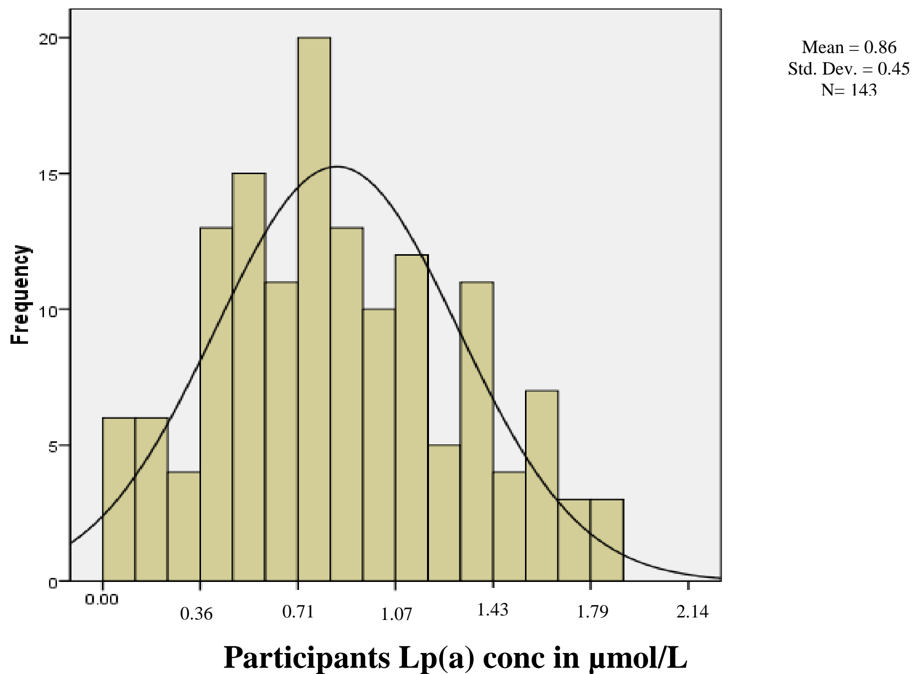
Table 4 shows that there was no difference in the Lp(a) and LDL-c concentration means between males and females. It also shows a significant association between age and Lp(a) concentration. Duration on ART was significantly associated with Lp(a) concentrations as well. All HAART combinations led to increase in Lp(a) in the long term.

4.2 Distribution of Lp(a) and LDL-c in the study population

A Shapiro-Wilk's test ($p > 0.05$) (Shapiro and Wilk 1965) (fig 3-6) showed that the Lp(a) & LDL-c concentrations were approximately normally distributed for both males and females (Doane & Seward 2011).

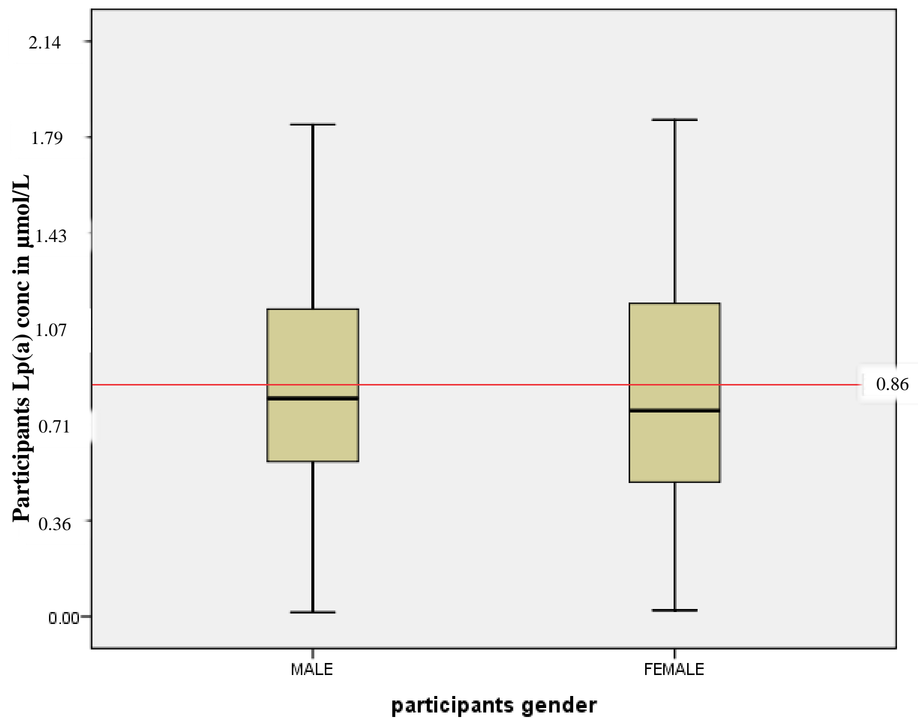
For details of the normality tests, kurtosis and skewness values for both independent and dependent variables, see appendix D

Fig. 3 Showing a Histogram of the distribution of Lp(a) in the study population



According to figure 3, Lp(a) was normally distributed in the population with mean 0.86µmol/L ±0.45µmol/L with the majority having levels below 1.07µmol/L. However, quiet a significant number had levels above 1.07µmol/L (considered high Lp(a) level)

Fig.4 Box plot showing the distribution of Lp(a) concentration levels in males and females



p=0.719

As shown in figure 4, Lp(a) distribution levels were similar in both males and females with means (±SD) 0.88µmol/L ±0.45µmol/L and 0.85µmol/L ±0.44µmol/L respectively. The median is shown by the dark horizontal lines demarcating each box. The line cutting through the two boxes was the overall mean (0.86µmol/L). The figure shows no significant difference between male and female distribution of Lp(a).

Fig. 5 Showing a Histogram of the distribution of LDL-c in the study population

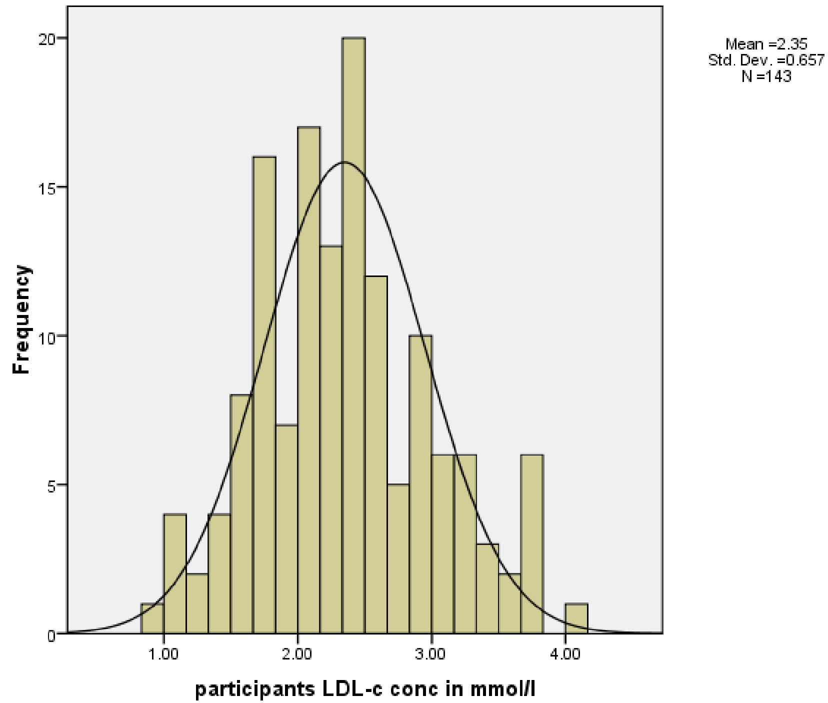
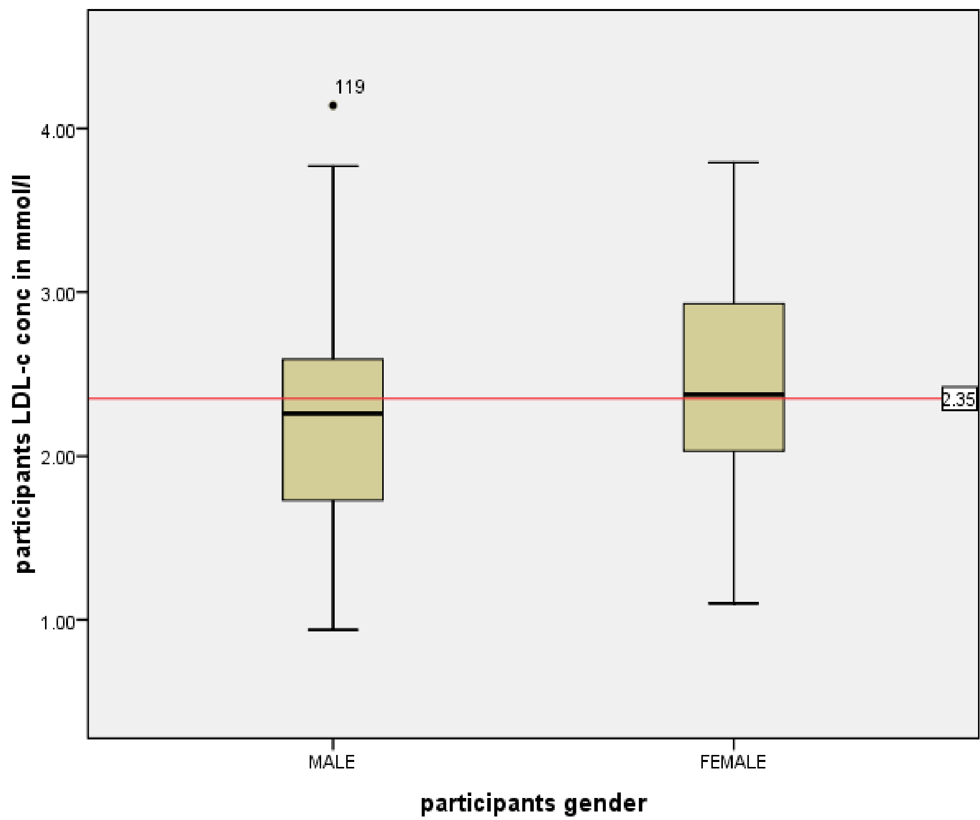


Figure 5 shows that LDL-c was normally distributed in the study population with mean 2.35mmol/L ±0.66mmol/L (SD). The majority had normal reference levels (below 3.36mmol/L). LDL-c was also similar across all age groups irrespective of gender as shown below.

Fig.6 Box plot showing the distribution of LDL-c concentration levels in males and females



p=0.078

Figure 6 shows that LDL-c was similar in both males and females with mean (±SD) 2.23mmol/L ±0.66mmol/L and 2.43mmol/L ± 0.65mmol/L. Note: the number 119 in the figure is an outlier, it represents the participant's study number.

