ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY

ANTIRETROVIRAL TREATMENT ASSOCIATED HYPERGLYCEMIA AND DYSLIPIDEMIA AMONG HIV INFECTED PATIENTS AT BURAYU HEALTH CENTER, ADDIS ABABA, ETHIOPIA

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A THESIS BE SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY, SCHOOL OF ALLIED HEALTH SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (CLINICAL CHEMISTRY TRACK)

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This is to certify that the thesis prepared by Molla Abebe, entitled: *Antiretroviral Treatment Associated Hyperglycemia and Dyslipidemia among HIV Infected Patients at Burayu Health Center, Addis Ababa, Ethiopia, 2012**:*** and submitted in partial fulfillment of the requirements of the degree of Masters of Sciences (Clinical Laboratory Sciences with a Specialty Track of Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Introduction: Development of HAART has brought significant suppression of viral replication, decreasing morbidity and mortality and dramatically transforming HIV into chronic disease. Unfortunately, the prospect of maintaining patients on HAART for long term may be restricted by a heterogeneous collection of unexpected metabolic abnormalities, including dysregulation of glucose metabolism, dyslipidemia and lipodystrophy.

Objective: To assess antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients at Burayu Health Center, Addis Ababa, Ethiopia.

Methodology: A cross-sectional study was conducted on adult HIV infected individuals at Burayu Health Center, Addis Ababa, Ethiopia from September, 2011 to May, 2012. Equal number of HAART naïve and HAART initiated patients (n=126 each) were included in the study. Demographic data were collected using a well-structured questionnaire. TC, TG, HDL-C, LDL-C and glucose were determined using COBAS INTERGA 400 chemistry analyzer. The data were analyzed using SPSS version 19 software. Chi-square, student-t-test and logistic regression were used to assess association between variables. P value < 0.05 was considered as statistically significant.

Result: Of 252 study participants, 72.2% were females, mean age was 35.3 years; mean BMI was 21.4; mean time with the virus was 20.6 months; 62.7% were married; 48.4% were at primary educational level; 52.4% were house wives; 15.5% were TB-HIV co-infected and 43.7% were categorized as WHO stage one. The prevalence of hyperglycemia, hypertriglyceridemia, hypercholesterolemia, decreased HDL-C and increased LDL-C was 7.9%, 22.8%, 42.1%, 50.8% and 23% in HAART initiated and 5.6%, 10.3%, 11.1%, 73% and 7.1% in non-HAART groups, respectively. ART regimens observed as a first line were only containing 2 nucleoside backbones (from AZT/D4T/3TC/TDF) with either NVP or EFV. Serum TG level ≥200 mg/dl was more common among patients who received D4T based than those with AZT based antiretrovirals (34% versus 16.4%, P = 0.029).

Conclusion: First-line HAART is associated with potentially atherogenic lipid profile levels in patients with HIV infection compared to untreated patients in our setting. This indicates glucose and lipid profile levels need to be monitored regularly in HIV infected patients taking antiretroviral treatment.

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I. TABLE OF CONTENTS

Contents **Page**

II. LIST OF FIGURES

III. LIST OF TABLES

IV. ABBREVIATIONS

3TC ---- Lamivudine

ABC ---- Abacavir

AIDS ---- Acquired Immune Deficiency Syndrome

ART ---- Antiretroviral Therapy

ARV ---- Anti-retrovirus

ATV/r ---- Atazanavir/ritonavir

AZT/ZDV ---- Zidovudine

BP ---- Blood Pressure

CART ---- Combination Antiretroviral Therapy

CD ---- Cluster of Differentiation

CVD ---- Cardiovascular Disease

D4T ---- Stavudine

ddI ---- Didanosine

EFV ---- Efavirenz

ESR ---- Erythrocyte Sedimentation Rate

FTC ---- Emtracitabine

GLUT-4 ---- Glucose Transporter Four

HAART ---- Highly Active Antiretroviral Therapy

HDL-C ---- High Density Lipoprotein Cholesterol

HIV ---- Human Immunodeficiency Virus

HK ---- Hexokinase

IL ---- Interleukin

LDL-C ---- Low Density Lipoprotein Cholesterol

LPV/r ---- Lopinavir/ ritonavir

MoH ---- Ministry of Health

mtDNA ---- Mitochondrial DNA

NCEP ---- National Cholesterol Education Program

NNRT ---- Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI ---- Nucleoside Reverse Transcriptase Inhibitor

NtRTI ---- Nucleotide Reverse Transcriptase Inhibitor

NVP ---- Nevirapine

PI ---- Protease Inhibitor

RNA ---- Ribonucleic Acid

SOCS-1 ---- Suppressor of Cytokine Signaling-1

SPSS ---- Statistical Package for Social Sciences

TC ---- Total Cholesterol

TDF ---- Tenofovir disoproxil fumarate

TG ---- Triglycerides

TNF ---- Tumor Necrosis Factor

VCT ---- Voluntary Counseling and Testing

VLDL-C ---- Very Low Density Lipoprotein Cholesterol

V. OPERATIONAL DEFINITIONS

Hyperglycemia: An abnormally high level of glucose in the blood (>115 mg/dl fasting glucose). This condition can exist due to several metabolic disorders such as diabetes mellitus.

Hyperlipidemia: A condition where there is an elevation of lipids, or fats, in the blood. This could be due to an increase in triglycerides, cholesterol, or both.

Dyslipidemia: A condition marked by abnormal concentrations of lipids or lipoproteins in the blood.

Lipodystrophy: Also called fat redistribution, is a disturbance in the way our body produces, uses and stores fat. There are two different kinds of lipodystrophy. In fat wasting, also known as lipoatrophy, fat is lost from particular areas of the body, especially the arms, legs, face and buttocks. The second kind of lipodystrophy is fat accumulation, also known as hyperadiposity. In fat accumulation, fat builds up in particular parts of the body, especially the belly, breasts and back of the neck.

Highly active antiretroviral therapy (HAART): A combination of different drugs commonly two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor or protease inhibitor that used to suppress HIV replication in human body.

Body mass index: A standardized estimate of an individual's relative body fat calculated from the subject's height and weight. It can be used to estimate or determine if subject is at a relatively normal weight, overweight, or obese. It is calculated based on the ratio of weight and square of the height (BMI = weight / (height X height), weight in kilograms and height in meters.

1. INTRODUCTION

1.1. Background Information

The acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) (1). The HIV virus targets the host immune system, making it a very difficult pathogen for the human body to fight. In addition HIV makes the host highly susceptible to secondary infections, rapid mutation rates within the viral genome make vaccine and drug development difficult (2).

The number of people living with HIV worldwide in 2008 was reaching an estimated 33.4 million (31.1 million–35.8 million). As of December 2008, approximately 4 million people in low- and middle-income countries were receiving antiretroviral therapy, a 10-fold increase over five years. In 2008, an estimated 2.7 million (2.4 million–3.0 million) new HIV infections occurred. It is estimated that 2 million (1.7 million–2.4 million) deaths due to AIDS-related illnesses occurred worldwide in 2008 (3).

Highly active antiretroviral therapy (HAART) is the mainstay of treatment for those infected with HIV (4). Since its introduction in 1996, mortality and morbidity rates in HIV-infected individuals in countries with widespread access to HAART have plummeted. The main effect of HAART is to suppress viral replication, allowing the individual's immune system to recover and protecting from the development of AIDS and death (5).

In recent years, provision of HAART to those in need has become an increasingly important and feasible global priority. The current strategy is focused on the roll out of HAART programs on the basis of strict medical need. Priority is therefore being given to the treatment of those at the highest risk of disease progression or death (6).

HAART, using multiple antiretroviral agents that are designed to attack the virus at different parts of the life cycle was found to be more effective than mono-therapy in significantly reducing rates of morbidity and mortality in patients with HIV. Since the three-drug combination regimen (HAART) was initiated in 1996, at least 23 drugs are now available as therapy for either the treatment-naive patient or the patient who has failed a regimen (7). At present, the different ARVs are classified under categories such as nucleoside reverse transcriptase inhibitors (NRTI),

nucleotide reverse transcriptase inhibitors (NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and more recently fusion and integrase inhibitors. These drugs are administered as combined therapy as in the case of HAART. Among the newer classes of drugs under investigation are the assembly and budding inhibitors, as well as the zinc finger inhibitors. Virus assembly and disassembly are particularly attractive candidate processes for antiviral intervention (8).

NRTIs were the first drugs used to treat patients with HIV; they are 'dideoxy-type' nucleoside analog drugs. They have antiretroviral activity via drug phosphorylation, incorporation into viral DNA (deoxyribonucleic acid), DNA termination, and inhibition of the viral reverse transcription enzymes (9). As with NRTI, NNRTI target enzyme is reverse transcriptase. However, NNRTI bind directly and non-competitively to the enzyme reverse transcriptase. Protease inhibitors exert their antiviral effect by inhibiting HIV protease as a crucial enzyme on the HIV life cycle (10). Fusion inhibitors block the last step in the three-step viral entry process consisting of attachment, co-receptor binding and fusion, thereby preventing viral capsid entry into the host cell and prevent uninfected cells from becoming infected (11).

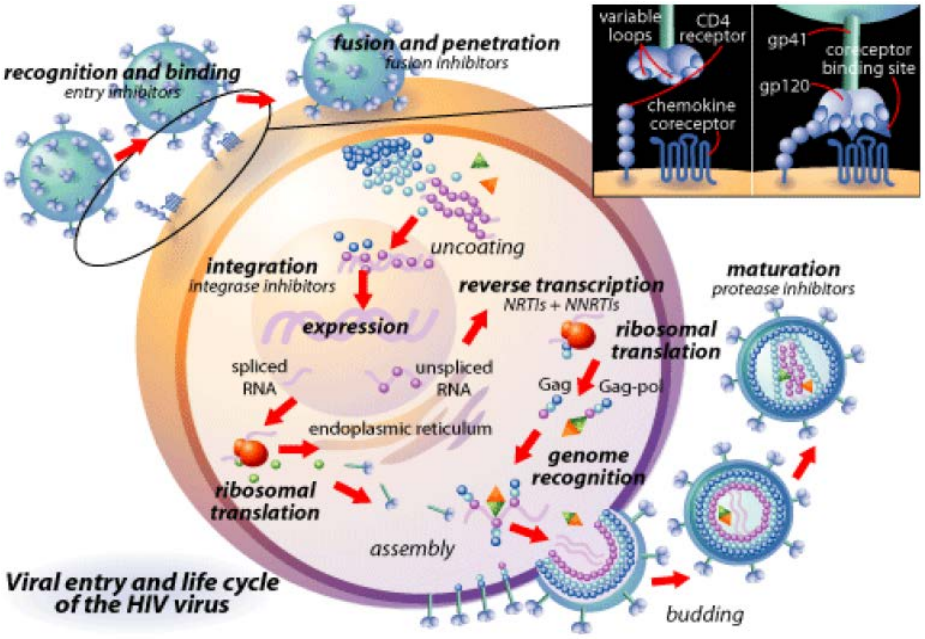


Figure 1: The life cycle of HIV and ARV's mechanism of action (2).

In Ethiopia, as ministry of health (MoH) reports in 2010, approximately there are about 1.2 million people live with HIV, with an adult HIV prevalence of 2.4% (7.7% urban and 0.9% rural) and male-female HIV prevalence of 1.9% and 2.9% respectively. A total of 397,818 people living with HIV are estimated to be in need of antiretroviral treatment (12). HIV has become the leading cause of mortality among 15-49 years of age that accounts for about 43% of all population in 2008. ART was started in the country in 2003 and free ART was launched in 2005. In 2009, 93 public hospitals, 47 private hospitals, 12 military hospitals and 292 health centers and 3 non-governmental organization clinics were providing HIV care and treatment services in all regions of the country (13).

In striving to achieve universal access to ART and further improve the lives of people living with HIV, the Ethiopian government through the MoH endorsed a policy on the supply and provision of antiretroviral drugs in 2002. Since the advent of the ART program, more than 200,000 people have started on treatment in about 500 facilities throughout the country. ART service expansion has been recent and fast from only four facilities in 2003 to 517 in 2009. Parallel with this, the number of people who have accessed ART has also increased substantially from 900 in 2003 to 211,000 in 2009 (14).