4.3 Prevalence of high Lp(a) and LDL-c concentration in the study population

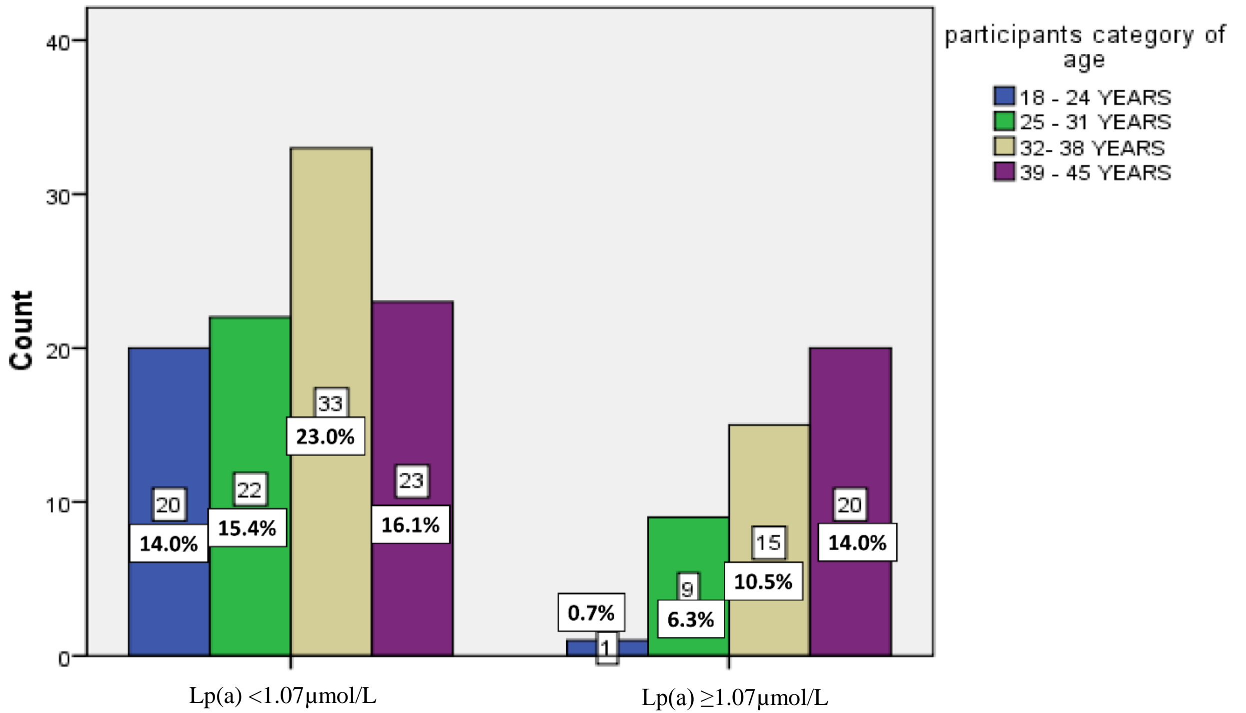
Table 5: comparison of Lp(a) concentration below or above normal with independent variables

characteristics	n (%) high LP(a)level (≥1.07µmol/L)	Low Lp(a) level (<1.07µmol/L)	p-value[c]
n	45(31.5%)	98 (68.5%)	
Sex			
Male	18 (31.6)	39 (68.4)	0.982
Female	27 (31.4)	59 (68.6)	
Age category(in years)			
18-24	1 (4.8)	20 (95.2)	0.009
25-31	9 (29.0)	22 (71)	
32-38	15 (31.3)	33 (68.8)	
39-45	20 (46.5)	23 (53.5)	
Duration category (in years)			
1-5 years	17 (19.3)	71 (80.7)	<0.001
6-10 years	28 (50.9)	27 (49.1)	
ART combination			
TDF/FTC/EFV	28 (27.7)	73 (72.3)	0.086
TDF/FTC/NVP	8 (44.4)	10 (55.6)	
D4T/3TC/NVP	2 (20)	8 (80)	
AZT/3TC/NVP	6(66.7)	3(33.3)	
D4T/3TC/EFV	1 (20)	4 (80)	

C= Chisquared test was used; TDF= Tenofovir disoproxil fumarate; FTC= Emtricitabine; EFV=Efavirenze; NVP=Nevirapine; D4T= Stavudine; 3TC= lamivudine; AZT= Zidovudine. Note: read percentages by row.

As shown in table 5, there was no association between having high Lp(a) levels and sex but high Lp(a) levels were more prevalent with age and increased duration on HAART. Specific ART combination was not associated with increased Lp(a), however, all combinations led to high Lp(a) in the long term.

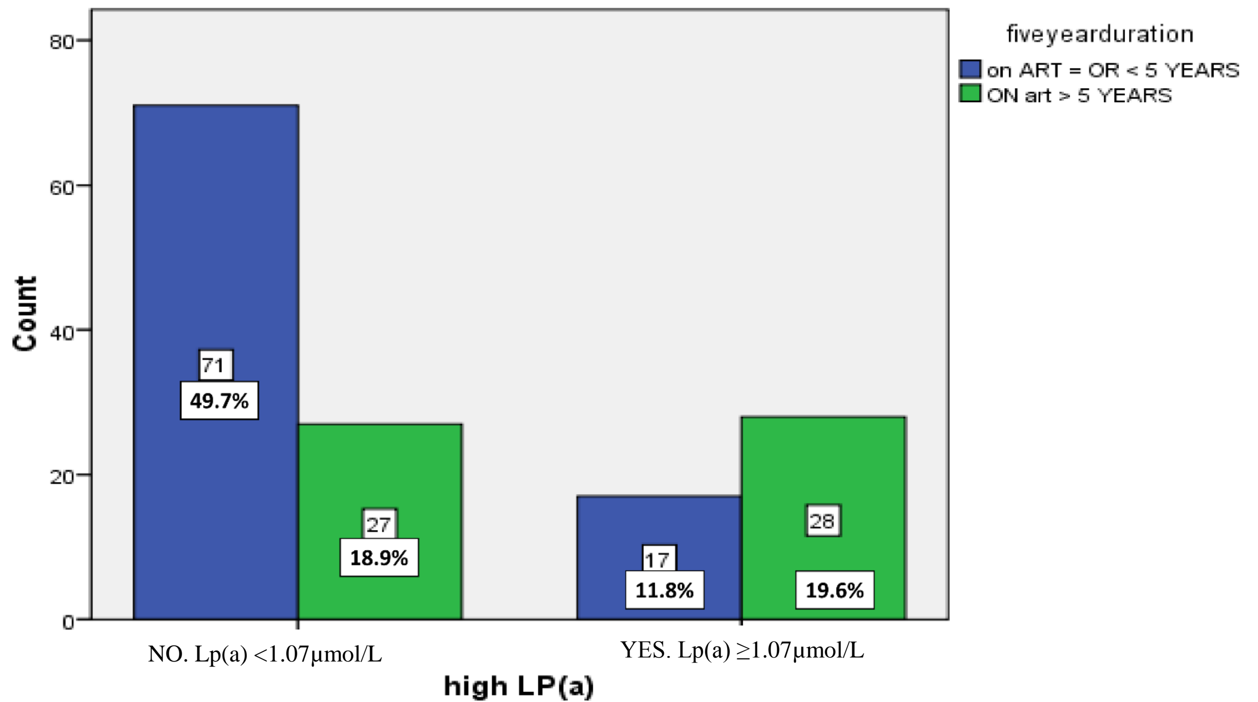
Fig 7. A bar chart showing high and low Lp(a) in different age categories



p=0.009

Figures 7 showed that as age increased, Lp(a) was higher. The number of participants increase with advance in age category especially for participants having high Lp(a) levels (≥1.07µmol/L). There is a significant difference between participant's age categories.

Fig 8. A bar chart showing high and low Lp(a) concentration level in different ART duration category (five year duration)



p<0.001

As seen from figure 8, the participants with longer duration on HAART, had high Lp(a) concentration levels compared to participants on short duration and this relationship is significant.

Table 6: comparison of LDL-c concentration below or above normal with the independent variables

Independent variable	n (%) high LDL-c concentration (≥3.36mmol/L)	n (%) Low LDL-c concentration (<3.36mmol/L)	p-value[c]
n	11(8%)	132(92.3%)	
Sex			
Male	3 (5.3)	54 (94.7)	0.375
Female	8 (9.3)	78 (90.7)	
Age category(in years)			
18-24	1 (4.8)	20 (95.2)	0.618
25-31	1 (3.2)	30 (96.7)	
32-38	5 (10.4)	43 (89.6)	
39-45	4 (9.3)	39 (90.7)	
Duration category (in years)			
1-5 years	6 (6.8)	82 (93.2)	0.620
6-10 years	5 (9.1)	50 (90.9)	
ART combination			
TDF/FTC/EFV	8 (7.9)	93 (92.1)	0.943
TDF/FTC/NVP	1 (5.6)	17 (94.4)	
D4T/3TC/NVP	1 (10)	9 (90)	
AZT/3TC/NVP	1 (11.1)	8 (88.9)	
D4T/3TC/EFV	0(0.0)	5(100.0)	

C= Chisquared test was used; TDF= tenofovir disoproxil fumarate; FTC= emtricitabine; EFV=efavirenze;NVP=nevirapine; D4T= stavudine; 3TC= lamivudine; AZT= zidovudine. Note: read percentages by row.

As shown in table 6, there was no difference between having high or low LDL-c levels and sex, age, duration on HAART or type of ART combinations.

4.4 Logistic Regression to determine the contribution of each independent variable in predicting high Lp(a) concentration levels.

Table 7. Predictors of High Lipoprotein(a) using univariate and multivariate logistic regression

Variable	Odds Ratio[u] (95% CI)	p-value	adjusted Odds[m] Ratio OR(95%CI)	p-value
Sex				
Male	1.00		1.00	
Female	0.99 (0.48,2.04)	0.982	0.62 (0.26,1.50)	0.297
Age category(in years)				
18-24	1.00		1.00	
25-31	8.18 (0.95,70.44)	0.056	10.14 (0.89,115.03)	0.062
32-38	9.09 (1.11,74.16	0.039	11.47 (1.06,124.04)	0.045
39-45	17.39 (2.13,141.33)	0.008	16.32 (1.46,181.98)	0.023
Duration category (in years)				
1-5 years	1.00		1.00	
6-10 years	4.33 (2.04,9.15)	<0.001	4.00 (1.66,9.64)	0.002
ART combination				
TDF/FTC/EFV	1.00		1.00	
TDF/FTC/NVP	2.08 (0.74,5.82)	0.161	1.20 (0.39,3.65)	0.738
D4T/3TC/NVP	0.65 (0.13,3.25)	0.602	0.39 (0.06,2.32)	0.306
AZT/3TC/NVP	5.21 (1.21,22.29)	0.026	9.02 (1.41,57.54)	0.020
D4T/3TC/EFV	0.65 (0.06,6.08)	0.707	0.69 (0.05,8.75)	0.780
Low LDL-c	1.00		1.00	
High LDL-c	1.26 (0.35,4.57)	0.716	1.10 (0.24,4.90)	0.895

U= univariate logistic regression was used; m= multivariate logistic regression was used; TDF= tenofovir disoproxil fumarate; FTC= emtricitabine; EFV=efavirenze;NVP=nevirapine; D4T= stavudine; 3TC= lamivudine; AZT= zidovudine

As shown in table 7, the ages 32-45 contributed significantly in predicting high Lp(a) levels with reference to the ages 18-24 years. The odds of having high Lp(a) were about 4 times more in participants on longer duration (6-10 years) compared to the participants on short duration on HAART (1-5 years). The odds of having high Lp(a) (AOR- in multivariate analysis) in participants on AZT/3TC/NVP, were 9.02 (p=0.020) times more than those on TDF/FTC/EFV.

Table 8: Association between Lp(a) and LDL-c concentration levels

	n(%)			
	low Lp(a) conc. (<1.07µmol/L)	**high Lp(a)conc. (≥1.07µmol/L)**	**total**	**p value**
LDL-c < 3.36mmol/l	91 (68.9)	41 (31.1)	132(100)	0.716
LDL-c ≥ 3.36mmol/l	7 (63.6)	4(36.4)	11(100)	
total	98 (68.5)	45(31.5)	143(100)	

As shown in table 8, there was no significant difference between having high or low Lp(a) levels and LDL-c concentration levels

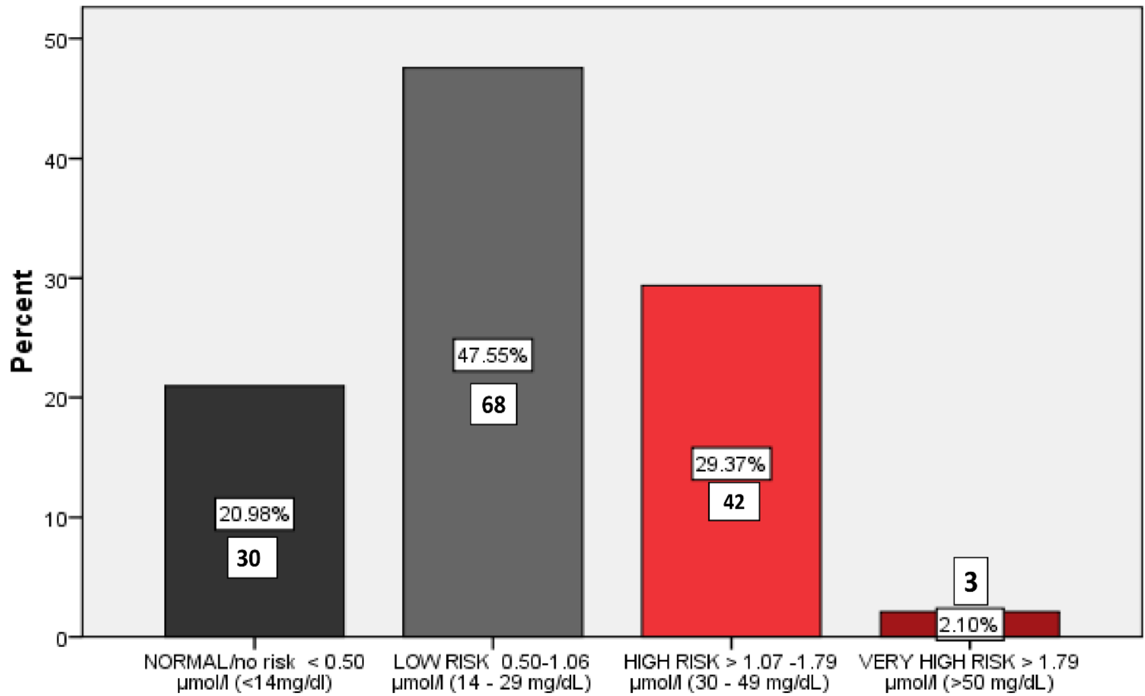
Pearson's correlation results

A correlation was done using Pearson's correlation in SPSS and the result showed a significant positive relationship between age r(141) = 0.32, p<0.001, duration on HAART r(141) =0.43, p<0.001 and Lp(a) concentration levels.

Pearson's correlation was used also to determine the relationship between age (as a continuous variable) and LDL-c concentration levels, the result showed a weak positive significant relationship r(141) = 0.180, p= 0.031.

4.5 Participant's risk category for developing CVD based on their Lp(a) and LDL-c concentrations.

Fig. 9 Bar chart showing the participant's risk category for developing a cardiovascular disease based on their Lp(a) serum concentration levels

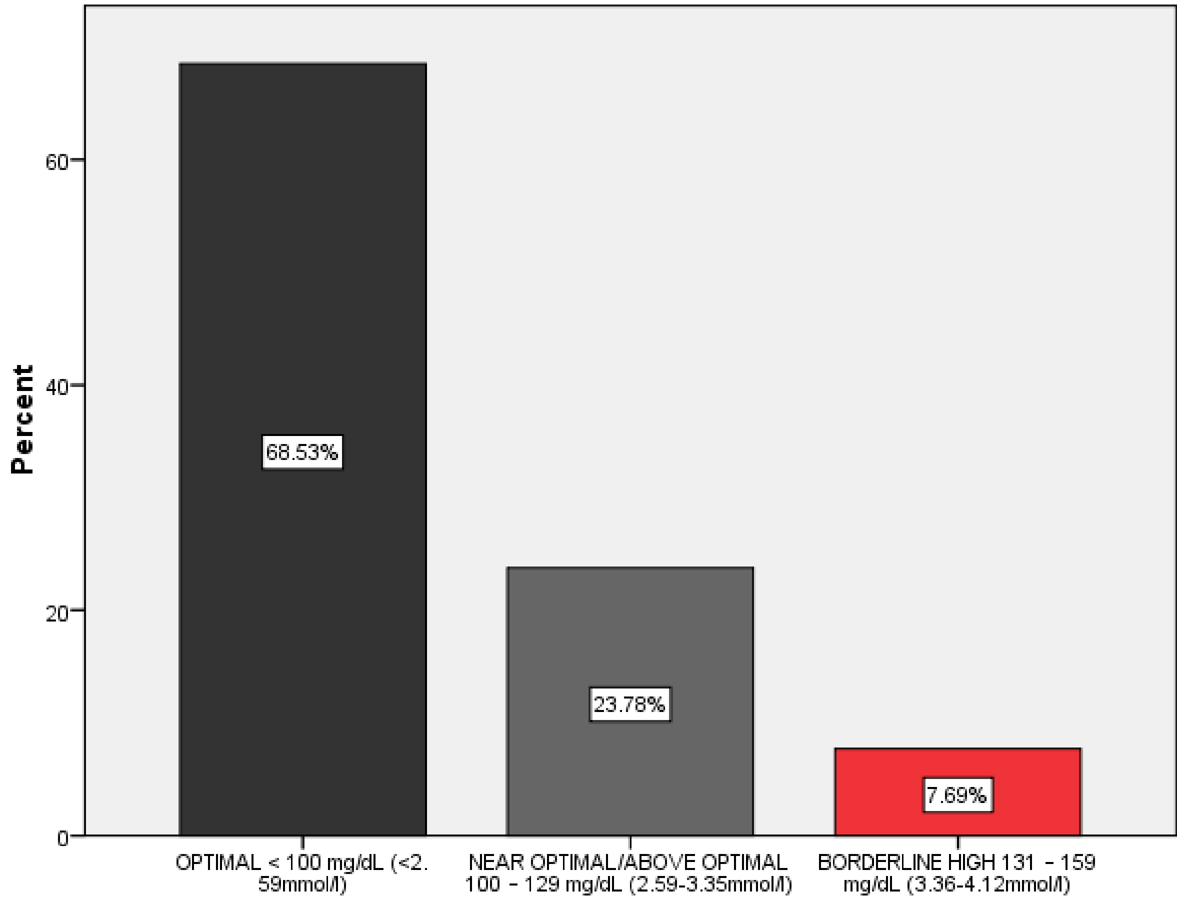


Risk category for developing a CVD using a Lp(a) conc. threshold of 0.50µmol/L

Reference values adopted from Ryan, GM and Julius, T (2005)

Figure 9 shows that 79% of the participants were at risk for CVD, however, of these, 48% were at low risk; 29% at high risk and 2% very high risk.

Fig. 10 Bar chart showing the participant's risk category for developing a cardiovascular disease according to their LDL-c levels



Risk category for developing a CVD using a LDL-c threshold of 3.36mmol/l

Adopted from National Cholesterol Education Program (NCEP) (2001).

Figure 10 shows that about 7.7% of the participants had borderline high risk for CVD based on their LDL-c level. The rest (92.3%) had no risk of which 68.5% had optimal or most desirable LDL-c level and 23.8% had a near optimal/above optimal LDL-c level.

CHAPTER FIVE

5.0 DISCUSSION

This was a cross sectional laboratory based study that was aimed at describing the distribution of Lp(a) and determining the prevalence of high Lp(a) levels in HIV positive individuals on HAART and also determining their risk categories for developing cardiovascular disease based on the Lp(a) and LDL-c concentrations. The study also determined the effect of duration on HAART, ART combination and age on Lp(a) serum concentrations.

Lp(a) distribution

Lp(a) concentration was normally distributed in the study population with mean Lp(a) levels of 0.88µmol/L ± 0.45µmol/L (SD) in males and 0.85µmol/L ± 0.44µmol/L in females. The overall mean in the study population was 0.86µmol/L ± 0.45µmol/L. This can possibly have both negative and positive clinical and laboratory implications considering that the means were above normal levels (<0.50µmol/L) but below the cut off threshold (1.07µmol/L). However, a follow up study would be required to ascertain the clinical implications of this, as many factors such as were elucidated by Siekmeier et al. (2008) and Paultre et al. (2000), like genetic, environmental, CVD incidence due to high Lp(a), dietary factors, apo(a) size isoform et cetera would need to be considered and also, due to scarce information on Lp(a) in HIV, it is difficult to ascertain from this study design, the actual clinical implications. Nevertheless, the mean distribution result was different from those reported by Sawadogo et al (2013) in Burkina Faso (West africa) where they reported the mean Lp(a) concentration of 1.72µmol/L ± 0.96µmol/L in HAART treated group. The difference was possibly due to their mean age (37.9±10.4 and 41± 7.6 years for participants treated with HAART for six and 12 months respectively) which was higher than the one reported in this study as well as due to genetic, ethnic and geographical variations in Lp(a) concentrations (Dube et al.2002, and Achim 2005). The reduction in sample size in this study could also contribute significantly to the differences seen, as a result, another study with a larger sample size is needed to establish clearly, the clinical and laboratory implications. The less skewed

distribution of Lp(a) (in blacks) reported in this study is similar to that reported in other studies (Helmhold et al.1991 and Achim 2005).