Standardized formulary for first and second-line ART, with the use of two NRTIs and one NNRTI as the standard first-line approach, reserving PIs for second-line is the center piece of the public health approach to ART. Recommended first line ARV Regimens for Adults and Adolescents in Ethiopia includes: preferred; TDF+FTC+EFV, ZDV+3TC+EFV and ZDV+3TC+NVP and alternatives; D4T+3TC+NVP, TDF+3TC+NVP, D4T+3TC+EFV, ABC+3TC+NVP, ABC+3TC+EFV, and ABC+3TC+ZDV. In the event of first-line treatment failure, there is indication to start second-line regimens. Apparently, the selection of the second-line regimen depends on the first-line regimen that patient has been taking. The second line regimen during treatment failure of first line combination of TDF+FTC or 3TC +EFV or NVP, ZDV or D4T+3TC+EFV or NVP, and ABC + 3TC + ZDV is ZDV ±3TC +LPV/r or ATV/r Or ZDV+ABC+LPV/r or ATV/r, TDF+3TC±ZDV+LPV/r or ATV/r Or ABC + ddI +LPV/r or ATV/r and EFV or NVP + LPV/r or ATV/r, respectively (15).

Glucose is the molecule that the cells of the human body use as an energy source. Cells use glucose to make ATP (adenosine triphosphate), which is the primary energy provider in the cell.

Maintaining a steady level of glucose in the blood is critical in the human body. If the amount of blood glucose is too much or too little, serious physical problems can occur. For example, low blood sugar can cause unconsciousness and excessively high blood sugar can cause nerve damage. Insulin helps cells take in glucose by making the cell membrane more permeable to the sugar. Severe hyperglycemia may lead to hyperosmolar syndrome and insulin deficiency to life-threatening ketoacidosis. Chronic hyperglycemia causes long-term damage, dysfunction and failures of various cells, tissues and organs (16).

Lipids are either compounds that yield fatty acids when hydrolyzed or complex alcohols that can combine with fatty acids to form esters. Lipids are carried in the bloodstream by complexes known as lipoproteins. This is because these lipids are not soluble in the plasma water. Thus they travel in micelle-like complexes composed of phospholipids and protein on the outside with cholesterol, cholesterol esters, and triglycerides on the inside. The four main types of lipoproteins are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoprotein (LDL) and high density lipoprotein (HDL). Each of these has some percentage of cholesterol or triglyceride associated with its apoproteins and phospholipids, to make each lipoprotein unique. Triglycerides are the major constituent of chylomicrons and VLDL, while cholesterol is the major lipid associated with LDL and HDL. Increased blood level of lipids (hyperlipidemia), especially excess LDL-cholesterol (LDL-C), are associated with cardiovascular problems (17).

1.2. Statement of the Problem

The development of HAART has brought about significant suppression of viral replication, dramatically decreasing morbidity and mortality and transforming the HIV into a chronic disease for many patients. However, the prospect of maintaining patients on long term HAART may be restricted by a heterogeneous collection of unexpected metabolic abnormalities, including dysregulation of glucose metabolism, dyslipidemia, and/or lipodystrophy (18,19).

Both HIV infection itself and antiretroviral therapy can cause or worsen lipid abnormalities (20,21). Metabolic effects of HIV infection such as hypertriglyceridemia are long recognized, and side effects of HAART such as dyslipidemia and insulin resistance were described very soon after its introduction. Prior to the introduction of HAART, it was recognized that HIV infection itself caused dyslipidemia. HIV-infected, untreated patients (particularly those with more advanced disease) are more likely to have low total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) and elevated serum triglyceride (TG) than HIV-negative, with lower HDL-C concentrations associated with higher circulating HIV RNA levels and longer duration of HIV infection (22). Hypertriglyceridemia is common in individuals with HIV infection, but the mechanisms responsible for increased plasma triglyceride (TG) concentrations are not clear (23). Many antiretrovirals (ARVs) have dyslipidemic properties. Use of PIs has been associated with hypertriglyceridemia and hypercholesterolemia. Although NNRTIs induce less dyslipidemia than PIs, it does appear that efavirenz, one of two commonly prescribed NNRTIs, has a deleterious effect on lipids when compared to the other commonly used NNRTI, nevirapine. Use of the NRTI stavudine has been associated with a worse lipid profile than the NtRTI tenofovir, with significantly larger increases in total cholesterol, LDL-C and triglycerides (21,22).

HIV/AIDS patients frequently present with diabetes and metabolic complaints. As treatment of HIV develops, and access to therapy improves, the incidence of HIV-associated diabetes is bound to grow (23). HAART has led to an increase in metabolic dysfunction, including insulin resistance, diabetes dyslipidemia and lipodystrophy (24-26). PI-based HAART has been associated with insulin resistance, hyperglycemia, overt type2 diabetes mellitus, peripheral lipoatrophy, visceral adiposity, and hyperlipidemia but less frequently associated with NRTI or NNRTI use, demographic, and virologic factors. Reports of insulin resistance and the

development of overt diabetes increased with the routine clinical use of HIV-1 PIs. This leads to the supposition that the use of this class of drugs induced hyperglycemia (27).

Some PIs, such as indinavir and ritonavir, block insulin-mediated glucose disposal by a direct blockade of GLUT-4, causing insulin resistance (27,28). Other PIs, such as amprenavir and atazanzvir, have no effect on this mechanism. Indinavir increases hepatic glucose production and release. With indinavir, insulin loses some of its ability to suppress hepatic glucose production; therefore, glycogenolysis and gluconeogensis increase (27). Other PIs cause upregulation of suppressor of cytokine signaling-1 (SOCS-1) expression in insulin-sensitive tissues. SOCS-1 is a known inducer of insulin resistance and diabetes and immunoblotting analyses revealed increases in SOCS-1 protein expression in adipose, skeletal muscle, and liver tissues of indinavir administered rats (29).

Other proposed mechanism(s) for the development of PI-induced insulin resistance and diabetes include: decreased conversion of proinsulin to insulin; increases in soluble type 2 TNFα receptors reflecting activation of the TNF system by TNFα, a known inducer of insulin resistance; reductions in the release of adipocyte-derived adiponectin, which enhances insulin-stimulated suppression of hepatic glucose production and increases in peripheral glucose disposal. Further, “lipotoxicity” due to (a) increased subcutaneous fat loss that results in increases in circulating fatty acids which when taken up by muscle and liver inhibit insulin-signaling (b) inhibition of adipogenesis leading to suppression of lipogenesis, and stimulation of lipolysis, (c) dysregulation of CD36, a facilitator of fatty acid uptake, or (d) decreases in adipocyte-derived perilipin, a regulator of lipid metabolism, leading to stimulation of lipolysis is also recognized to act in concert with other metabolic perturbations to promote PI-induced insulin resistance and type 2 diabetes (29,30).

Circulating cytokine levels, particularly TNF-α and IL-6, are frequently elevated in HIV positive patients. It is well established that such elevations in TNF-α can cause significant impairment of insulin action. The molecular mechanism by which elevations in TNF-α and other inflammatory cytokines lead to insulin resistance is incompletely understood. However, these cytokines can influence both normal suppression of hepatic glucose production and insulin stimulated glucose uptake. In addition, TNF-α can indirectly influences peripheral insulin sensitivity by stimulating triglyceride and free fatty acid production in the liver (30).

Additional mechanism focused on mitochondrial toxicity of NRTIs on adipocytes suggested that NRTI-induced inhibition of DNA polymerase-γ leads to depletion of cellular mitochondrial DNA (mtDNA) content through inhibition of mtDNA synthesis (31). Depletion of mtDNA has been demonstrated in subcutaneous fat samples taken from patients with lipodystrophy (32,33).

The NRTI has the pharmacodynamic capability to enter the target cells and subsequently to enter the mitochondrion in either its free-drug or phosphorylated form. The target cell possesses activated cellular nucleoside kinases to mono-, di- and subsequently triphosphorylate the NRTI, so that the active triphosphate form of the drug is present within the mitochondrion. The triphosphorylated NRTI can inhibit DNA polymerase-γ either by serving as a competitive or alternative but ineffective substrate or by chain termination of the nascent mtDNA strand (noncompetitive). Then it will cause mtDNA depletion and dysfunction (34). The thymidine analogues stavudine and zidovudine decreased lipid content, mitochondrial activity, and adipocyte survival in vitro (35).

This hypothesis and the results of experimental studies suggest that differences within the NRTI class variably affect different organs. Clinical signs and symptoms are related to the specific organ and function involved; for example, mitochondrial dysfunction in adipose tissue leads to lipoatrophy, mitochondrial dysfunction in the liver leads to lactic acidosis and hepatic steatosis, and mitochondrial dysfunction in nerves leads to neuropathy. Depending on the level of mitochondrial dysfunction, energy metabolism is affected at multiple points, with cell death by apoptosis an ultimate outcome (36).

HIV patients are often associated with aberration of biochemical parameters like renal profile, liver profile, thyroid profile, thrombocytopenia and severe anemia with high erythrocyte sedimentation rate (ESR). Patients with HIV infection were reported to have hypocholesterolemia with or without hypertriglyceridemia, but the mechanism of decrease in cholesterol levels is not known (37). Studies have been observed when HIV patients are treated with protease inhibitors. They tend to exhibit hyperlipidemia with increase in total cholesterol, triglycerides, low density lipoproteins, and concomitant decrease in high density lipoprotein cholesterol (37,38). Infection can increase serum triglycerides levels by decreasing clearance of circulating lipoproteins levels as process seems to inhibit the lipoprotein lipase activity or

stimulating hepatic lipid synthesis through increase in either hepatic fatty acid synthesis or reesterification of fatty acids derived from lipolysis (37).

Patients with HIV are subjected to dyslipidemia and other complications secondary to HAART that are often referred to as HIV metabolic syndrome (39,40). In Uganda, recent reports showed that HIV infected patients have infrequent elevations in serum TC, LDL and TG (41). In another study, HIV-infected HAART-naïve subjects had lower concentrations of LDL and HDL and a higher concentration of TG than healthy controls. After receiving PI-based HAART, LDL-C and TG concentrations increased, while HDL-C concentrations remained unchanged. However, Nevirapine based regimen has been shown to have increase TC and HDL-C (39).

The increase of plasma lipid concentrations may involve up to 70-80% of HIV-positive subjects treated with a PI-containing regimen and are frequently (but not always) associated with the fat redistribution or the lipodystrophy syndrome. The potential clinicopathological consequences of HIV-associated hyperlipidaemia are not completely known, but some observations report an increased risk of premature coronary artery diseases in young HIV-positive individuals receiving PIs, besides peripheral atherosclerosis and acute pancreatitis (42).

Premature coronary atherosclerosis is now a growing problem because antiretroviral drugs can lead to serious metabolic disturbances resembling those in the metabolic syndrome (43,44). Lipodystrophy, a clinical syndrome of peripheral fat wasting, central adiposity, dyslipidemia, and insulin resistance, is most prevalent among patients treated with protease inhibitors. These patients should thus be screened for hyperlipidemia, hyperglycemia, and hypertension, and they may be candidates for lipid-lowering therapies (43).

Use of HAART has been linked to hyperglycemia, dyslipidemia and increased risk of cardiovascular disease (CVD) in HIV-infected patients in industrialized countries. The effects of HAART on glucose and lipid metabolism among sub-Saharan Africans, for whom access to antiretroviral therapy is expanding, remain largely unknown (41). This is specially a major gap that should be given high emphasis in our county Ethiopia because of increments of usage of HAART by HIV patients from day to day.

1.3. Significance of the Study

Highly active antiretroviral therapy is likely to cause diabetes and dyslipidemia as indicated in different researches conducted elsewhere. However, data about the patterns of glucose and lipid profile in HAART naïve and those on HAART is limited in Ethiopian population. Metabolic effects may differ from place to place due to some host and environmental factors. In addition, the HAART regimens taken as first line, second line and type of ARV drugs available in different countries differ because of affordability of the patient or interest of the organization that supports poor countries. Thus, it is essential to assess the pattern of hyperglycemia and dyslipidemia on patients put under highly active antiretroviral therapy to determinant risk status in our population. Of all, the data generated from this study will be used as baseline for future large scale studies in the country.

1.4. Literature Review

The employment of therapeutic regimens with the combination of antiretroviral drugs has shown an impact on the survival rate of patients with acquired immune deficiency syndrome in recent years. Despite the advances obtained, starting with the earliest use of PIs in 1995 there began to emerge the first reports of alterations in the lipid metabolism related to this group of drugs. Insulin resistance, increase in plasmatic lipids**,** and fat redistribution have been indicated as metabolic complications related to the use of ARV regimens (45).

A cohort study in USA in 2003 indicated that, among the 50 men, notable declines in mean serum TC (-30mg/dl), HDL-C (-12 mg/dl), and LDL-C values (-22 mg/dl) were observed after HIV infection. Following HAART initiation, there were large increases in mean TC and LDL-C values (50 and 21 mg/dl respectively); however, the mean changes from the preseroconversion values were 20 mg/dl (95% CI, -1 to 41) and -1 mg/dl (95%CI, -25 to 22), respectively. The median value (interquartile range) of triglycerides was 225 mg/dl (147-331 mg/dl) (46).