Prevalence of High Lp(a) concentration

According to data from prospective studies showing a higher cardiovascular risk in patients with Lp(a) levels ≥1.07µmol/L (Cantin et al.2002,Glader et al.2002, Stubbs et al.1999 and Shai et al.2005), the same ≥1.07µmol/L cut off value was used in this study at which Lp(a) concentration level was considered high.

As shown in table 5, the prevalence of high Lp(a) among HIV positive individuals in the study population was high, 31.5% (45) of which 18 were male, representing 31.6% of all the males (57) in the study population. 27 females had high Lp(a) Concentration level and only 59 (68.6%) out of all the females (88) in the study population had low Lp(a) concentration. The prevalence of high Lp(a) in the age group 18-24 years was 4.9% out of a total of 21 participants in that group. The prevalence in the age group 25-31 years was 29.0% (n=31), 31.3% (n=48) in the age group 32-38 years and 46.5% (n=43) in the group aged 39-45 years.

From the above prevalence figures, this implies that even though Lp(a) prevalence was similar in both males and females, levels tended to be raised with an increase in age. This is important and it means the Lp(a) levels of patients with advancing age should be closely monitored for raising trends. The distribution and prevalence levels that we found in this study, considering the age limit being younger (≤ 45 years old), seem to suggest negative implications based on the cut-off value for CVD risk owing to the fact that Lp(a) is associated with enhanced atherogenic potential, particularly at levels ≥ 1.07µmol/L, and has generally been shown to be an independent predictor (odds ratio ≈ 1.5 to 2) of cardiovascular risk particularly in younger (<60 years old) subjects (Ariyo et al. 2003 and Danesh et al. 2000).

The prevalence of high Lp(a) in HIV positive individuals in this study (31.5%) was lower, compared to the findings of Shahmanesh et al. (2001) who reported that Lp(a) higher than 1.07µmol/L was observed in 41% receiving non-nucleoside reverse

transcriptase inhibitors (NNRTI). However, Niehues et al. (2005) reported a prevalence of 22%. The differences again may be due to factors such as geographical location, ethnic group, Genetic orientation, Duration on HAART and age of participants. The reduction in sample size from 245 to 143 in this study could be the reason for the difference as well.

Age and duration on HAART

It was shown from the results that age and duration played an important role in increasing the levels of Lp(a) concentration. The older the participant ($p= 0.009$) and the longer the duration ($p<0.001$) the more the Lp(a) concentration level. Using univariate and multivariate logistic regression, it was shown that the level of Lp(a) was likely to be higher as age advanced ((ages 32-38 AOR 11.47(95% CI[1.06,124.04]) and 38-45 AOR 16.32(95%CI[1.46,181.98]) in comparison with age category 18-24 years). A correlation was done using Pearson's correlation in SPSS and the result showed a significant positive relationship between age $r(141) = 0.32$, $p<0.001$, duration on HAART $r(141) =0.43$, $p<0.001$ and Lp(a) concentration levels.

This means that, even though, the patient may positively benefit clinically from the advantages of longer duration on HAART such as reduced viral load under given circumstances, they can still suffer negative effects of raised serum Lp(a) that comes with longer duration on HAART. Hence, the need for close follow up of patients who have been on HAART for a longer duration as well as advancing age as shown below.

As shown in table 7, there was a significant association between age category and having high Lp(a) levels. A logistic regression conducted showed that in comparison to the reference group (18-24 age category) the odds of having high Lp(a) in the ages 32-38 years and 39-45 years were 9.09 (95% CI [1.11,74.16]) and 17.39 (95% CI[2.13,141.33]) respectively and this relationship was significant. Further adjusted (multivariate analysis), the results showed that the participants aged 32-38 years were 11.47 AOR (95% CI [1.06, 124.04]) times more likely to have high Lp(a) serum levels compared to participants aged 18-24 years and the participants aged 39-45 years were 16.32 AOR (95% CI[1.46,181.98]) times more likely to have high Lp(a) serum levels compared to the participants aged 18-24 years. Another significant finding from the table using the

adjusted ratio was that the group that was on ART for six to ten years were 4.00 AOR (95% CI[1.66,9.64]) times more likely to have a Lp(a) ≥ 1.07µmol/L compared to those participants who were on ART only for one to five years (reference group).

The relationship between age, duration on HAART and Lp(a) levels was similar to a study done by Sawadogo et al.(2013) where they reported that participants who were on HAART for 12 months had higher Lp(a) levels (1.96µmol/L ± 1.22µmol/L) compared to the group treated for six months (1.42µmol/L ± 0.91µmol/L) (p=0.034).They also reported that an increase of Lp(a) with age (p=0.025) was found particularly in the group of 35 to 50 years (2.10µmol/L ± 1.20µmol/L). Similarly, in a follow up study by Mauss et al. (2008), they reported an absolute change in Lp(a) concentration after six months of ART showing a positive correlation with baseline concentration of Lp(a) (spearman p=0.367, p< 0.01).

As shown in Table 6, 11(8%) out of the total population (143) had LDL-c levels equal to or above 3.36mmol/L of which 3 were male and 8 were female. There was no association between age category (p=0.618), duration on HAART (p=0.620) and having high (≥ 3.36mmol/L) LDL-c levels. This was contrary to a study done in Zambia by Kiage et al.(2013) assessing the early effects of combination antiretroviral therapy (cART) on CVD risk markers where they reported a significant increase in LDL-c (P=0.02) in the group on D4T +3TC +NVP after three months of treatment. The same was reported by Van der Valk et al.2001. However, in the current study, when Pearson's correlation was used to determine the relationship between age (as a continuous variable) and LDL-c concentration levels, the result showed a weak positive significant relationship r(141) = 0.180, p= 0.031. The differences can be explained by the transformation or use of the categorical variable 'high LDL-c' for LDL-c which reduces the power of statistical test used. But when used as a continuous variable as shown by the Pearson's correlation, there is a significant positive association between LDL-c levels and age. However, the differences in LDL-c may be hard to explain as the study was limited. Some information like participant's diet, life style which has an impact on LDL-c were

not obtained to ascertain why the distribution tended to be thus. The reduced sample size may be another factor responsible for the observed differences.

Sex

Sex of the participants had no correlation with Lp(a) levels, this was similar to the report by Sawadogo et al.(2013), p=0.34.This may be due to the 90% genetic predisposition of Lp(a) levels in individuals (Boerwinkle et al.1992).

There was no significant association between sex (p=0.375) and having high (≥ 3.36mmol/L) LDL-c levels. However, the mean LDL-c levels in men (2.23mmol/L) were lower than those of females (2.43mmol/L) which is similar to a study by Agete et al. (2012) where they reported that the females had proportionally raised LDL-c, slightly higher when compared to males. However, they did not report the mean LDL-c levels but reported percentages; 23.7% of 38 males had LDL-c ≥3.36mmol/L while in 75 females, 38.7% had LDL-c ≥ 3.36mmol/L. This report is similar to the findings in this current study were 5.3% out of 55 males had LDL-c ≥ 3.36mmol/L and 9.3% out of 88 females had LDL-c ≥ 3.36mmol/L. The reasons for these differences may be due to the mean age of the participants on HAART which was 37.2 ±8.7 years while the mean age and SD of the participants in this current study was 34.0 ± 7.6 years, as LDL-c in some cases is positively associated with increased age. As a matter of fact, the LDL-c in their study was significantly associated with increased age (p=0.004) while in this current study LDL-c was not associated with age (p=0.129). However, other reasons are likely to explain this difference which were beyond the scope of this study.

HAART combinations

In this study, there were more participants (70.6%) on tenofovir disoproxil fumarate (TDF), emtricitabine (FTC) and efavirenz (EFV) ART combination with regard to other NRTI & NNRTI based regimen. However, there was no association between type of ART combination and mean Lp(a) concentration levels (p=0.136) and there was no specific ART combination associated with raised Lp(a) concentrations (p= 0.086) though the group receiving AZT+3TC+NVP (6.3%) were 9.02 (AOR) (95% CI[1.41,57.54]) times more likely to have high Lp(a) levels compared to the group receiving

TDF+FTC+EFV (70.6%). All combinations increased Lp(a) in the long term. In a study done by Mauss et al.(2008), they also reported that no difference was found between patients receiving efavirenz (EFV) and those receiving nevirapine (NVP) (P=0.23) or between those receiving tenofovir (TDF) and those receiving zidovudine (p=0.74).

There was no association between ART combination (p=0.943) and having high (≥ 3.36mmol/L) LDL-c levels. This was contrary to a study by Gallant et al. (2004) which showed that some antiretroviral drugs, such as stavudine (d4T) increase the blood levels of LDL-cholesterol. Nevirapine (NVP) was associated with increases in LDL-cholesterol as well (Van der Valk et al.2001). This difference in the findings can be attributed to the fact that in our study, a combined analysis (ANOVA) was employed for ART combination and not for each specific combination (within groups). Also, logistic regression was not analyzed on the contribution of specific ART combinations in predicting high LDL-c levels. It is important to also mention that the number of patients on stavudine in this study was not sufficient to detect what others found in their studies as cited above.

LDL-c

The odds of having high Lp(a) serum levels in participants with high LDL-c levels was 1.10 (95%CI[0.24,4.90]) times compared to those with low LDL-c but as shown in table 7, this finding was not significant (p=0.895). Furthermore, using chi-square as shown in table 8, high levels of Lp(a) were not significantly (p=0.716) associated with LDL-c serum levels.

In this study, the levels of Lp(a) concentration were not associated with LDL-c levels (p=0.716).This was contrary to a study done by Koppel et al.(2000) where they reported that the elevation of Lp(a) did not relate to any other clinical or laboratory parameter than to LDL-cholesterol. The reason for this was not clear. However, though Lp(a) was not associated with LDL-c, they are both risk factors for CVD and a meta-analysis of prospective studies demonstrated that elevated levels of Lp(a) was an independent risk factor for CVD and that the pro-atherogenic influence of Lp(a) seemed to be particularly

enhanced in subjects with elevated levels of LDL cholesterol (Danesh et al.2000 and Suk et al.2006).

Lp(a) and established risk factor for cardiovascular disease, LDL cholesterol, has been evidently demonstrated to interact in increasing the risk for CVD (Maher et al. 1995 and von Eckardstein et al. 2001), in particular, given high levels of both lipoproteins. However, in our study, LDL-c levels were particularly not elevated, suggesting a rather reduced interaction in increasing the risk for CVD. Nonetheless, in support of a pro-atherogenic role, Lp(a) has been detected in the vessel wall, where it appears to be retained more avidly than LDL-c and independently, potentially predicting CVD owing to its highly atherogenic properties (Beisiegel et al. 1990 and Siekmeier et al 2008).