Similar cohort study on 212 HIV-infected patients receiving a protease inhibitor-based antiretroviral therapy in Italy in 2003 showed that, at the end of their 12-month follow-up, mean serum lipid levels were the following: serum TG levels, 306.4 mg/dl; TC levels, 258.7 mg/dl; LDL-C, 165.2 mg/dl; HDL-C, 40.1 mg/dl. Thus, statistically significant increase in serum levels of TG ($P<0.005$) and total Cholesterol and LDL-C ($P<0.05$) were observed after 1 year of new PI-containing treatment. Seen as a whole, at the end of the 12-month study period, hypertriglyceridemia was reported in 81 of 212 treated patients (38.2%), hypercholesterolemia in 53 (25%), mixed hypertriglyceridemia and hypercholesterolemia in 32 (15.1%), increased LDL-C level in 57 (26.7%), and decreased HDL-C level in 20 (9.4%). Severe hypertriglyceridemia and hypercholesterolemia were registered in 30 (14.1%) and 18 cases (8.5%), respectively. No clinical occurrence of cardiovascular diseases or acute pancreatitis was observed during the entire 1-year follow-up (47).

Another cohort study done in Switzerland in 2005 found that, non-HDL-C levels increase with increasing exposure to either PI- or NNRTI-based therapy, HDL-C levels increase and TG levels decrease with increasing exposure to NNRTI-based therapy; whereas TG levels increase with increasing exposure to PI-based therapy. Between NNRTI-based therapies, there is a slight

difference in TG levels, which tend to increase with increasing exposure to efavirenz and to decrease with increasing exposure to nevirapine. Of the three common PI-based therapies, nelfinavir appears to have a relatively favourable lipid profile, with little change with increasing exposure. Of the other two PI therapies, lopinavir with ritonavir has a more favourable profile than indinavir with ritonavir, with smaller increases in both non-HDL-C and TG and an increase in HDL-C. Increasing exposure to abacavir is associated with a decrease in the level of triglycerides (48).

A cross sectional study done in Brazil in 2007 on 372 both ART and pre-ART patients showed that, 362 (97.2%) individuals had normal glucose levels. Seven (out of ten) of those in the group with elevated glycaemia were patients in ARV treatment without PIs, one and two patients were with hyperglycemia on never had ARV and treatment with PIs, respectively. Most of patients (79.9%) had normal TC levels. Among those who exhibited elevated levels, 51.4% (37/72) were receiving ARV treatment with PIs and 22.2% (16/72) were ART naïve patients. Regarding the TG, 52% of the individuals sampled presented normal levels, 20.2% presented borderline levels and 27.8% presented elevated levels. Concerning the levels of HDL-C, 57.1% of the sample was composed of individuals with levels below normal. Of those, 32.7% (67/205) were without treatment and the rest were post ART individuals. There was a statistically significant association between the levels of TC and TG and the use of the ARV treatment (45).

The cohort study done in Thailand in 2008 conducted on 200 HIV patients who have been treated with HAART regimen for average 39.35 ± 17.58 months concluded that, prevalence of hyperlipidemia in men was higher than women ($p = 0.004$) but there was no difference in blood glucose level between two groups. Patients developed Diabetes Mellitus, hypercholesterolemia, hypertriglyceridemia and high LDL-C (10.5%, 34.0%, 35.5% and 6.5% respectively). There was no difference of dyslipidemia complication among HAART regimens because NRTI and NNRTI regimens were mostly prescribed over than PI regimens (49).

According to the study which recruited 150 confirmed HAART naïve patients living with HIV/AIDS and 100 HIV sero-negative controls in Ghana between May to December 2010, Apart from serum triglycerides ($p<0.01$) which showed a significant increase in the subjects compared to the control group, serum total cholesterol ($p<0.001$), HDL-cholesterol ($p<0.001$) and LDL-cholesterol ($p<0.001$) showed significant decreases compared to the control group. HDL-C in

subjects with CD4 <200 cells/ mm^{-3} was significantly reduced when compared to the control group while HDL-C in subjects with CD4 between 200-499 cells/ mm^{-3} and CD4 $\geq$500 showed no statistically significant difference in comparison to the control group. From the correlation analysis, serum total cholesterol (R=0.67), HDL-cholesterol (R=0.27) and LDL- cholesterol (R=0.27) showed a positive significant correlation to the CD4 count. Serum triglycerides (R= -0.27) correlated negatively to CD4 count (50).

A cohort study conducted in India in 2011on 168 HIV infected patients found, after 6 months, TC levels increased by 49 mg/dl, LDL-C levels by 30 mg/dl, and HDL-C levels increased by 18 mg/dl (P < .001 for all). At baseline and at 12 months, TC was >200 mg/dl for 1% and 26% of patients, respectively; LDL-C level was >130 mg/ dl for 3% and 23%, respectively; HDL-C level was <40 mg/dl for 91% and 23%, respectively; and blood glucose level was >110 mg/dl for 14% and 13%, respectively. TC level >200 mg/dl was more common among patients who received efavirenz than among those who received nevirapine (32% versus 16%; P = 0.04) (51).

2. HYPOTHESIS

Different combinations of antiretroviral therapy have hyperglycemic and dyslipidemic effect among patients receiving HAART as compared to naïve patients.

3. OBJECTIVES OF THE STUDY

3.1. General objective

To evaluate antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients at Burayu health center, Addis Ababa, Ethiopia

3.2. Specific objectives

- To determine the prevalence of hyperglycemia and dyslipidemia among HIV infected patients with or without HAART
- To compare glucose and lipid profile levels among HIV infected patients with and without antiretroviral therapy
- To evaluate the intra-variability of glucose and lipid profile levels of patients taking different regimens of HAART

4. MATERIALS AND METHODS

4.1. Study Setting and Period

The study was conducted at voluntary counseling and testing (VCT) center of Burayu Health Center in collaboration with Ethiopian Health and Nutrition Research Institute (EHNRI) Clinical Chemistry Laboratory, Addis Ababa, Ethiopia, from September 2011 to May 2012. As of September 15, 2011, there were 964 HIV infected cases (682 pre- ART and 282 ART) on care at the Health Center.

4.2. Study Design

A cross-sectional study was conducted to assess antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients at Burayu Health Center, Addis Ababa, Ethiopia.

4.3. Population

4.3.1. Source Population

All HIV-infected patients who visited Burayu Health Center for VCT.

4.3.2. Study Population

All adult HIV infected individuals who fulfilled the inclusion criteria and came for medical care at Burayu Health Center during the study period.

4.4. Sample Size

Since it was not possible to get the prevalence of HAART associated hyperglycemia and dyslipidemia in Ethiopia (up to our knowledge), we used the prevalence of similar study done outside whose average hyperglycemia and dyslipidemia prevalence was taken as 9% (49). Considering 95% confidence interval, 5% margin of error and 9% proportion, the sample size was calculated using the following standard formula. $n = \frac{Z\,(\frac{\alpha}{2})^2\,P(1-P)}{d^2}$

n = Sample size

z $(\frac{\alpha}{2})^2$ = At 95% confidence interval Z value ($\alpha = 0.05$) = 1.96

P = Proportion of occurrence of the event to be studied (prevalence) 9% (0.09)

d = Margin of error (5%) (0.05)

$$n = \frac{(1.96)^2\ 0.09\ (1-0.09)}{(0.05)^2}$$

$$n = \frac{3.8416 \text{ x } 0.09 \text{ x } 0.91}{0.0025} = 126$$

A total of 252 study subjects, 126 HAART initiated and 126 non-HAART control groups were included in the study.

4.5. Sampling Techniques

Consecutive sampling technique was employed to include study participants who met the inclusion criteria and consented to participate in the study. Thus, all patients who visited the VCT center during the sample collection period for their routine follow-up or diagnosis were included until the expected sample size was fulfilled.

4.6. Inclusion Criteria

- Patients equal to or older than 18 years
- Above or equal to six months since started antiretroviral treatment for HAART initiated patients because hyperglycemia and dyslipidemia is the chronic effect of HAART and it is supported by different similar literatures.
- All HAART naïve patients who visited the VCT center during the study period

4.7. Exclusion Criteria

- Patients with hyperglycemia or dyslipidemia at baseline(for HAART initiated patients)
- Pregnant women and nursing mother

4.8. Data Collection Procedures

After received a clear explanation of the objectives of the study and signed the informed consent, patients answered a questionnaire; gave information regarding, age, gender, marital status, educational level, occupation, period with HIV infection, ART exposure in the previous six months and the ARV regimen used. Medical records were also used to confirm the information, baseline glucose and lipid profile. Height and weight were assessed for calculating the Body

Mass Index (BMI). Blood was drawn for laboratory testing, requested either for routine evaluation or investigation.

Appropriate amount (6-10ml) of blood sample was collected by venipuncture from fasting individuals using an evacuated tube system and serum was separated within one hour. Finally, the sample was transported to EHNRI Clinical Chemistry Laboratory with Ice Box for analysis.

The levels of glucose, TC, HDL-C, LDL-C and TG were estimated by COBAS INTEGRA 400 random access full automated auto analyzer. Glucose was determined by Hexokinase (HK) method, TG and total TC were evaluated with enzymatic colorimetric method and HDL-C and LDL-C were analyzed by homogenous enzymatic colorimetric method (Annex II).

The cutoff point for the categorization of the glucose, TC, HDL-C, LDL-C and TG levels was based on the COBAS INTEGRA 400 method manual and National Cholesterol Education Program (NCEP) guideline of USA (52,53).

4.9. Study variables

4.9.1. Dependent variables

- Glucose
- Triglyceride
- Total cholesterol
- HDL-C
- LDL-C levels

4.9.2. Independent variables

- Socio-demographic characteristics
- BMI
- Duration with HIV
- Duration with HAART
- ARV regimen
- TB co-infection

4.10. Data Management and Quality Assurance

Instruction was given for all nurses who were in charge of interviewing and collecting clinical data from the medical records on how to collect appropriate data excluding identifiers. Data quality was ensured through use of pretested data collection materials and intensive supervision by the principal investigator. For laboratory analysis two level quality control samples (Annex I)

were run to assess the functionality of the instrument and reagents and the user manual of the equipment was strictly followed. In addition, well trained and experienced laboratory professionals were participated in the analysis procedure.

4.11. Data Processing, Presentation and Analysis

Data were entered and analyzed using SPSS window version 19. Frequencies and proportions were used to describe the characteristics of study participants in relation to relevant variables. Tables and figures were used to present results of calculated frequencies and proportions. Statistical analysis like chi-square, student-t-test and logistic regression were used to see the association between socio-demographic characteristics, duration with HIV/HAART, BMI and HAART regimens with serum glucose and lipid profile levels. P- Value <0.05 was considered to be statistically significant.

4.12. Dissemination of Results

The study report was submitted to the School of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. The abstract will be submitted to local associations to present the results of the study during continuous medical education events organized through these associations. Moreover, it will be submitted to international or national peer reviewed journal for publication.

4.13. Ethical Consideration

Ethical clearances were obtained from the Department of Medical Laboratory, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, Oromia Regional Health Bureau, EHNRI and National Ethical committee. A letter was also submitted to Burayu Health Center to get permission to undergo the study. VCT center counselors (nurses) from the Health Center collected patient data to keep the confidentiality of the participants. Patients received their results through clinicians for further diagnosis and treatment, accordingly.

4.14. Limitations of the Study

This study is cross-sectional, which does not have a follow up to see the effects of HAART at individual level. In addition, this paper does not include all potential confounders of hyperglycemia and dyslipidemia such as nutritional status and physical exercise.

5. RESULT

5.1. General Characteristics of the Study Subjects

Over a period of seven months, September, 2011 to May, 2012, data were collected from 252 HIV infected patients who seek medical care at Burayu Health Center. Of these, 126 (50%) were initiated HAART (HAART group) and 126 (50%) were not (non-HAART group). Majority of the study participants were females (182 (72.2%)). Eighty six females (68.3%) and 40 (31.7%) males had started HAART but 96 (76.2%) females and 30 (23.8%) males had not. The overall mean age was 35.3±10.2 years (range: 18 to 75 years). The mean age of HAART group and non-HAART group was 37.1±9.8 and 33.4±10.2 years, respectively. Age 30 to 39 was the highest in number age group having 110 (43.7%) frequency.

The mean total body mass index (BMI) was 21.4±3.1 (kg/m^2). It was 21.9±3.2 in HAART group and 20.8±2 in non-HAART group. One hundred eighty eight (74.6%) individuals had a normal body mass index. Three (2.4%) and 9 (7.1%) HAART and 1 (0.8%) and 24 (19.0%) non-HAART groups were obese and underweight, respectively. BMI was significantly associated with HAART initiation ($p = 0.023$).