Risk category for developing CVD based on Lp(a) and LDL-c concentrations.

As shown in figure 9, 21%(30) had Lp(a) concentration level below 0.50µmol/L which is the normal reference value with no known risk for CVD, 48%(68) were in the low risk category (Lp(a) concentration levels ranging from 0.50µmol/L-1.04µmol/L) for developing a CVD, 29%(42) were in the high risk category (Lp(a) levels ranging from 1.07-1.75µmol/L) for developing a CVD and only 2% (3) were in the very high risk category (Lp(a) ≥ 1.79µmol/L) for CVD. 79% of the participants were at risk for CVD based on Lp(a) levels. This is clinically critical and significant, warranting close monitoring of these patients.

According to figure 10, based on LDL-c levels, only 11(8%) were in the borderline high risk category for CVD (LDL-c concentration from 3.36-4.12mmol/L). 98(68%) of the total population were in the optimal category (LDL-c concentration < 2.59mmol/L) and 34 (24%) were in the near optimal/above optimal category (LDL-c concentration from 2.59-3.35mmol/L). There were no participants in the high risk category for CVD (LDL-c concentration from 4.14-4.90mmol/L) and very high risk category for CVD with LDL-c concentration levels above 4.92mmol/L. This means that the burden of high LDL-c levels was lower compared to the prevalence of high Lp(a) levels in these patients.

Studies on the risk categories for developing CVD based on Lp(a) and LDL-c in HIV positive patient on HAART are scarce and we did not find any specific study in literature in this respect to compare with. This study could be one of the few to categorize participant’s risk for developing CVD using Lp(a) and LDL-c concentration levels.

5.1 CONCLUSION

Since Lp(a) tended to be raised with increasing age ($p=0.009$) and longer duration on HAART ($p < 0.001$), this implies that age & duration on HAART were risk factors for CVD risk and contributed significantly to high prevalence levels of high Lp(a) in HIV patients on HAART at Livingstone General Hospital. This entails that in the management of HIV, patients on HAART must also be monitored for Lp(a) levels especially those with longer duration and advancing age. Lp(a) can also be used to categorize patients according to their risk for developing CVD, this helps in identification and application of appropriate preventive measures.

5.2 LIMITATIONS OF THE STUDY

Even though the study sample size (n=143) was normally distributed, we cannot rule out a possibility that a different picture of results would be seen if the original sample size was used, so the sample size in this study was a limitation.

High cost of reagents resulted in a small sample size, the results of this study cannot therefore be generalized to the whole population. A follow-up study would probably be required if we were to generalize the results to the whole population.

5.3 RECOMMENDATIONS

i. Periodic serum Lp(a) check-ups in HIV positive patients at Livingstone General hospital is recommended.
ii. Determining the baseline Lp(a) concentrations across the country (Zambia) for HIV positive patients on HAART and HIV positive treatment naïve patients will be important so that the picture at large scale can be established.

iii. Conducting baseline Lp(a) tests before initiating HIV treatment naïve patients on ART.
iv. Since Lp(a) levels in the normal population is limited to genetic variations with inter individual variations of 1000, and variations due to ethnic as well as geographical location, it will be important to establish what the levels are in the HIV negative population as well.
v. Screening of high risk patients and their families for plasma Lp(a) may permit early detection and preventive treatment of CAD (Coronary Artery Disease).

5.4 IMPLICATIONS FOR FUTURE RESEARCH

I. There was only one study in literature that reported that those with high Lp(a) levels at baseline before initiating HAART had significant increase compared to those with low baseline Lp(a) levels. Further study is needed to determine if baseline Lp(a) is the basis of significant Lp(a) increase in HIV positive patients initiating HAART.
II. Experimental research is needed to determine the mechanism by which HAART raises Lp(a) in HIV positive patients.
III. There is need for a follow-up study with a larger sample size to determine specific ART regimen associated with significant Lp(a) increase, if it exists, by comparing the effect of ART combinations on Lp(a) levels.
IV. Genetic studies are required to determine carriers of LPA allele genetic variants in Zambians independently associated with coronary artery disease and Comparison of these LPA genetic variants between HIV positive patients with low Lp(a) and high Lp(a) levels on HAART.
V. To identify modifiable metabolic risk factors associated with Lp(a) increase given the current difficulties in therapeutically reducing Lp(a) levels in individuals predisposed to high Lp(a).

5.5 CONTRIBUTION TO SCIENCE AND CHEMICAL PATHOLOGY

This is the first study known to us that has determined and categorized CVD risk in HIV positive patients on HAART based on Lp(a) levels. This study has therefore

determined an easy way of estimating prevalence burden of high Lp(a) that can be used to identify high risk patients.

It is also the first study in literature, based on our knowledge, to assess serum Lp(a) levels in HIV positive patients over longer duration on HAART (ten years).

5.6 CLINICAL AND LABORATORY USE

Lp(a) is likely to be a potential surrogate for LDL-c and other lipoproteins owing to its highly thrombotic and atherogenic properties compared to the existing lipid biomarkers.

Due to the elusive nature of Lp(a) attributable to its 90% genetic predisposition and ethnic variability, more studies are required to ascertain the actual clinical and laboratory implications.

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APPENDICES

APPENDIX A: BUDGET

Item	Detail	Cost estimate ZMW	Quantity needed	Total ZMW
UNZABREC submission fee		**500**		**500**
Stationery and printing	Data collection forms	**100**	**250**	**100**
Transport	**LSK-L/STONE**	**500**	**2**	**500**
Lp(a) reagent	**LP3403** **R1 1x10ml** **R2 X 1X6ml**	**K2,847 ($438)**	**3**	**8,541**
Lp(a) calibrator	**LP3404** **5x 1ml**	**K3,113 ($479)**	**1**	**3,113**
Lp(a) Control	**LP3406** **Level 3 3x1ml**	**K2,080 ($320)**	**1**	**2,080**
Lipid Control	**LE2668** **Level 1 5x1ml**	**K689 ($106)**	**1**	**689**
Admin & freight	**From Ireland, UK**	**K1,488.5 ($229)**		**1,488.5**
CUSTOMS CLEARANCE		**900**		**900**
LDL-c reagent	**OSR183**	**4,270.25**	**1**	**4,270.25**
LDL-c Control	**ODC0005**	**3,865.70**	**1**	**3,865.70**
LDL-c Calibrator	**ODC0012**	**3,101.55**	**1**	**3,101.55**
Thesis Preparation costs		**500**		**500**
TOTAL				**K29,649**

APPENDIX B: GANNT CHART

	July-Aug 2014	sept 2014	October-november 2014	12th Dec 2014	March 2015	April 2015	May 2015
Presented to Department							
Submitted proposal to Asst Dean (PG) office							
Presented at GPPF							
Submit proposal to UNZA REC review and approval							
Approved by UNZA REC							
collect data							
Analyze data							
Write dissertation							
Presented results							
Submit final dissertation							

APPENDIX C: DATA COLLECTION FORM

Lipoprotein(a) assessment data collection tool
Study number:
Date

Participant's Identification

Identification number

Age of participant

Sex of Participant: 1.Female [ ] 2. Male [ ]

Study site (Specify name) ………………………………………………………….

SECTION 1: ART & DRUG HISTORY

1. Is the patient on ART? YES [ ] NO [ ]
2. What is the patient's current drug combination?
…………………………….. + …………………………………. + …………………
3. How long have they been on this combination?
…………………………………………
4. Has the physician changed their drug combination before? If yes, what combination were they on?
……………………………………………………………………………………..
5. How long have they been on ART? ………………………

:

SECTION 2: LABORATORY RESULTS

ART Number : [ ]
Specimen container used / type:
Green (serum) [ ] plain/ plain (plasma) [ ]

Lp(a) concentration value: [ ]
LDL Cholesterol value: [ ]

END OF DATA COLLECTION FORM

APPENDIX D: INDEPENDENT AND DEPENDENT VARIABLE TESTS OF NORMALITY OF DISTRIBUTION IN THE STUDY POPULATION

Dependent variable	**Independent variable**	**Skewness(SE)**	**±1.96**	**Kurtosis(SE)**	**±1.96**	**(Shapiro-Wilk's test of normality) significant at p-value > 0.05**	**COMMENT**
Lp(a) conc.by	**Sex**						
	male	0.219(0.316)	0.69	-0.411(0.623)	-0.660	0.309	Approx. normal
	female	0.285(0.260)	1.09	-0.624 (0.514)	-1.21	0.181	Approx. normal
Lp(a) conc.by	**ART Combination**						Approx. normal
	TDF/FTC/EFV	0.292(0.240)	1.21	-0.389(0.476)	-0.81	0.174	Approx. normal
	TDF/FTC/NVP	-0.94(0.536)	-1.75	-1.083(1.038)	-1.04	0.323	Approx. normal
	D4T/3TC/NVP	0.900(0.687)	1.31	-0.244(1.334)	-0.18	0.217	Approx. normal
	AZT/3TC/NVP	-0.567(0.717)	-0.79	-0.132(1.400)	-0.09	0.915	Approx. normal
	D4T/3TC/EFV	0.113(0.913)	0.123	-0.531(2.000)	-0.26	0.842	Approx. normal
Lp(a) conc.by	**Age (years)**						
	18-24	0.645(0.501)	1.28	0.465(0.972)	0.47	0.543	Approx. normal
	25-31	0.246(0.421)	0.58	-0.598(0.821)	-0.72	0.625	Approx. normal
	32-38	0.247(0.343)	0.72	-0.419(0.674)	0.62	0.401	Approx. normal
	39-45	0.191(0.361)	0.52	-0.646(0.709)	-0.91	0.499	Approx. normal
Lp(a) conc.by	**Duration (years)**						
	1-5	0.359(0.257)	1.39	0.051(0.508)	0.10	0.260	Approx. normal
	6-10	-0.080(0.322)	0.24	-0.990(0.634)	1.56	0.159	Approx. normal
	age	-0.407(0.203)		-0.670(0.403)			Approx. normal
Lp(a) conc. levels		0.256(0.203)	1.26	-0.564(0.403)	-1.39		Approx. normal
LDL-c conc. levels		0.342(0.203)	1.68	-0.202(0.403)	-0.50		Approx. normal
LDL-c conc. by	**sex**						
	Male	0.482(0.316)	1.52	0.362(0.623)	0.58	0.475	Approx. normal
	female	0.281(0.260)	1.08	-0.428(0.514)	0.48	0.91	Approx. normal

APPENDIX E : ETHICAL APPROVAL LETTER

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

12th December, 2014.

Our Ref: 007-10-14.

Mr. Sepiso Masenga,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P.O Box 50110,
Lusaka.

Dear Mr. Masenga,

RE: RESUBMITTED RESEARCH PROPOSAL: **"SERUM LEVELS OF LIPOPROTEIN (a) IN HIV POSITIVE PATIENTS ON HAART AT LIVINGSTONE GENERAL HOSPITAL, SOUTHERN PROVINCE, ZAMBIA" (REF. No. 007-10-14)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 24th November, 2014. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Mrs. M. Mbewe
CHAIRPERSON

Date of approval: 12th December, 2014. **Date of expiry:** 11th December, 2015.

APPENDIX F: PERMISSION LETTERS

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

Telephone : +260211252641
Telegram: UNZA, Lusaka
Telex: UNZALU ZA 44370

P.O Box 50110
Lusaka, Zambia

Email: assistantdeanpgmedicine@unza.zm

23rd September, 2014

Mr Sepiso K Masenga
Department of Pathology & Microbiology
School of Medicine
UNZA
LUSAKA

Dear Mr. Masenga,

RE: GRADUATE PROPOSAL PRESENTATION FORUM

Having assessed your dissertation proposal titled **"Assessment of Serum LipoproteinA in HIV Patients on ART at Livingstone Genreal Hospital, Southern Province (Zambia) 2014",** we are satisfied that all the corrections to your research proposal have been done. The proposal meets the standard as laid down by the Board of Graduate Studies.

You can proceed and present to Research Ethics Committee.

Yours faithfully,

Dr. S.H. Nzala
ASSISTANT DEAN, POSTGRADUATE
CC: HOD, Pathology & Microbiology

APPROVED

Department of pathology and Microbiology
School of Medicine
University of Zambia
P.O. Box 50110
Lusaka, Zambia.

22nd September, 2014.

The senior Medical superintendent,
University Teaching Hospital,
P.O Box RW1X,
Lusaka, Zambia.

UFS:

The Head of department,
Department of pathology and Microbiology,
University Teaching Hospital,
P.O Box RW1X,
Lusaka, Zambia.

No objection from Dept

Dear sir/madam,

RE: REQUEST FOR PERMISION FOR THE USE OF U.T.H LABORATORY BECKMAN COULTER AU480 CHEMISTRY ANALYSER FOR LDL-c AND LIPOPROTEIN(a) SAMPLE ANALYSIS FOR MY ACADEMIC RESEARCH

The above matter refers.

I am an MSc student pursuing a degree In Chemical Pathology at the University of Zambia, School of medicine. As part of the academic requirement , I will be conducting a research at Livingstone General Hospital (L.G.H) and intend to analyze samples from U.T.H due to the unavailability of a chemistry analyzer that is able to analyze Lipoprotein(a) .

I hereby ask for permission from you for the use of Beckman coulter AU480 chemistry analyzer for determination of **LDL-c and Lipoprotein(a) levels (for 245 samples)**. I will use my own reagents, Controls and calibrators for the same purpose. The sample analysis time for the said tests is under 3hrs and can be run anytime in 24hrs as will be accorded.

Please kindly note that L.G.H has already granted me permission to conduct the study. However, I will only be allowed to conduct this research following your approval which I must submit to the University of Zambia Biomedical Research Ethics committee (UNZABREC) for approval to conduct the research. I will also furnish you with an approval letter from UNZABREC prior to the research.

Your assistance will be highly appreciated.

Find attached the study information and approval letter from L.G.H.

Yours faithfully,

Mr. Sepiso K. Masenga

All correspondence should be addressed
To the Executive Director
Telephone: 03 – 320221
Fax: 03 – 321365

In reply please quote:

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

LIVINGSTONE GENERAL HOSPITAL
P.O BOX 60091
LIVINGSTONE

27th August, 2014.

Mr. Sepiso Masenga,
Department of pathology and Microbiology,
School of Medicine,
University of Zambia,
P.O. Box 50110,
Lusaka, Zambia.

Dear Mr. Masenga,

RE: PERMISION TO CONDUCT A RESEARCH AT LIVINGSTONE GENERAL HOSPITAL: MSc PATHOLOGY (CHEMICAL PATHOLOGY).

We are in receipt of your letter dated 8th August 2014 on the above subject.
Approval has been granted to you Mr. Sepiso K Masenga, NRC 242633/75/1, Biomedical scientist pursuing a Master's degree in pathology (chemical pathology) at the University of Zambia, to conduct a research on **"Assessment of serum Lipoprotein(a) in HIV Patients on ART at Livingstone General Hospital, Southern Province (Zambia) 2014."**

However, the research should only commence upon the production of a copy of UNZA REC approval.

You will also be required to furnish Livingstone General Hospital with a summary of your research findings at the completion of the study.

Yours sincerely,

Dr. N. H. Monze
Medical Superintendent
Livingstone General Hospital

Cc. Head Clinical Care
Cc. File

APPENDIX G: LDL-CHOLESTEROL PROTOCOL (OSR6196 and OSR6296)

BECKMAN COULTER © Beckman Coulter, Inc. 2010; Rev #:1, Dec 01, 10

This procedure is valid for the following chemistry analyzers:

- AU400/AU400e
- AU480
- AU600
- AU640/AU640e
- AU680
- AU2700/AU5400

PRINCIPLE:

LDL-Cholesterol (LDL-C) plays a causal role in the development of coronary heart disease (CHD). In 1988, the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) developed recommendations for the diagnosis and treatment of patients with hypercholesterolemia[1]. These recommendations defined LDL Cholesterol as the primary target of therapy.
The 2001 update of these guidelines (NCEP-ATP III)[2] put further emphasis on better risk identification and more aggressive cholesterol-lowering treatment.
The guidelines classify LDL- Cholesterol levels as follows

1. < 100 mg/dL: Optimal
2. 100 – 129 mg/dL: Near optimal/above optimal
3. 131 – 159 mg/dL: Borderline high
4. 160 – 189 mg/dL: High
5. ≥ 190 mg/dL: Very high

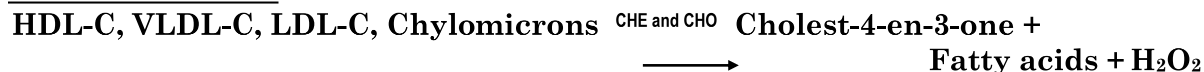
INTENDED USE:

System reagent for the quantitative determination of LDL-Cholesterol in human serum or plasma on Beckman Coulter AU Clinical Chemistry analyzers.

METHODOLOGY:

The Beckman Coulter AU System LDL-Cholesterol test is a two reagent homogenous system. The assay is comprised of two distinct phases. In phase one, a unique detergent solubilizes cholesterol from non-LDL-lipoprotein particles. This cholesterol is consumed by cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrine to generate a colorless end product.
In phase two, a second detergent in the R2 reagent releases cholesterol from the LDL-lipoproteins. This cholesterol reacts with cholesterol esterase, cholesterol oxidase, and a chromogen system to yield a blue color complex which can be measured bichromatically at 540 / 660nm. The resulting increase in absorbance is directly proportional to the LDL-C concentration in the sample.

Reaction Phase 1:

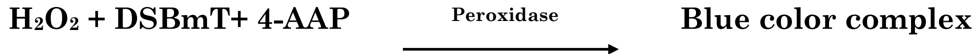


HDL-C, VLDL-C, LDL-C, Chylomicrons CHE and CHO → **Cholest-4-en-3-one + Fatty acids + H_2O_2**

H_2O_2 – 4-AAP Peroxidase → **LDL-C + Colorless end product**

Reaction Phase 2:

LDL-C CHE and CHO → **Cholest-4-en-3-one + Fatty acids + H_2O_2**



H_2O_2 + DSBmT+ 4-AAP $\xrightarrow{\text{Peroxidase}}$ Blue color complex

SPECIMEN:

PATIENT PREPARATION:

A twelve to fourteen-hour fast is recommended, but not required.[10]

TYPE:

Serum, EDTA, or heparinized plasma free from hemolysis is the recommended specimen. Separate samples from red blood cells as soon as possible (within 3 hours). Plasma using anticoagulants such as oxalate and citrate is not recommended.[3]

HANDLING CONDITIONS:

Use fresh sample for analysis when possible. If analysis is delayed, specimens are stable 5 days when stored at 2-8°C. If stored for more than 5 days, specimens are stable for up to 3 months when stored at -70°C.
Avoid repeated freezing and thawing of the sample. Samples should only be frozen once.[4]

EQUIPMENT AND MATERIALS:

EQUIPMENT:

Beckman Coulter AU400/AU400e, AU480, AU600, AU640/AU640e, AU680, AU2700, and AU5400 analyzers.

MATERIALS:

Beckman Coulter AU System LDL-Cholesterol Reagent
Final concentration of reactive ingredients:

Ingredient	Concentration
MES Buffer (pH 6.3)	
Cholesterol esterase (Pseudomonas)	1875 U/L
Cholesterol oxidase (Nocardia sp.)	1125 U/L
Peroxidase (Horseradish)	975 U/L
Detergent 1	0.75 %
Detergent 2	0.25 %
DSBmT	0.25 mmol/L
4-aminoantipyrine	0.375 mmol/L
Ascorbate Oxidase	2250 U/L

Also contains preservatives.
Test tubes 12 -16 mm in diameter or sample cups (Cat No. AU1063).
Beckman LDL-Cholesterol Calibrator (Cat. No. ODC0024)
Storage location of the calibrator in this laboratory:

Precautions:

1. The Beckman LDL-Cholesterol Assay and Calibrator are for *in vitro* diagnostic use.
2. Do not ingest reagents or calibrators. Harmful if swallowed.
3. The LDL Reagent contains sodium azide as a preservative, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
4. The LDL Calibrator is manufactured from human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products derived from

human blood should be treated as potentially infectious. Therefore adequate safety precautions are recommended.

PREPARATION:

The Beckman Coulter AU System LDL-Cholesterol Reagent is liquid, ready for use. No preparation is needed.
For the LDL-C Calibrator, accurately add 1.0 mL of deionized water to the calibrator vial. Mix thoroughly. Avoid foaming.

Storage Requirements:

1. The unopened reagents and calibrator are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. The reconstituted calibrator is stable for 14 days when stored 2 - 8°C. It may be divided into aliquots and frozen once. The frozen reconstituted calibrator is stable for 30 days at -70°C.
4. Do not use reagents that have been frozen.
5. Protect the reagents from direct sunlight.

Indications of Deterioration:

Discoloration of the reagent, visible signs of microbial growth, turbidity or precipitation in reagent may indicate degradation and warrant discontinuance of use.

PERFORMANCE PARAMETERS:

The following data was obtained using this LDL-Cholesterol Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained at individual facilities may differ.