The total time from the serological diagnosis of HIV infection was 20.6±17.3 months (Mean ±SD). It was longer in HAART groups compared to those non-HAART groups (mean: 29.5±17.1 versus 11.8±12.3 months, $p < 0.0001$). Majority of the study participants, 78 (31%) had a relatively long period of time since diagnosed as HIV positive with the range of 25 to 41 months stayed. Fifty nine (46.8%) of the HAART and 19 (15.1%) non-HAART groups were under this category. Among those HAART groups, 9 (3.6%) of the patients were lived with HIV for more than 60 months (5 years).

Of all the subjects in both groups, about two third, 158 (62.7%), of them were married and 20 (7.9%) were separated. Higher number of patients, 122 (48.4%) were at primary educational level but 8 (3.2%) reached tertiary level. Most (132 (52.4%)) of the individuals were house wives. Students, farmers, drivers, mechanics and family member were among others.

Of all the subjects, 39 (15.5%) of them showed TB-HIV co infection. Twenty one (16.7%) of the HAART and 18 (14.3%) non-HAART groups were TB-HIV co-infected. Majority of the study

participants, 110 (43.7%) were categorized under WHO stage one class and very few of the patients, 10 (4%) were grouped under WHO stage four. Seventy nine (62.7%) non-HAART and 60 (47.6%) HAART groups respectively were classified as WHO stage one and three clinically (Table.1).

Table 1. Distribution of study participants according to socio-demographic characteristics and body mass index at Burayu Health center, Addis Ababa, Ethiopia, 2012.

Variable		**Group of Patients**		**Total**
		HAART Initiated	Non-HAART	
		N (%)	N (%)	N (%)
Gender	Female	86(68.3)	96(76.2)	182(72.2)
	Male	40(31.7)	30(23.8)	70(27.8)
Age Group (years)	18-29	25(19.8)	51(40.5)	76(30.2)
	30-39	61(48.4)	49(38.9)	110(43.7)
	40-49	25(19.8)	16(12.7)	41(16.3)
	≥50	15(11.9)	10(7.9)	25(9.9)
BMI	Underweight (<18.5)	9(7.1)	24(19)	33(13.1)
	Normal (18.5-24.9)	97(77)	91(72.2)	188(74.6)
	Overweight (25-29.9)	17(13.5)	10(7.9)	27(10.7)
	Obese (≥30)	3(2.4)	1(0.8)	4(1.6)
Time in months since diagnosis of HIV infection	<6	0(0)	57(45.2)	57(22.6)
	6-12	22(17.5)	21(16.7)	43(17.1)
	13-24	22(17.5)	27(21.4)	49(19.4)
	25-41	59(46.8)	19(15.1)	78(31)
	>42	23(18.3)	2(1.6)	25(9.9)
Marital Status	Single	18(14.3)	16(12.7)	34(13.5)
	Married	78(61.9)	80(63.5)	158(62.7)
	Divorced	6(4.8)	7(5.6)	13(5.2)
	Widowed	19(15.1)	8(6.3)	27(10.7)
	Separated	5(4)	15(11.9)	20(7.9)
Educational Level	No education	32(25.4)	51(40.5)	83(32.9
	Primary	68(54)	54(42.9)	122(48.4
	Secondary	21(16.7)	18(14.3)	39(15.5)
	Tertiary	5(4)	3(2.4)	8(3.2)
Occupation	Gov't employee	23(18.3)	16(12.7)	39(15.5)
	Merchant	17(13.5)	16(12.7)	33(13.1)
	House wife	61(48.4)	71(56.3)	132(52.4)
	Daily labor	10(7.9)	13(10.3)	23(9.1)
	Others	15(11.9)	10(7.9)	25(9.9)
TB Co-infection	Present	21(16.7)	18(14.3)	39(15.5)
	Not present	105(83.3)	108(85.7)	213(84.5)
WHO Stage	I	31(24.6)	79(62.7)	110(43.7)
	II	25(19.8)	30(23.8)	55(21.8)
	III	60(47.6)	17(13.5)	77(30.6)
	IV	10(7.9)	0(0)	10(4)
Total		126(100)	126(100)	252(100)

5.2. Quality Control Sample Result

Precinorm Universal (PNU) normal level and Precipath Universal (PPU) pathological level control samples were used for glucose, triglyceride (TG) and total cholesterol (TC). Precipath HDL-C/LDL-C (PPHL) pathological level control was used for high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). All control samples were run repeatedly before the analysis of the sample and during each run of the sample daily. The calculated mean and standard deviation of repeatedly ran control samples by COBAS INTEGRA 400 analyzer were compared to the assigned mean and standard deviation of the kit insert for the glucose and lipid profile reagents. These values and coefficient of variation (CV≤5%) were under the accepted range according to quality control rules (Table.2).

Table 2. The comparison of repeatedly done quality control sample values by COBAS INTEGRA 400 with kit inserts' assigned values for glucose/lipid profile reagents.

Analyte	Control	Assigned mean	Assigned SD	N	Calculated mean	Calculated SD	Calculated %CV
Glucose	PNU	94	5	20	91.35	4.57	5
	PPU	254	13	20	257.4	9.65	3.75
TG	PNU	98	5	20	99.1	4.4	4.44
	PPU	213	11	20	216.05	8.71	4.03
TC	PNU	97	5	20	97	2.9	2.99
	PPU	193	10	20	193.65	4.59	2.37
HDL-C	PPHL	30	2	10	26.8	1.03	3.85
LDL-C	PPHL	200	16	13	205.69	3.01	1.46

5.3. Serum Fasting Glucose and Lipid Profile Levels

The distribution of serum glucose and lipid profile levels in both non-HAART and HAART initiated HIV infected study participants were nearly normally distributed except TG which is not normally distributed in both groups of patients. The skewness and kurtosis of glucose, TG, TC, HDL-C and LDL-C levels were 0.133 and 0.511, 2.267 and 7.461, 0.813 and 1.015, 0.577 and 0.348 and 0.371 and 0.345, respectively (Fig.2).

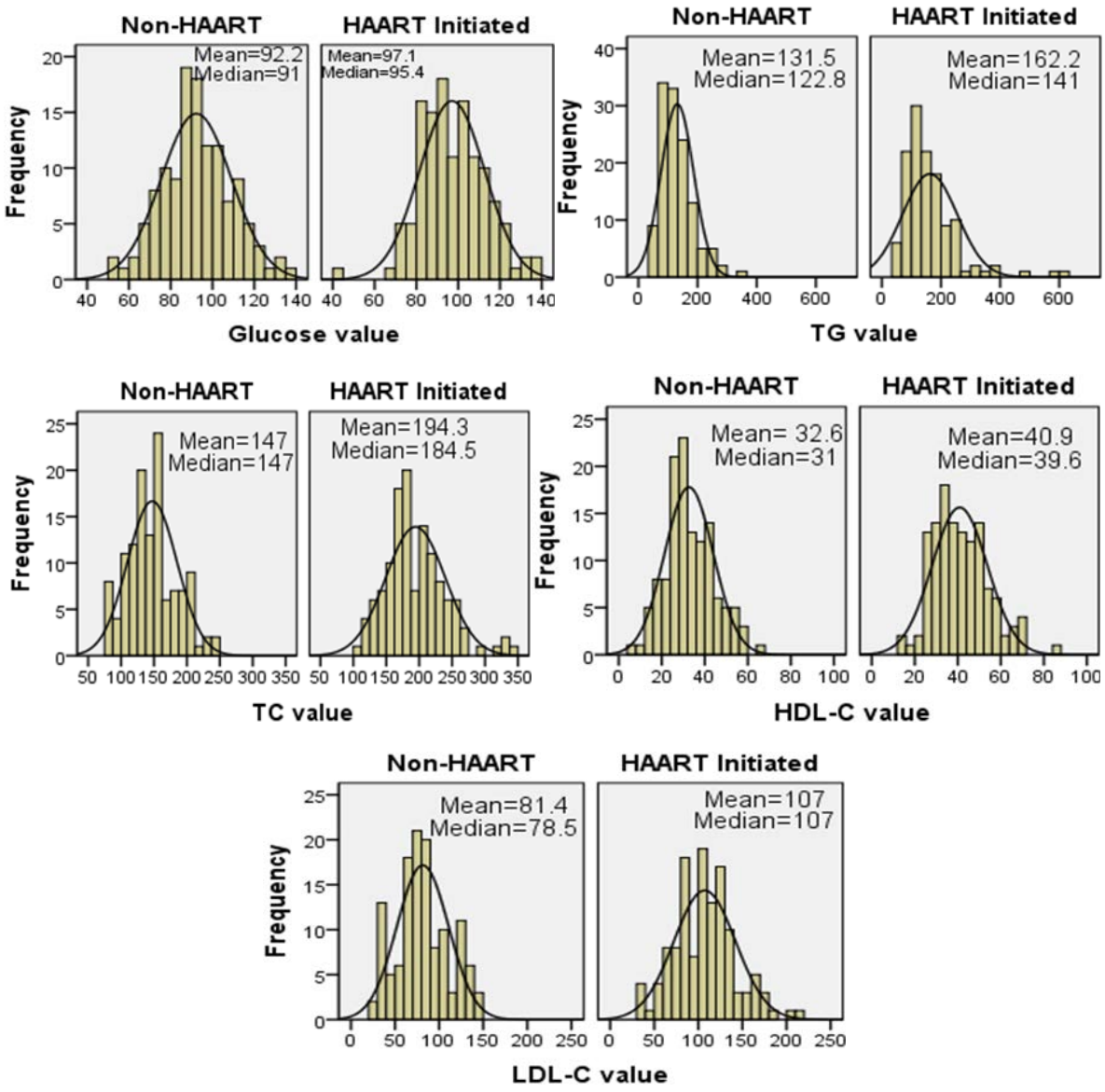


Figure 2. The distribution of serum glucose and lipid profile levels among non-HAART and HAART initiated individuals at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Totally, 17 (6.7%) individuals' serum glucose level was abnormally high. There was no association between serum glucose level and HAART initiation (P = 0.45) in our study participants. However, there was statistically significant increase in serum lipid profile levels of HAART initiated patients than HAART naïve individuals (p = 0.01 for TG and <0.001 for others) (Table.3).

Table 3. Distribution of glucose and lipid profiles on HAART initiated and non-HAART initiated study participants at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Glucose/Lipid		**Group of Patients**		**Total**	**X^2**	**P value**
		HAART Initiated	Non-HAART			
		N (%)	N (%)	N (%)		
Glucose	Normal	116(92.1)	119(94.4)	235(93.3)	0.568	0.45
	High	10(7.9)	7(5.6)	17(6.7)		
Triglyceride	Normal	98(77.8)	113(89.7)	211(83.7)	6.554	0.010
	High	28(22.2)	13(10.3)	41(16.3)		
Total cholesterol	Normal	73(57.9)	112(88.9)	185(73.4)	30.923	<0.0001
	High	53(42.1)	14(11.1)	67(26.6)		
HDL-Cholesterol	Low	64(50.8)	92(73)	156(61.9)	13.192	<0.0001
	Normal	62(49.2)	34(27)	96(38.1)		
LDL-Cholesterol	Normal	97(77)	117(92.9)	214(84.9)	12.395	<0.0001
	High	29(23)	9(7.1)	38(15.1)		
Total		126(100)	126(100)	252(100)		

From all study subjects in both groups, the overall mean fasting serum glucose, TG, TC, HDL-C and LDL-C levels were 94.7±16.4 mg/dl, 146.9±77.7 mg/dl, 170.8±47.8 mg/dl, 36.7±12.8 and 94.3±34.7 mg/dl (Mean±SD), respectively. HAART initiated patients showed higher mean fasting serum glucose, TG, TC, HDL-C and LDL-C levels than non-HAART groups (p = 0.019, 0.002, <0.0001, <0.0001and <0.0001, respectively) (Table.4).