PRECISION:[9]

Estimates of precision, based on CLSI recommendations[8], are consistent with typical performance. The within run precision is less than 3%CV and total precision is less than 5%CV. Assays of control sera were carried out and data reduced following CLSI guidelines.

N=60	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
52.91	0.41	0.77	1.12	2.12
98.97	0.56	0.57	2.27	2.30
125.08	0.92	0.74	2.85	2.28

METHOD COMPARISON:[9]

Patient samples were used to compare this LDL-Cholesterol Reagent. Representative performance data on AU analyzers is shown in the next table.

Y Method	AU640/AU640e
X Method	Method 2
Slope	0.960
Intercept	-8.1
Correl. Coeff. (r)	0.965
No. of Samples (n)	115
Range (mg/dL)	16 – 188

Sensitivity:

Typical change in absorbance for 1 mg/dL of LDL-Cholesterol is 1.8 mAbsorbance.

CALIBRATION:

STANDARD PREPARATION:

Perform a one-point calibration (AB) using a water blank (blue rack) and the Beckman LDL Cholesterol Calibrator (Cat No ODC0024). Calibration stability is 7 days. The assigned value for this calibrator is traceable to the US CDC LDL Cholesterol reference method. Any instrument or reagent modification may invalidate the assigned value.[11]

CALIBRATION PROCEDURE:

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL:

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

ANALYZER PARAMETERS:

A complete list of test parameters and operating procedures can be found in the appropriate User's Guide and at www.beckmancoulter.com.

CALCULATIONS:

For SI Units (mmol/L), multiply the results by 0.0258.

REPORTING RESULTS:

REFERENCE RANGES:

Adults[7]: 75 - 193 mg/dL

The NCEP guidelines classify LDL- Cholesterol levels as follows

1. < 100 mg/dL: Optimal
2. 100 – 129 mg/dL: Near optimal/Above optimal
3. 131 – 159 mg/dL: Borderline high
4. 160 – 189 mg/dL: High
5. ≥ 190 mg/dL: Very high

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

PROCEDURES FOR ABNORMAL RESULTS:

Abnormal results are flagged by the listed analyzers according to the normal values entered by the user into the instrument parameters.

REPORTING FORMAT:

Results are automatically printed for each sample in mg/dL at 37°C.

LIMITATIONS:

The Beckman Coulter AU System LDL-Cholesterol procedure is linear from 7 to 400 mg/dL. Samples exceeding the upper limit of linearity should be diluted with physiological saline and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN. Samples with triglyceride values up to 1293 mg/dL do not interfere with the results of the LDL-Cholesterol assay. Samples with triglyceride levels greater than this should not be diluted.

INTERFERING SUBSTANCES:

Results of studies[5] show that the following substances interfere with this LDL-Cholesterol procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 20 mg/dL Ascorbate
Bilirubin:	No significant interference up to 40 mg/dL conjugated Bilirubin
	No significant interference up to 40 mg/dL unconjugated Bilirubin
Globulin:	No significant interference up to 5 g/dL added Gamma Globulin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 900 mg/dL Intralipid*
Triglyceride:	No significant interference up to 1500 mg/dL Triglyceride**

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

**Triglyceride concentrate manufactured by Miles Pentex, Cat No 96-051-6, was used to measure triglyceride interference.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[6] for a compilation of reported interferences with this test.

REFERENCES:

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APPPENDIX H RANDOX LABORATORIES QUALITY CONTROL, CALIBRATION & REAGENT INSERT PROTOCOLS

LIPOPROTEIN (a) (Lp (a))

RX SERIES

INTENDED USE

Immunoturbidimetric assay for the quantitative *in vitro* determination of Lipoprotein (a) in human serum or plasma. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and RX **imola**.

Cat. No.

LP 3403	R1. Buffer	1 x 10 ml
	R2. Latex Reagent	1 x 6 ml

CLINICAL SIGNIFICANCE(1)

Lipoprotein (a) determination is intended for use in conjunction with clinical evaluation, patient risk assessment and other lipid tests to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations.

PRINCIPLE

Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample.

Note: This product is licensed from Denka Seiken.

SAMPLE COLLECTION AND PREPARATION(2)

Collect serum using standard sampling tubes and plasma using tubes containing Li heparin, Na heparin, Na EDTA, K EDTA, citrate.

SAMPLE STORAGE AND STABILITY(3)

Samples may be stored at 4°C for 14 days without significant decrease. For long term storage the samples should be stored at -20°C or -70°C.

REAGENT COMPOSITION

Contents	Initial Concentration
R1. Buffer	
Glycine	0.17M
Sodium Chloride	1.08M
Sodium ethylenediamine tetra acetic acid disodium salt dihydrate	0.05M
Sodium azide	≤0.09% w/v
R2. Latex Reagent	
Glycine	0.17M
Sodium Chloride	0.1M
Suspension of latex particles coated with anti-lp(a) antibodies	0.5%
Sodium azide	≤0.09% w/v

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer

Buffer is ready for use and is stable up to the expiry date when stored at +2°C to +8°C protected from light.

R2. Latex Reagent

Latex Reagent is ready for use and stable up to the expiry date when stored at +2°C to +8°C protected from light. Invert several times before use, avoiding the formation of foam.

Reagent 1 = Buffer
Reagent 2 = Latex Reagent

MATERIALS PROVIDED

Buffer
Latex Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Lipoprotein (a) Calibrator Series Cat. No. LP 3404
Randox Lipoprotein (a) Control Level 3 Cat. No. LP 3406
Randox Lipid Controls:-

Level 1	LE 2661 or LE 2668
Level 2	LE 2662 or LE 2669
Level 3	LE 2663 or LE 2670

NB all 3 levels of lipid control contain Lp(a) with concentrations in the normal range.

PROCEDURE NOTES

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue contact Randox Laboratories RX Support, Northern Ireland (028) 94451070.

CALIBRATION

The use of Randox Lp(a) Calibrator Series is recommended for calibration. Saline is used as S1 and Cal 1-5 as S2-S6. A multi point calibration is recommended, with change of reagent lot/bottle or as indicated by quality control procedures.

This assay uses an **exponential** calculation and **no reagent blank**. Ensure that on the Calibration Checks screen the following are selected for this test:

Reagent Blank Measurement
- **Disable reagent blank**

QUALITY CONTROL

Randox Lipoprotein(a) Control Level 3 and a Randox Lipid control are recommended for quality control to monitor accuracy and precision. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants ie bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories RX Support, Northern Ireland (028) 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

SPECIFICITY/INTERFERENCE

The following analytes were tested up to the noted levels and did not cause interferences:

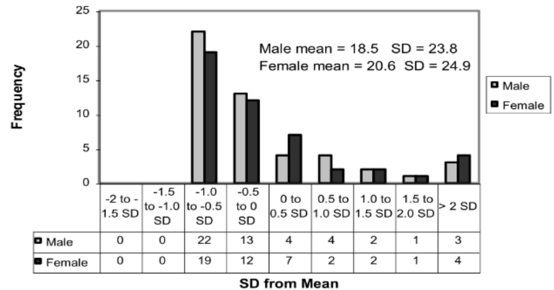
Intralipid®	5%
Bilirubin	35 mg/dl
Haemoglobin	1040 mg/dl
Ascorbic Acid	50 mg/dl
Triglycerides	493mg/dl
Plasminogen	200 mg/dl
Apolipoprotein B	200 mg/dl

NORMAL RANGE [1,4,5]

ADULTS < 30 mg/dl

The above reference range was established based on a sample of 96 Caucasian individuals comprising 49 males (age range 17-90 years; mean = 55 years) and 47 females (age range 13-84 years; mean = 55 years) resident in Northern Ireland. The population tested was an ambulatory population with no history of coronary disease. Results showed a mean Lp(a) value of 18.5 mg/dl for males and 20.6 mg/dl for females. Reference ranges have not been established for this assay for different ethnic populations or disease states.

Lipoprotein (a) In 96 N. Ireland Individuals



	-2 to -1.5 SD	-1.5 to -1.0 SD	-1.0 to -0.5 SD	-0.5 to 0 SD	0 to 0.5 SD	0.5 to 1.0 SD	1.0 to 1.5 SD	1.5 to 2.0 SD	> 2 SD
Male	0	0	22	13	4	4	2	1	3
Female	0	0	19	12	7	2	2	1	4

Lp(a) concentrations have been shown to be genetically determined and to vary with ethnic populations. One study carried out in the United States showed that mean plasma levels of Lp(a) were approximately twice as high in African people or people of African descent compared to levels in Caucasians[4]. Also, the distribution of Lp(a) is less skewed in African people or people of African descent than in Caucasians[4]. Other studies have also shown no difference in Lp(a) levels between men (mean = 14mg/dl) and women (mean =15mg/dl)[5]. Levels of Lp(a) have been shown not to differ significantly between pre- and post menopausal caucasian women[5].

It is therefore recommended that each laboratory establish its own reference range to reflect the age, race, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data was obtained using a RX **daytona** analyser at 37°C.

ASSAY RANGE

The range of this assay is approximately 3 - 90 mg/dl. In the event of a rerun the upper limit is extended to approximately 180 mg/dl.

Alternatively if the sample concentration exceeds the assay range, dilute the sample 1+2 with 0.9% NaCl solution and reassay. Multiply the result by 3.

These values are dependent on the lot specific values of the calibrators in use.

PROZONE EFFECTS

Antigen excess effects are not noted up to 341 mg/dl.

SENSITIVITY

The minimum detectable concentration with an acceptable % coefficient of variation was determined as 3.4 mg/dl.

PRECISION

Within run precision

	Level 1	Level 2	Level 3
Mean (mg/dl)	19.9	27.4	59.1
SD	0.46	0.69	1.02
CV(%)	2.3	2.54	1.72
n	20	20	20

Between run precision

	Level 1	Level 2	Level 3
Mean (mg/dl)	22.8	27.5	57.7
SD	1.39	1.17	1.73
CV(%)	6.09	4.14	2.99
N	20	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 0.97 X + 2.17
and a correlation coefficient of r = 0.995

29 patient samples were analyzed spanning the range 3.49 to 81.29 mg/dl.

The Randox Lp (a) test kit shows minimum apo (a) size related bias. Size heterogeneity of apo (a) can affect to varying degrees the outcome of other commercially available kits.[6]

SERUM/PLASMA COMPARISON

The Randox method was used to compare serum samples (X) to plasma samples (Y) collected into tubes containing Li heparin, Na heparin, Na EDTA, K EDTA or citrate. 56 samples were tested. The data was subjected to linear regression analysis.

Results:

(i) Serum/Plasma (Li heparin):

Sample range:	2 – 77.3 mg/dl
Linear regression analysis:	y = 0.956x – 1.199
Correlation coefficient r:	0.996

(ii) Serum/Plasma (Na heparin)

Sample range:	2.3 – 78.3 mg/dl
Linear regression analysis:	y = 0.958x – 0.522
Correlation coefficient:	0.996

(iii) Serum/Plasma (Na EDTA)

Sample range:	1.7 – 78.8 mg/dl
Linear regression analysis:	y = 0.972x + 0.023
Correlation coefficient:	0.999

(iv) Serum/Plasma (K EDTA)

Sample range:	1.8 – 79.5 mg/dl
Linear regression analysis:	y = 0.981x + 0.085
Correlation coefficient:	0.999

(v) Serum/Plasma (Citrate)

Sample range:	2.0 – 79.4 mg/dl
Linear regression analysis:	y = 0.963x + 0.065
Correlation coefficient:	0.999

LIMITATIONS

1. Performance of this assay was not tested with age - matched pairs in a diseased population.
2. Normal range values for this assay have not been established for African - American populations.
3. This assay has not been tested for interference by Statin Therapy.
4. Intake of alcohol, aspirin, niacin and estrogen supplements have the potential of causing a misrepresentation of the true LP(a) concentrations.

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4. Marcovina, S.M., Albers, J.J., Wijsman, E., Zhang, Z., Chapman, N.H. and Kennedy, H. (1996). Differences in Lp(a) concentrations and apo(a) polymorphs between black and white Americans. Journal of Lipid Research **37**: 2569 – 2585
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Revised 02 Sep 11 ck
Rev. 003

RANDOX

CE

LIPOPROTEIN (a) CONTROL (Lp (a) CONTROL 3)

QUALITY CONTROL LEVEL 3

CAT. NO. LP 3406 **LOT NO.** 920LP
SIZE: 3 x 1 ml **EXPIRY:** 2015-10

INTENDED USE
For use in the quality control of Lipoprotein(a) assays.

CHARACTERISTICS
Randox Lipoprotein(a) level 3 control is based on lyophilised human serum containing Lipoprotein(a).

SAFETY PRECAUTIONS AND WARNINGS
For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

This material has been tested for the HIV (Human Immunodeficiency Virus) Antibody, HBs Ag and HCV antibody and found to be non-reactive using FDA approved methods. However, as no method can offer complete assurance as to the absence of infectious agents, this material should be handled as though capable of transmitting infectious disease.

Dispose of this material according to local regulations.

PREPARATION
The Controls must be reconstituted using the following procedure:

1. Open each vial carefully.
2. Reconstitute by pipetting exactly 1 ml of redistilled water into each vial.
3. Replace the rubber stopper and leave to stand for 30 minutes.
4. Dissolve contents completely by swirling or rotating.
5. Prior to use, mix contents by inverting the vials. Ensure that no lyophilised material remains unreconstituted.
6. The control is then ready for use.

STABILITY AND STORAGE
Unreconstituted controls are stable up to the expiry date shown on the side of each individual bottle when stored at +2°C to +8°C. Once reconstituted, the Randox LP(a) control is stable for 14 days at +2°C to +8°C in the absence of bacterial contamination.

VALUE ASSIGNMENT

MEAN (mg/dl)	RANGE (mg/dl)	MEAN (nmol/l)	RANGE (nmol/l)
46.0	36.8 - 55.2	112.4	89.9 - 134.9

Each lot of control is assayed immunoturbidimetrically by Randox Laboratories Ltd. with reference to a master lot of Lipoprotein(a) Control.

01 Nov '13 ne

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RANDOX CE

LIPOPROTEIN(a) CALIBRATOR SERIES (Lp(a) CAL SET)

CAT. NO. LP 3404
SIZE: 5 x 1 ml

INTENDED USE
For use in the calibration of Lipoprotein(a) assays.

CHARACTERISTICS
Randox Lipoprotein(a) Calibrator Series is based on lyophilised human serum containing Lipoprotein(a). Lp(a) concentrations are present at 5 levels.

SAFETY PRECAUTIONS AND WARNINGS
For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

This material has been tested for the HIV (Human Immunodeficiency Virus) Antibody, HBsAg and HCV antibody and found to be non-reactive using FDA approved methods. However, as no method can offer complete assurance as to the absence of infectious agents, this material should be handled as though capable of transmitting infectious disease.

Dispose of this material according to local regulations.

STABILITY AND STORAGE
Unreconstituted calibrators are stable up to the expiry date shown on the side of each individual bottle when stored at +2°C to +8°C. Once reconstituted, the Randox Lp(a) calibrator series is stable for 14 days at +2°C to +8°C in the absence of bacterial contamination.

PREPARATION
The Calibrators must be reconstituted using the following procedure:
1. Open each vial carefully.
2. Reconstitute by pipetting exactly 1 ml of redistilled water into each vial.
3. Replace the rubber stopper and leave to stand for 30 minutes.
4. Dissolve contents completely by swirling or rotating.
5. Prior to use, mix contents by inverting the vials. Ensure that no lyophilised material remains unreconstituted.
6. The calibrators are then ready for use.

VALUE ASSIGNMENT
Randox Lipoprotein (a) calibrator values are traceable to WHO Reference Material SRM2B.

LOT NO.	LIPOPROTEIN(a) (mg/dl)	EXPIRY DATE
1019LP	9.0	2017-03
1020LP	15.1	2017-03
1021LP	29.2	2017-03
1022LP	55.4	2017-03
1023LP	82.3	2017-03

Instrument specific nmol/l values are available on request. Please contact Randox Laboratories - Technical Services, Northern Ireland, tel: +44 (0) 28 9445 1070 or email Technical.Services@randox.com, with details of the instrument you are using.

08 July '14 ne

RANDOX

LIPID CONTROL - LEVEL 1 (LPD CONTROL 1)

Cat. No. LE2668 **Lot No.** 2219CH
Size: 5 x 1ml **Expiry:** 2016-10

INTENDED USE

This product is intended for *in vitro* use in the quality control of Direct HDL, Direct LDL, Lipoprotein (a), Apolipoprotein A-1, Apolipoprotein B, Cholesterol and Triglyceride methods on clinical chemistry systems.

SAFETY PRECAUTIONS AND WARNINGS

Human source material, from which this product has been derived, has been tested at donor level for the Human Immunodeficiency Virus (HIV1 & HIV2) antibody, Hepatitis B surface antigen (HbsAg) and the Hepatitis C virus (HCV) antibody and found to be NON-REACTIVE. FDA approved methods have been used to conduct these tests. However, since no method can offer complete assurance as to the absence of infectious agents, this material and all patient samples should be handled as though capable of transmitting disease. For *in vitro* diagnostic use only.

STORAGE AND STABILITY

Unopened Lipid Control is stable until the expiry date printed on the product label when stored between +2°C and +8°C. Once reconstituted, the components of the serum are stable for 7 days at +2°C to +8°C, and 4 weeks at -20°C when frozen once. The following exceptions apply: LP(a) is stable for 16 weeks at -20°C when frozen once. Values may drop by up to 10% for Direct LDL Cholesterol, when stored for 4 weeks at -20°C.

PREPARATION FOR USE

Open the vial carefully, avoiding any loss of the material and reconstitute with 1 ml of distilled water. Replace the rubber stopper, close the vial and leave to stand for 30 minutes before use. Ensure that all traces of dry material are dissolved by swirling gently.

MATERIALS PROVIDED

Lipid Control - Level 1 5 x 1ml

MATERIALS REQUIRED BUT NOT PROVIDED

Volumetric pipette
Distilled water

VALUE ASSIGNMENT

Each batch of Lipid Control is submitted to a number of external laboratories. Values are assigned from a consensus of results obtained by these laboratories and internal testing conducted at Randox Laboratories Ltd.

If a method is unavailable, contact Randox Laboratories - Technical Services, Northern Ireland, tel: +44 (0) 28 9445 1070 or email Technical.Services@randox.com

23 Apr '14 ne

RANDOX

LIPID CONTROL - LEVEL 1 (LPD CONTROL 1)

Cat. No. LE2668 Lot No. 2219CH

Size: 5 x 1ml Expiry: 2016-10

Analyte	unit	target	Range low	Range high	methods
Apolipoprotein A-1	g/l	1.01	0.83	1.19	Immunoturbidimetric
	mg/dl	101	82.8	119	
	g/l	0.88	0.72	1.03	Nephelometric
	mg/dl	87.5	71.8	103	
Apolipoprotein B	g/l	0.86	0.70	1.01	Immunoturbidimetric
	mg/dl	85.7	70.3	101	
	g/l	0.73	0.60	0.86	Nephelometric
	mg/dl	73.2	60.0	86.4	
Cholesterol	mmol/l	3.61	3.14	4.08	Cholesterol Oxidase
	mg/dl	139	121	157	
	mmol/l	3.44	2.99	3.89	Siemens Dimension
	mg/dl	133	115	151	
HDL - Cholesterol	mmol/l	0.74	0.63	0.85	Direct Clearance Method
	mg/dl	28.6	24.3	32.9	
	mmol/l	0.36	0.25	0.47	Phosphotungstic acid pptn.
	mg/dl	13.9	9.73	18.1	
	mmol/l	0.74	0.63	0.85	Direct HDL Immunoseparation
	mg/dl	28.7	24.4	33.0	
	mmol/l	0.75	0.64	0.87	Direct HDL PEGME
	mg/dl	29.0	24.7	33.3	
	mmol/l	0.81	0.69	0.94	Direct HDL PPD
	mg/dl	31.4	26.7	36.1	
	mmol/l	0.77	0.66	0.89	Direct HDL Roche 3rd generation
	mg/dl	29.8	25.3	34.3	
	mmol/l	0.72	0.61	0.83	PEG Precipitation
	mg/dl	27.8	23.5	32.1	
	mmol/l	0.82	0.70	0.94	HDL - Ultra
	mg/dl	31.7	27.0	36.4	
LDL - Cholesterol	mmol/l	2.04	1.73	2.35	Direct Clearance Method
	mg/dl	78.7	66.8	90.8	
	mmol/l	2.08	1.77	2.39	Selective detergent methods
	mg/dl	80.3	68.3	92.3	
Lipoprotein (a)	mg/dl	12.1	9.68	14.5	Immunoturbidimetric
	nmol/l	26.0	20.8	31.2	
Triglycerides	mmol/l	1.27	1.07	1.47	Lipase/GPO-PAP no correction
	mg/dl	112	94.7	129	
	mmol/l	1.29	1.08	1.50	Lipase/GK UV no correction
	mg/dl	114	95.6	132	
	mmol/l	1.30	1.09	1.51	Lipase/GPO-PAP 0.11mmol/l correction
	mg/dl	115	96.5	134	

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APPENDIX I: PUBLICATIONS

1. **Masenga SK**, Kaile T, Kantenga T (May 2015), Predisposing factors of high Lipoprotein(a) in HIV positive patients on HAART at Livingstone General Hospital, Zambia. *Jour of Med Sc & Tech*; 4(2); Page No: 125 – 132
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