Table 4. The comparison of mean serum glucose and lipid profile levels with independent t-test between non-HAART and HAART initiated study participants at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Glucose/lipid profile	**Group of Patients**	**Mean**	**SD**	**Mean difference**	**T**	**95%CI of the difference**		**P value**
						Lower	Upper	
Glucose	HAART	97.0714	15.688	4.83175	2.353	0.78713	8.87636	0.019
	Non-HAART	92.2397	16.889					
Triglyceride	HAART	162.1849	92.746	30.66508	3.188	11.72046	49.60970	0.002
	Non-HAART	131.5198	55.283					
Total cholesterol	HAART	194.3444	45.214	47.11032	8.983	36.78094	57.43969	<0.0001
	Non-HAART	147.2341	37.702					
HDL-Cholesterol	HAART	40.8516	12.862	8.23810	5.400	5.23369	11.24250	<0.0001
	Non-HAART	32.6135	11.303					
LDL-Cholesterol	HAART	107.1563	34.987	25.79762	6.347	17.79311	33.80213	<0.0001
	Non-HAART	81.3587	29.276					

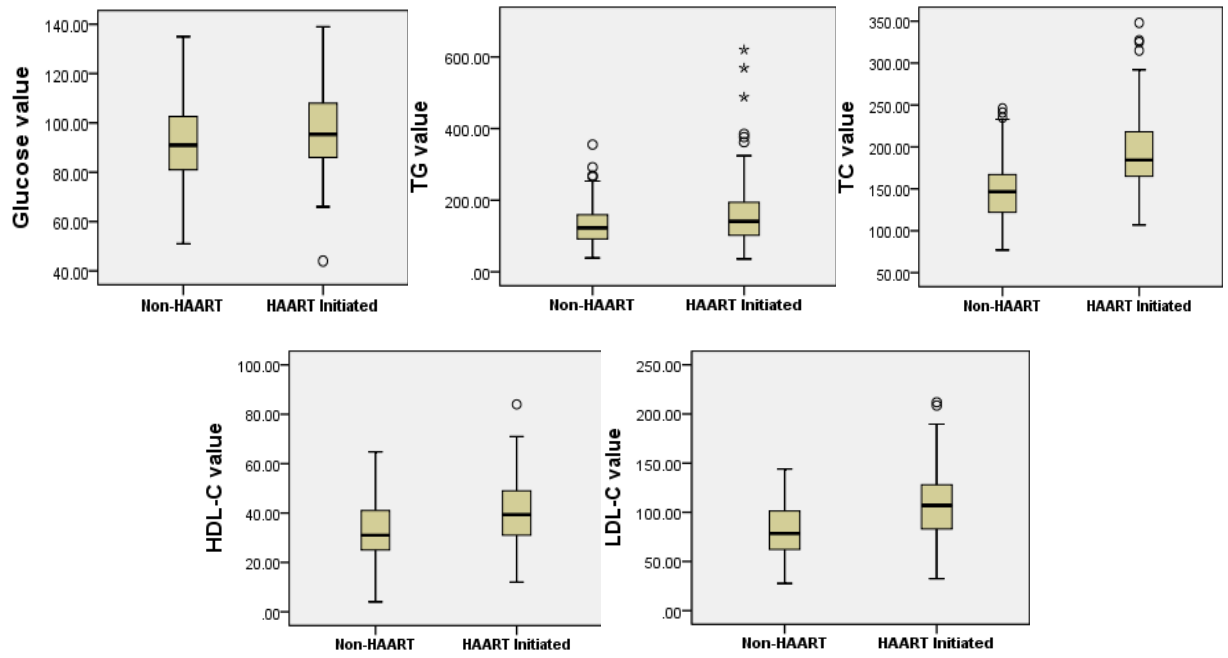


Figure 3. The comparison of glucose and lipid profile levels among non-HAART and HAART initiated HIV infected individuals at Burayu Health Center, Addis Ababa, Ethiopia, 2012. The box represents 25% and 75% Interquartile ranges (IQRs), the solid line in the boxes showed median and the lines indicate 95% confidence interval. Circles outside this range are classified as outliers.

There was no statistically significant association between serum glucose/lipid profile levels and gender (p value >0.05). However, there was statistically significant association between TG, TC and LDL-C and age group (p = 0.047, 0.004 and 0.01, respectively). Regardless of glucose and HDL-C levels, TG, TC and LDL-C levels have showed statistically significant association with duration of HIV infection, (p = 0.012, 0.019 and 0.039 respectively). Except serum TC and HDL-C levels (p = 0.006 and 0.027, respectively), glucose and other lipid profile levels had no statistically significant correlation with body mass index (BMI).Only serum LDL-C level showed statistically significant difference with TB-HIV co-infection (p = 0.045). In addition, TG level showed independent statistically significant association with WHO stage classification (p = 0.036) (Table.5).

Table 5. The distribution of glucose and lipid profiles among age group and duration with HIV of study participants at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Glucose/Lipid		**Age Group**		**Total**	**X^2**	**P value**
		<35	≥35			
		N (%)	N (%)	N (%)		
Glucose	Normal	121(93.5)	114(92.4)	235(93.3)	0.675	0.411
	High	7(5.5)	10(8.1)	17(6.7)		
Triglyceride	Normal	113(88.3)	98(79)	211(83.7)	3.955	0.047
	High	15(11.7)	26(21)	41(16.3)		
Total cholesterol	Normal	104(81.3)	81(65.3)	185(73.4)	8.186	0.004
	High	84(18.8)	72(58.1)	67(26.6)		
HDL-Cholesterol	Low	44(34.4)	52(41.9)	156(61.9)	1.527	0.217
	Normal	64(34.4)	32(48.5)	96(38.1)		
LDL-Cholesterol	Normal	116(90.6)	98(79)	214(84.9)	6.610	0.01
	High	12(9.4)	26(21)	38(15.1)		
Total		128(100)	124(100)	252(100)		
Glucose/Lipid		**Time since HIV diagnosis (months)**		**Total**	**X^2**	**P value**
		<20	≥20			
		N (%)	N (%)	N (%)		
Glucose	Normal	117(93.6)	118(92.9)	235(93.3)	0.047	0.828
	High	8(6.4)	9(7.1)	17(6.7)		
Triglyceride	Normal	112(89.6)	99(78)	211(83.7)	6.273	0.012
	High	13(10.4)	28(22)	41(16.3)		
Total cholesterol	Normal	100(80)	85(66.9)	185(73.4)	5.514	0.019
	High	25(20)	42(33.1)	67(26.6)		
HDL-Cholesterol	Low	82(65.6)	74(58.3)	156(61.9)	1.436	0.231
	Normal	43(34.4)	53(41.7)	96(38.1)		
LDL-Cholesterol	Normal	112(89.6)	102(80.3)	214(84.9)	4.241	0.039
	High	13(10.4)	25(19.7)	38(15.1)		
Total		125(100)	127(100)	252(100)		

5.4. Association among TC, HDL-C and LDL-C and HAART Initiation, Adjusting for the Potential Confounding Factors Through a Binary Logistical Regression

Total Cholesterol: Adjusting for the effect of one another, HAART initiation, age group, duration with HIV and BMI by logistical regression, it was found that two variables remained, indicating HAART initiation and BMI had an independent association with serum TC level; Odds Ratio (95%CI for odds ratio) and p-value were 5.493 (2.578-11.702) and <0.0001 and 1.883 (1.019-3.479) and 0.043, respectively).

HDL-C: Adjusting for the effect of each other, HAART initiation and BMI by logistic regression, it was also found that only HAART initiation remained and this showed that it was the independent indicator which could affect serum HDL-C level; Odds Ratio (95%CI for odds ratio) and p-value were 2.479 (1.457-4.218) and 0.001).

LDL-C: Adjusting for the effect of one another, HAART initiation, age group, duration with HIV and TB co-infection by multiple logistical regression, it was observed that one variable remained in the final model, indicating HAART initiation had an independent association with serum LDL-C level; Odds Ratio (95%CI for odds ratio) and p-value were 3.264 (1.326-8.031) and 0.01 (Table.6).

Table 6. Association among TC, HDL-C and LDL-C and HAART initiation adjusted mainly for age group, duration with HIV infection and BMI in HIV positive individuals at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Triglyceride				
Variable	**Odds Ratio**	**95%CI for odds ratio**		**P value**
		Lower	**Upper**	
HAART initiation	1.507	0.623	3.644	0.363
Age group	0.612	0.299	1.251	0.178
Duration with HIV	1.724	0.763	3.897	0.190
WHO Stage	0.734	0.341	1.582	0.430
Total Cholesterol				
HAART initiation	5.493	2.578	11.702	**<0.0001**
Age group	0.574	0.308	1.071	0.081
Duration with HIV	0.822	0.408	1.656	0.583
BMI	1.883	1.019	3.479	**0.043**
HDL-Cholesterol				
HAART initiation	2.479	1.457	4.218	**0.001**
BMI	1.603	0.945	2.719	0.080
LDL-Cholesterol				
HAART initiation	3.264	1.326	8.031	**0.01**
Age group	0.510	0.237	1.097	0.085
Duration with HIV	1.098	0.478	2.524	0.826
TB Co-infection	2.138	0.906	5.046	0.083

5.5. Highly Active Antiretroviral Therapy (HAART) Drugs

Most of the HAART initiated patients (43(34.1%)) were between 13-24 months since started antiretroviral treatment and followed by 42 individuals whose HAART duration was between 25-41 months. The rest 28(22.2%) and 13 (10.3%) were between 6-12 months and above 3.5 years, respectively since HAART initiated. Duration of HARRT was not significantly associated with serum glucose/lipid profile levels.

A total of 4 nucleoside reverse transcriptase inhibitors (Lamivudine (3TC), Zidovudine (AZT), Stavudine (D4T) and Tenofovir (TDF)) and 2 non-nucleoside reverse transcriptase inhibitors (Nevirapine (NVP) and Efavirenz (EFV) with 5 combinations (D4T-3TC-NVP, AZT-3TC-EFV, D4T-3TC-EFV, AZT-3TC-NVP and TDF-3TC-EFV) were used to treat patients. NVP and EFV based combinations were used by almost equivalent number of individuals (65(51.6%) versus 61(48.4%), respectively). In addition, D4T and AZT based ARVs were taken by 47 (37.3%) and 67 (53.2%) individuals, respectively. AZT-3TC-NVP combinations of antiretrovirals were the most frequently used drugs among the HAART initiated study participants, 36 (28.6%) and relatively small number of patients 12 (9.5%) were using TDF-3TC-EFV combinations (Fig.4).

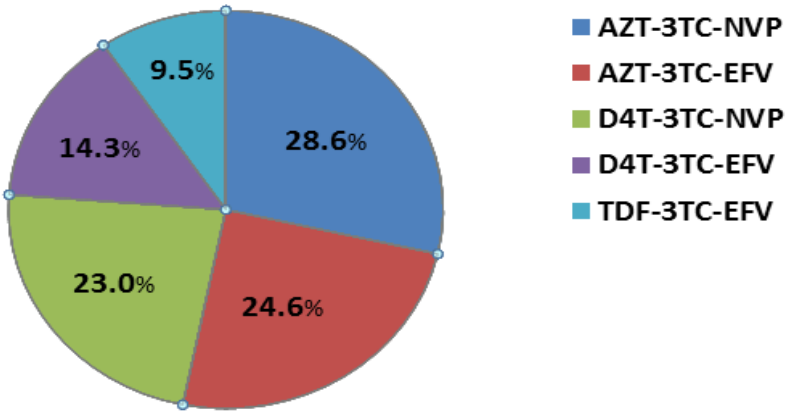


Figure 4. The frequency of different combinations of antiretroviral drugs taken by HIV infected patients at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

There was no association between serum glucose/lipid profile levels and different combinations of antiretrovirals except level of triglyceride with D4T based combinations compared to AZT based combinations ($p = 0.029$) (Table.7).

Table 7. Serum glucose and lipid profile levels distribution among NVP/EFV and D4T/AZT based ARVs taken by study participants at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Glucose/Lipid		**NVP versus EFV based Combination of ARVs**		**Total**	**X^2**	**P value**
		NVP	EFV			
		N (%)	N (%)	N (%)		
Glucose	Normal	60(92.3)	56(91.8)	116(92.1)	0.011	0.917
	High	5(7.7)	5(8.2)	10(7.9)		
Triglyceride	Normal	48(73.8)	50(82)	98(77.8)	1.201	0.273
	High	17(26.2)	11(18)	28(22.2)		
Total cholesterol	Normal	37(56.9)	36(59)	73(57.9)	0.057	0.812
	High	28(43.1)	25(41)	53(42.1)		
HDL-Cholesterol	Low	28(43.1)	36(59)	64(50.8)	3.199	0.074
	Normal	37(56.9)	25(41)	62(49.2)		
LDL-Cholesterol	Normal	48(73.8)	49(80.3)	97(77)	0.746	0.388
	High	17(26.2)	12(19.7)	29(23)		
Total		65(100)	61(100)	126(100)		
Glucose/Lipid		**D4T versus AZT based Combination of ARVs**		**Total**	**X^2**	**P value**
		D4T	AZT			
		N (%)	N (%)	N (%)		
Glucose	Normal	40(85.1)	64(95.5)	104(91.2)	3.745	0.053
	High	7(14.9)	3(4.5)	10(8.8)		
Triglyceride	Normal	31(66)	56(83.6)	87(76.3)	4.747	0.029
	High	16(34)	11(16.4)	27(23.7)		
Total cholesterol	Normal	28(59.6)	39(58.2)	67(58.8)	0.021	0.884
	High	19(40.4)	28(41.8)	47(41.2)		
HDL-Cholesterol	Low	26(55.3)	33(49.3)	59(51.8)	0.407	0.524
	Normal	21(44.7)	34(50.7)	55(48.2)		
LDL-Cholesterol	Normal	37(78.7)	53(79.1)	90(78.9)	0.002	0.961
	High	10(21.3)	14(20.9)	24(21.1)		
Total		47(100)	67(100)	114 (100)		

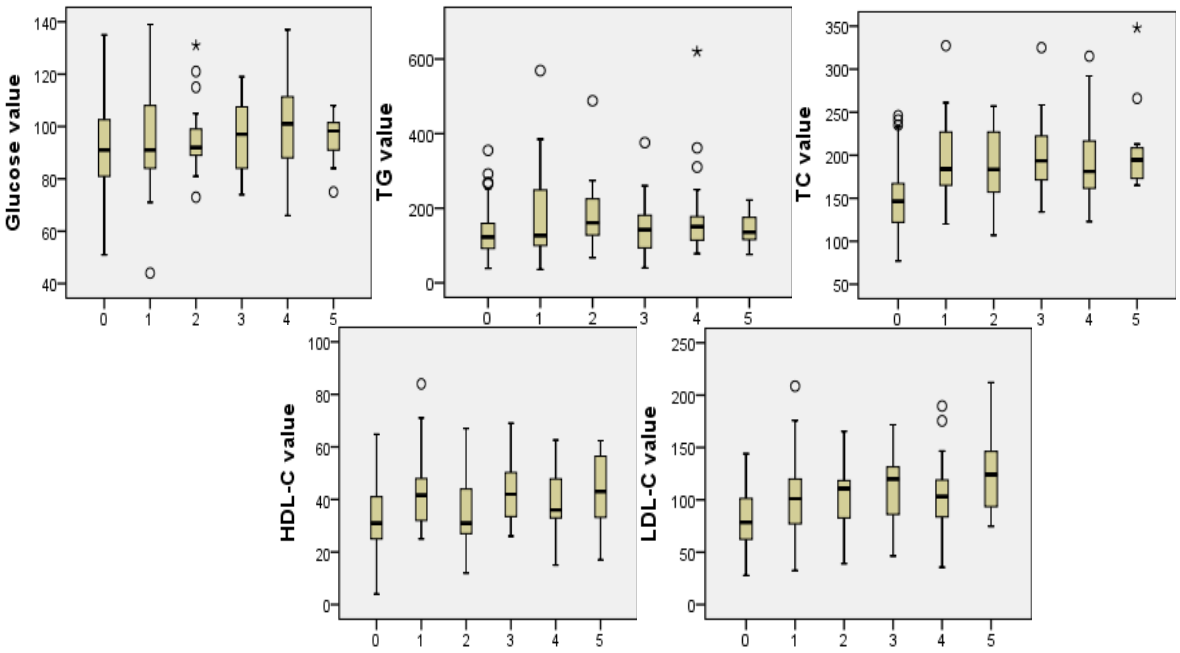
Compared to the non-HAART group, AZT-3TC-EFV combination of HAART treated patients showed a significant increase in mean serum glucose level ($p = 0.02$). In addition, mean serum TG level showed significant increase with D4T-3TC-EFV and AZT-3TC-EFV combinations ($p = 0.016$ and 0.022, respectively). However, there was no difference in mean serum TG level between NVP based combination treated patients and non- HAART group. Mean serum HDL-C level was higher in NVP, D4T and AZT based combinations treated groups (p, <0.0001, 0.005 and 0.001, respectively) than non-HAART, but there were variations in EFV based

combinations. Moreover, mean serum TC and LDL-C levels showed significant increase in HAART group than non-HAART group with all of the above ARV combinations (Table.8).

Table 8. The Association of mean glucose/lipid profile levels among different ARV drugs and non-HAART group patients at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

Glucose/lipid profile		**HAART Group**									**Non-HAART** (n=126)
		D4T based (n=47)	AZT based (n=67)	NVP based (n=65)	EFV based (n=61)	D4T-3TC-NVP (n=29)	D4T-3TC-EFV (n=18)	AZT-3TC-NVP (n=36)	AZT-3TC-EFV (n=31)	TDF-3TC-EFV (n=12)	
Glucose	Mean±sd	96±19	98±14	96±17	98±14	96±21	96±14	96±13	100±16	96±9	92± 17
	P value	0.27	0.02	0.15	0.025	0.37	0.43	0.19	0.02	0.30	
TG	Mean±sd	174±109	157±87	157±93	168±93	170±116	180±96	147±69	170±104	143±44	132± 55
	P value	0.03	0.03	0.15	0.002	0.28	0.016	0.26	0.022	0.27	
TC	Mean±sd	190±47	195±43	196±44	193±47	192±48	187±45	199±40	191±47	207±53	147± 38
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	<0.0001	
HDL-C	Mean±sd	40±14	41±12	43±13	39±13	42 ±14	35±14	43±11	39±11	43±14	33±11
	P value	0.005	<0.0001	<0.0001	0.002	0.0007	0.60	<0.0001	0.003	0.016	
LDL-C	Mean±sd	102±34	107±34	107±35	107±35	101±37	104±30	112±33	102±35	126±39	81± 29
	P value	0.0004	<0.0001	<0.0001	<0.0001	0.0102	0.004	<0.0001	0.002	0.0002	

All p-values are given compared to the non-HAART group



(Where, **0**=Non-HAART, **1**=D4T-3TC-NVP, **2**=D4T-3TC-EFV, **3**=AZT-3TC-NVP, **4**=AZT-3TC-EFV, **5**=TDF-3TC-EFV)

Figure 5. The comparison of glucose and lipid profile levels among non-HAART and 5 different combinations of first line ARVs initiated individuals at Burayu Health Center, Addis Ababa, Ethiopia, 2012.

6. DISCUSSION

In this study, data were collected from equal number of 126 HAART initiated and 126 non-HAART HIV infected individuals at Burayu Health Center. Of all, 72.2% were females. The overall mean age was 35.3±10.2 years (Mean ±SD). The mean total BMI was 21.4±3.1 (kg/m^2). The total time from the serological diagnosis of HIV infection was 20.6±17.3 months (Mean ±SD). Of all the subjects in both groups, about two third, 62.7%, of them were married. Higher number of patients, 48.4% were at primary educational level. Of all the participants, 15.5% of them showed TB-HIV co-infection. Majority of the study participants, 43.7%, were categorized under WHO stage one class.

Our results showed an increased prevalence of hyperglycemia, hypercholesterolemia and hypertriglyceridemia in patients receiving ARV drugs than non-HAART study participants. The prevalence of hyperglycemia (>115 mg/dl), hypertriglyceridemia (≥200 mg/dl), total cholesterol (TC) hypercholesterolemia (≥200 mg/dl), high density lipoprotein cholesterol (HDL-C) hypocholesterolemia (<40 mg/dl) and low density lipoprotein cholesterol (LDL-C) hypercholesterolemia (≥ 130 mg/dl) was 6.7%, 16.3%, 26.6%, 61.9% and 15.1% in total study participants, respectively. However, the prevalence was 7.9%, 22.8%, 42.1%, 50.8% and 23% in HAART initiated and 5.6%, 10.3%, 11.1%, 73% and 7.1% in non-HAART groups, respectively.

The percentage of HAART initiated individuals who had an increase in their glucose level were almost equivalent to non- HAART group, 10 (7.9%) and 7 (5.6%) respectively, and there was no statistically significant association between serum glucose level and HAART initiation ($p = 0.45$). However, HAART group showed higher mean glucose level than non-HAART group (97.1±15.7 versus 92.2±16.9 mg/dl, respectively, $p = 0.019$). In agreement with this finding, a cross-sectional study in Brazil in 2007 observed elevated glucose levels in 6.8% (7/103) patients treated without PIs, 1.5% (2/134) patients receiving PIs and 0.9% (1/112) non-HAART individuals but the small number limit their conclusions (45).

The result of our findings showed as increased serum lipid profile levels were associated to HAART initiation ($p = 0.01$ for TG and <0.0001 for others). In line with our findings, a cross-sectional study from India in 2005 showed, the prevalence of dyslipidemia was significantly higher in the first line treatment groups (54). Moreover, according to a study from the same

country in 2011, at baseline and at 12 months, TC was >200 mg/dl for 1% and 26% of patients; LDL-C level was >130 mg/ dl for 3% and 23%; HDL-C level was <40 mg/dl for 91% and 23% and blood glucose level was >110 mg/dl for 14% and 13%, respectively (51). This result is almost similar with our finding in which the prevalence of elevated level of TC, HDL-C, LDL-C and glucose was 11.1% and 42.1%, 73% and 50.8%, 7.1% and 23% and 5.6% and 7.9% for non-HAART and HAART groups, respectively. We considered increased glucose level as >115 mg/dl; otherwise, the lipid profiles classification was similar to the above study as described by the National Cholesterol Education Program guideline of USA (53).

Reports from a follow up study in Canada in 2002, among 745 ARV treated patients, 10% and 16% showed increased TG and TC levels, respectively (55). Unlike to the above and in agreement with ours a cross-sectional study from Cameroon in 2011 showed the prevalence of TC $\geq$ 200 mg/dl as 37.6% and 24.6% respectively in ART groups and ART naive groups ($p = 0.019$). The equivalents for LDL-C $\geq$ 130 mg/dl were 46.4% and 21% ($p \leq 0.001$). However, from this study, unlike ours the distribution of HDL-C and TG was similar between the two groups (56).

A long term analysis on plasma lipid concentrations was performed in patients starting first-line antiretroviral therapy in Netherlands in 2006 and showed concentrations of TC, LDL-C and TG continued to increase with slight decrease in HDL-C (57). Similarly, a study from Cameroon in 2005 showed TC, LDL-C and HDL-C levels increased significantly ($P<0.05$) but TG remained unaltered with first line ARV therapy for 3 months (58). This might be due to short treatment period.

A reduction of mean serum levels of TC, HDL- C and LDL-C observed from HAART to non-HART groups ($P <0.0001$ for all) in our study subjects was supported by a study from USA in 2003; following HAART initiation there were a large increase in mean TC and LDL-C values (50 and 21 mg/dl, respectively) (46). However, unlike ours study, in their study, the mean HDL-C remained below the baseline levels throughout follow-up period which might be due to the use of PIs (have lowering effect) in addition to first line ARVs.

In our HAART initiated study participants the mean serum TG, TC, LDL-C and HDL-C levels were 162.2, 194.3, 107.2, and 40.9 mg/dl, respectively. The result of another 1 year follow up

study in Italy in 2003 showed elevated mean levels of serum TG, 306.4 mg/dl; TC, 258.7 mg/dl; LDL-C, 165.2 mg/dl and HDL-C, 40.1 mg/dl than ours. These mean differences might be probably due to population difference in Ethiopia and Italy. They found Hypertriglyceridemia 38.2%, hypercholesterolemia 25%, increased LDL-C level in 26.7%), and decreased HDL-cholesterol level in 9.4% (47) which was almost comparable with our results. Moreover, a 5 year cohort study in Switzerland in 2005 found that non-HDL-C (VLDL-C+LDL-C) levels increased with increasing exposure to either PIs or NNRTI based therapy, HDL-C level increased and TG level decreased with increasing exposure to NNRTI based therapy; whereas TG levels increased with increasing exposure to PI-based therapy (48).

Among our study subjects, there was no association between serum glucose/lipid level and gender. However, according to a study from Thailand in 2008 on 200 HAART treated patients for an average of 39.35 months, the prevalence of hyperlipidemia was higher in men than women ($p = 0.004$) but there was no difference in blood glucose level between the two. Among those reported patients, they developed Diabetes Mellitus, hypercholesterolemia, hypertriglyceridemia and high LDL-C (10.5%, 34.0%, 35.5% and 6.5%, respectively) (49). Compared to this study, the prevalence of high serum levels of TG and glucose were lower and TC and LDL-C were higher in our study subjects.

In logistic regression adjusted for age group, duration with HAART and BMI, HAART initiation was significantly associated with serum TC, HDL-C and LDL-C levels. The adjusted odds ratio (95% CI, p value) HAART group versus non-HAART group was 5.493 (2.578–11.702, $p < 0.0001$) for TC, 2.479 (1.457–4.218, $p = 0.001$) for HDL-C and 3.264 (1.326–8.031, $p = 0.01$) for LDL-C in our result. Similarly, a study conducted in Cameroon in 2011, in multivariable analysis adjusted for age, sex, BMI, CD4 count and co-infection with tuberculosis, being on ART was significantly and positively associated with raised TC and LDL-C. The adjusted odd ratios (95% CI, p-value) ART-treated versus ART-naïve was 1.82 (1.06–1.12, $p = 0.02$) for TC $\geq$ 200 mg/dl and 2.99 (1.74–5.15), $p < 0.0001$) for LDL-C $\geq$ 130 mg/dl (56).

A combination of at least three drugs including NRTI, NNRTI and PIs, HAART is currently used to control the replication of HIV and AIDS (45). Even though, the national ART guideline for adolescents and adults in Ethiopia include PIs as second line regimens (15), only first line regimens, NRTI and NNRTI, were taken by the study participants at Burayu Health Center. Two

nucleoside backbones (from AZT/D4T/3TC/TDF) with either NVP or EFV NNRTIs combinations were used. AZT-3TC-NVP combination was the most frequently used drug in study participants, 36 (28.6%).

As indicated in the introduction, ARV drugs have been associated with an abnormal fat redistribution syndrome that might raise cholesterol and triglycerides levels, as well as cause insulin resistance (18,19). In line with this, our study showed significant increase of serum lipid levels in HAART group than non-HAART group. There was significant association of increased serum TG level with D4T based ARV treatment compared to AZT based regimens ($p = 0.029$). Similarly, a study from India (54) and Canada (55) showed a significant association of lipoatrophy with D4T use. Especially in Canada, an incident lipoatrophy was associated with duration of D4T (55). Unlike to these, studies form Spain and France showed the absence of difference in lipid profiles between D4T and AZT treated patients (59,60). However, from the France study, TG level was significantly higher in the D4T group than in the treatment naive controls, but did not differ between the zidovudine and the control group.

Even though, the serum glucose level was not associated (marginally associated) with either D4T or AZT based combinations of drugs ($p = 0.053$), compared to each other, its mean serum level was higher in patients taking AZT based combinations ($p = 0.02$) but not D4T based combinations compared to non-HAART patients. Other studies in Belgium showed D4T (mainly) and AZT were significantly associated with diabetes after adjusting for risk factors for diabetes and lipids. This might suggest the two thymidine analogs probably contribute directly to insulin resistance, potentially through mitochondrial toxicity (61).

Compared to each other, the independent effect of the use of NVP and EFV based combinations on serum lipid profile level was not seen among our study participants. However, compared to non-HAART groups, both of them showed an increase in mean serum lipid level except TG whose mean serum level was increased in EFV based combinations only ($p = 0.002$). Similarly, a 48 week follow up study in Australia in 2004 found that, the increase of HDL-C was significantly larger for patients receiving NVP (42.5%) than for patients receiving EFV (33.7%; $p = 0.036$), while the increase in TC was lower (26.9% and 31.1%, respectively; $p = 0.073$). The increase of non-HDL-C was smaller for patients receiving NVP (24.7%) than for patients receiving EFV (33.6%; $p = 0.007$), as were the increases TG (20.1% and 49.0%, respectively; p=

0.001) and LDL-C (35.0% and 40.0%, respectively; p = 0.378) (62). Another study done in Spain in 2004 on 10 individuals showed, after 48 week treatment with efavirenz, mean HDL-C level was significantly increased, from 38.5 to 52.5 mg/dl (95% CI for the increment, 8.4–19.4 mg/dl; P = 0.0001) (63). In addition, a study in India in 2011 found that TC level >200 mg/dl was more common among patients who received efavirenz than among those who received nevirapine (32% versus 16%; P = 0.04) (51). Moreover, a study in USA and Europe in 2004 found that Efavirenz was associated with higher levels of TC and TG than was nevirapine (64).

The result of our study showed as serum glucose level was not associated with either NVP or EFV based combinations of drugs (p= 0.917), compared to each other. However, its mean level was higher in AZT-3TC-EFV combination received patients (p = 0.02) compared to treatment naïve patients but there was no difference between NVP based combinations and non-HAART patients. Correspondingly, according to a 12 month follow-up study in Cameroon in 2007, blood glucose level increased significantly in HAART patients (45.56±7.86%). The increase was associated with EFV or AZT use. It is possible that EFV or AZT use in a combination therapy may exaggerate an underlying tendency to develop mitochondrial toxicity or insulin resistance (65). In case of lipid profile levels, a study conducted only on women in USA in 2007 showed that, 3TC, NVP, and EFV were independently associated with higher HDL-C (P <0.001 for all). D4T was associated with higher TG (P < 0.05), and Tenofovir was associated with lower TG (P = 0.009) (66).

7. CONCLUSION

First-line HAART with regimens NRTIs and NNRTIs are associated with potentially atherogenic lipid profile levels compared to untreated HIV infected patients in our setting. Even though, increased levels of serum glucose and lipid profile were observed in some patients not exposed to antiretrovirals, they were much more frequent after initiation of HAART therapy. Our study showed an independent association between first-line antiretroviral use and increased serum levels of total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol but not triglyceride and glucose. There is increased prevalence of hyperglycemia, hypertriglyceridemia and hypercholesterolemia in HAART initiated patients than non-antiretroviral initiated HIV infected patients at Burayu Health center. This might lead to metabolic complications particularly diabetes mellitus and dyslipidemia which potentially increase risk of cardiovascular diseases.

8. RECOMMENDATIONS

From the findings that we observed, serum fasting glucose and lipid profile levels needs to be monitored regularly in HIV infected patients on or without antiretroviral therapy to rule out unwanted effects that can be optimally managed. To this end, the clinical management of HIV-infected patients with dyslipidemia and/or hyperglycemia could emphasizes the importance of monitoring and optimizing lipid and glucose levels through lifestyle changes (such as doing physical exercise), switching antiretrovirals and lipid-lowering treatments. Future follow up studies to see the effects of protease inhibitors in addition to first line NRTI-NNRTI combinations on treated patients are warranted to address further the clinical implications of the increased levels of serum glucose and lipid profiles that might help for the better management of patients.

9. REFERENCES

1. Hoy J, Lewin S, Post JJ, Street A. HIV Management in Australasia:a guide for clinical care. 4 ed. Australia: ashm; 2009:9.
2. Lythgo PA. Molecular Virology of HIV-1 and Current Antiviral Strategies. *BioTeach J.* 2004;2:81-87.
3. UNAIDS/WHO. AIDS epidemic update. Geneva: 2009.
4. Jayasuriya A, Robertson C, Allan P. Twenty-five years of HIV management. *J. R. Soc. Med.* 2007;100(**8**):363-366.
5. Sabin CA, Phillips AN. Should HIV therapy be started at a CD4 cell count above 350 cells/[mu] l in asymptomatic HIV-1-infected patients? *Curr Opin Infect Dis.* 2009;22(**2**):191.
6. Lima VD, Johnston K, Hogg RS, Levy AR, Harrigan PR, Anema A, *et al.* Expanded access to highly active antiretroviral therapy: a potentially powerful strategy to curb the growth of the HIV epidemic. *JID.* 2008;198(**1**):59-67.
7. Orsega S. Treatment of adult HIV infection: Antiretroviral update and overview. *JNP.* 2007;3(**9**):612-624.
8. Ojewole E, Mackraj I, Naidoo P, Govender T. Exploring the use of novel drug delivery systems for antiretroviral drugs. *Eur. J. Pharm. Biopharm.* 2008;70(**3**):697-710.
9. Dagan T, Sable C, Bray J, Gerschenson M. Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: what is the evidence? *Mitochondrion.* 2002;1(**5**):397-412.
10. Lee YK. Mechanisms of highly active antiretroviral therapy (HAART) associated metabolic complications, UNIVERSITY OF HAWAI'I AT MANOA; 2007.
11. Greenberg ML, Cammack N. Resistance to enfuvirtide, the first HIV fusion inhibitor. *JAC.* 2004;54(**2**):333-340.
12. Federal Democratic Republic of Ethiopia MoH. HIV/AIDS and the Health-related Millennium Development Goals: The Experience in Ethiopia. 2010.
13. Abera B, walle F, Tewabe T, Alem A, Yessin4 M. ART-naive HIV patients at Feleg-Hiwot Referral Hospital Northwest, Ethiopia. *Ethiop. J. Health Dev.* 2010;24(**1**).
14. Sanders R. Equity and Access to ART in Ethiopia: Activity Report. 2010.
15. HAPCO/MoH. Guidelines for Management of Opportunistic Infections and Anti-Retroviral Treatment in Adolescents and Adults in Ethiopia. 2008.
16. Reinauer H, Home PD, Kanagasabapathy AS, Heuck C-C. Laboratory diagnosis and monitoring of diabetes mellitus. Geneva: WHO; 2002:5-8.

17. Arneson W, Brickell J. Clinical Chemistry: A Laboratory Perspective. 1 ed. Philadelphia: F.A. Davis Company; 2007:267-277.
18. Larson R, Capili B, Eckert-Norton M, Colagreco JP, Joyce K. Anastasi P, DrNP. Disorders of glucose metabolism in the context of human immunodeficiency virus infection. *J AANP.* 2006;18(**3**):92-103.
19. Gallagher DM. Current clinical issues impacting the lives of patients living with HIV/AIDS. *J Assoc of Nurses in AIDS care.* 2007;18(**1**):S11-S16.
20. Aberg J. Management of dyslipidemia and other cardiovascular risk factors in HIV-infected patients: case-based review. *Top HIV Med.* 2006;14(**4**):134-139.
21. Tungsiripat M, Aberg JA. Dyslipidemia in HIV patients. *Cleveland Clinic J Med.* 2005;72(**12**):1113-1120.
22. Feeney ER, Mallon PWG. HIV and HAART-Associated Dyslipidemia. *Open Cardiovasc Med J.* 2011;5:49.
23. Reeds D, Mittendorfer B, Patterson B, Powderly W, Yarasheski K, Klein S. Alterations in lipid kinetics in men with HIV-dyslipidemia. *Am J Physiol Endocrinol Metab.* 2003;285(**3**):E490-E497.
24. Spollett GR. Hyperglycemia in HIV/AIDS. *Diabetes Spectrum.* 2006;19(**3**):163-166.
25. Willard S. The nurse practitioner's role in managing dyslipidemia and other cardiovascular risk factors in HIV-infected patients: impact of antiretroviral therapy. *JANAC.* 2006;17(**1**):7-17.
26. Norris A, Dreher HM. Lipodystrophy syndrome: the morphologic and metabolic effects of antiretroviral therapy in HIV infection. *JANAC.* 2004;15(**6**):46-64.
27. Kalra S, Kalra B, Agrawal N, Unnikrishnan A. Understanding diabetes in patients with HIV/AIDS. *Diabetol Metab Syndr.* 2011;3:2.
28. Monroe A. HIV/AIDS and diabetes: minimizing risk, optimizing care. *BETA.* 2009;21(**2**):38-44.
29. Carper MJ, Cade WT, Cam M, Zhang S, Shalev A, Yarasheski KE, *et al.* HIV-protease inhibitors induce expression of suppressor of cytokine signaling-1 in insulin-sensitive tissues and promote insulin resistance and type 2 diabetes mellitus. *Am J Physiol Endocriol Metab.* 2008;294(**3**):E558-E567.
30. Hruz PW. Molecular mechanisms for altered glucose homeostasis in HIV infection. *Am. J. Infect. Dis.* 2006;2(**3**):187-192.
31. Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet.* 1999;354(**9184**):1112-1115.

32. Shikuma CM, Hu N, Milne C, Yost F, CarolWaslien, Shimizu S, *et al.* Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS.* 2001;15(**14**):1801-1809.
33. Nolan D, Hammond E, Martin A, Louise Taylor, Herrmann S, McKinnon E, *et al.* Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS.* 2003;17(**9**):1329-1338.
34. Nolan D, John M, Mallal S. Antiretoviral therapy and the lipodystrophy syndrome, part 2: concepts in aetiopathogenesis. *Antiviral Therapy.* 2001;6:145-160.
35. Caron M, Auclair M, Lagathu C, Lombes A, Walkerc UA, Kornprobst M, *et al.* The HIV-1 nucleoside reverse transcriptase inhibitors stavudine and zidovudine alter adipocyte functions in vitro. *AIDS.* 2004;18:2127-2136.
36. Gerschensona M, Brinkman K. Mitochondrial dysfunction in AIDS and its treatment. *Mitochondrion*. 2004;4(**5-6**):763-777.
37. Kumar A, Sathian B. Assessment of lipid profile in patients with human immunodeficiency virus (HIV/AIDS) without antiretroviral therapy. *Asian Pacific Journal of Tropical Disease.* 2011;1(**1**):24-27.
38. Colagreco JP. Cardiovascular considerations in patients treated with HIV protease inhibitors. *J. Assoc. Nurses AIDS Care.* 2004;15(**1**):30-41.
39. Adewole O, Eze S, Betiku Y, Anteyi E, Wada I, Ajuwon Z, *et al.* Lipid profile in HIV/AIDS patients in Nigeria. *African health sciences.* 2010;10(**2**):144-149.
40. Dubé MP, Stein JH, Aberg JA, Fichtenbaum CJ, Gerber JG, Tashima KT, *et al.* Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: recommendations of the HIV Medicine Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. *Clin. Infect. Dis.* 2003;37(**5**):613-627.
41. Buchacz K, Weidle PJ, Moore D, Were W, Mermin J, Downing R, *et al.* Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based AIDS care program in rural Uganda. *JAIDS.* 2008;47(**3**):304.
42. Calza L, Manfredi R, Chiodo F. Hyperlipidaemia in patients with HIV-1 infection receiving highly active antiretroviral therapy: epidemiology, pathogenesis, clinical course and management. *Int. J. Antimicrob. Agents.* 2003;22(**2**):89-99.
43. Sudano I, Spieker LE, Noll G, Corti R, Weber R, Lüscher TF. Cardiovascular disease in HIV infection. *Am. Heart J.* 2006;151(**6**):1147-1155.

44. Calza L, Manfredi R, Pocaterra D, Chiodo F. Risk of premature atherosclerosis and ischemic heart disease associated with HIV infection and antiretroviral therapy. *J. Infect.* 2008;57(**1**):16-32.
45. Araújo PSR, Ximenes RAA, Lopes CFC, Duarte JY, Silva MM, Carneiro EM. Antiretroviral treatment for HIV infection/AIDS and the risk of developing hyperglycemia and hyperlipidemia. *Rev. Inst. Med. Trop. Sao Paulo.* 2007;49(**2**):73-78.
46. Riddler SA, Smit E, Cole SR, Li R, Chmiel JS, Dobs A, *et al.* Impact of HIV infection and HAART on serum lipids in men. *JAMA.* 2003;289(**22**):2978-2982.
47. Calza L, Manfredi R, Farneti B, Chiodo F. Incidence of hyperlipidaemia in a cohort of 212 HIV-infected patients receiving a protease inhibitor-based antiretroviral therapy. *Int. J. Antimicrob. Agents.* 2003;22(**1**):54-59.
48. Young J, Weber R, Rickenbach M, Furrer H, Bernasconi E, Hirschel B, *et al.* Lipid profiles for antiretroviral-naive patients starting PI-and NNRTI-based therapy in the Swiss HIV Cohort Study. *Antiviral therapy.* 2005;10:585-591.
49. Luatngoen J. HAART-associated hyperlipidemia and hyperglycemia in HIV patients. *Khon Kaen Hospital Medical Journal.* 2008;32(**4**):60-67.
50. Obirikorang C, Quaye L. Serum lipid profiling in highly active antiretroviral therapy-naive HIV positive patients in Ghana; any potential risk? *Infectious Diseases.* 2010;1(**10**).
51. Padmapriyadarsini C, Ramesh Kumar S, Terrin N, Narendran G, Menon PA, Ramachandran G, *et al.* Dyslipidemia among HIV-infected patients with tuberculosis taking once-daily nonnucleoside reverse-transcriptase inhibitor–based antiretroviral therapy in India. *Clin. Infect. Dis.* 2011;52(**4**):540-546.
52. Roche. COBAS INTEGRA 400 method manual. Germany: 2004.
53. NCEP. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). 2002.
54. Pujari SN, Dravid A, Naik E, Bhagat S, Tash K, Nadler JP, *et al.* Lipodystrophy and dyslipidemia among patients taking first-line, WHO-recommended highly active antiretroviral therapy regimens in western India. *JAIDS.* 2005;39(**2**):199-202.
55. Heath KV, Hogg RS, Singer J, Chan KJ, O'Shaughnessy MV, Montaner JSG. Antiretroviral treatment patterns and incident HIV-associated morphologic and lipid abnormalities in a population-based cohort. *JAIDS.* 2002;30(**4**):440-447.

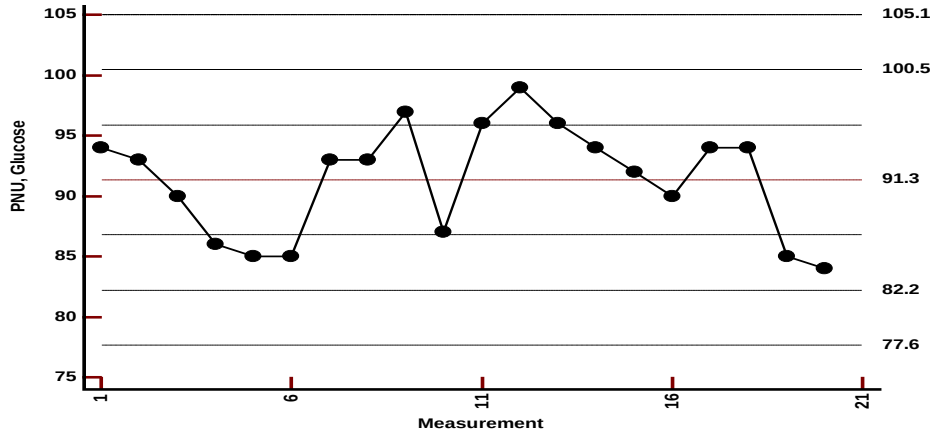
56. Yone EWP, Betyoumin AF, Kengne AP, Folefack FJK, Ngogang J, Pefura Yone E, *et al.* First-line antiretroviral therapy and dyslipidemia in people living with HIV-1 in Cameroon: a cross-sectional study. *AIDS research and therapy.* 2011;8:33.
57. Van Leth F, Hall D, Lange J, Reiss P. Plasma lipid concentrations after 1.5 years of exposure to nevirapine or efavirenz together with stavudine and lamivudine. *HIV Med.* 2006;7(**6**):347-350.
58. Ngogang Y. Effect of antiretroviral therapy on lipid metabolism in HIV/AIDS subjects in Cameroon. *J. Med. Sci.* 2005;5(**2**):78-82.
59. Domingo P, Sambeat MA, Pérez A, Ordoñez J, Rodríguez J, Vázquez G. Fat distribution and metabolic abnormalities in HIV-infected patients on first combination antiretroviral therapy including stavudine or zidovudine: role of physical activity as a protective factor. *Antiviral therapy.* 2003;8(**3**):223-232.
60. Saint-Marc T, Partisani M, Poizot-Martin I, Bruno F, Rouviere O, Lang JM, *et al.* A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. *AIDS.* 1999;13(**13**):1659-1667.
61. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, *et al.* Incidence and risk factors for new-onset diabetes in HIV-infected patients. *Diabetes Care.* 2008;31(**6**):1224-1229.
62. Van Leth F, Phanuphak P, Stroes E, Gazzard B, Cahn P, Raffi F, *et al.* Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLoS medicine.* 2004;1(**1**):e19.
63. Negredo E, Ribalta J, Ferré R, Salazar J, Rey-Joly C, Sirera G, *et al.* Efavirenz induces a striking and generalized increase of HDL-cholesterol in HIV-infected patients. *AIDS.* 2004;18(**5**):819-821.
64. Fontas E, van Leth F, Sabin CA, Friis-Møller N, Rickenbach M, d'Arminio Monforte A, *et al.* Lipid Profiles in HIV-Infected Patients Receiving Combination Antiretroviral Therapy: Are Different Antiretroviral Drugs Associated with Different Lipid Profiles? *J. Infect. Dis.* 2004;189(**6**):1056-1074.
65. Judith LN, Nouemsi APK, Julius O. The long-term effect of different combination therapies on glucose metabolism in HIV/AIDS subjects in Cameroon. *J. Med. Sci.* 2007;7(**4**):609-614.
66. Anastos K, Lu D, Shi Q, Tien PC, Kaplan RC, Hessol NA, *et al.* Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. *JAIDS.* 2007;45(**1**):34-42.

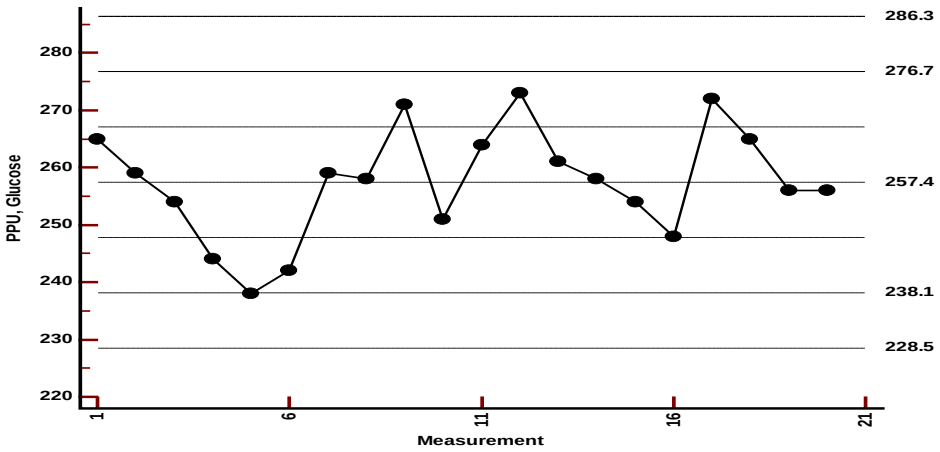
10. ANNEXS

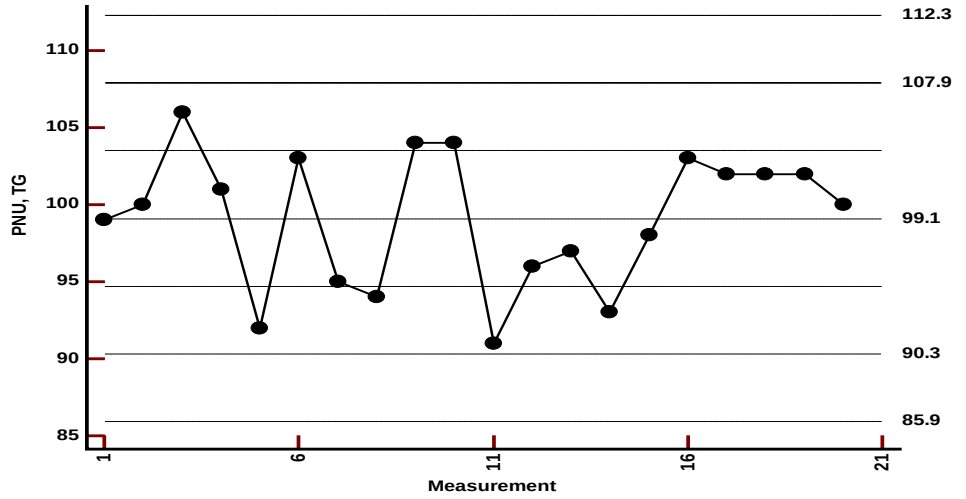
I. Quality Control Graph

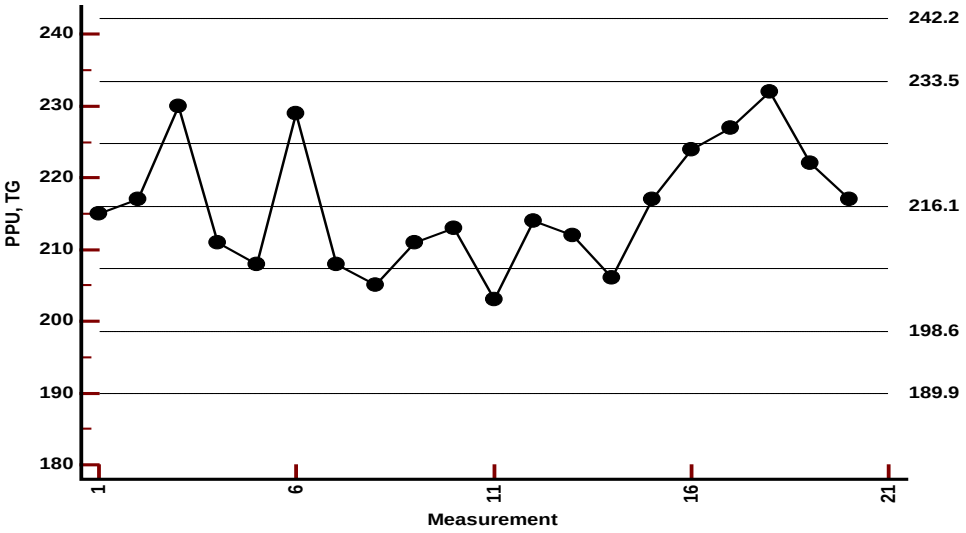
Precinorm Universal (PNU) normal level and Precipath Universal (PPU) pathological level control samples were used for glucose, triglyceride (TG) and total cholesterol (TC). Precipath HDL-C/LDL-C (PPHL) pathological level control was used for high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). The PNU and PPU were run 20 times and PPHL was run 10 times for HDL-C and 13 times for LDL-C. All quality control results were under accepted range according to Levy Jennings' quality control rules.

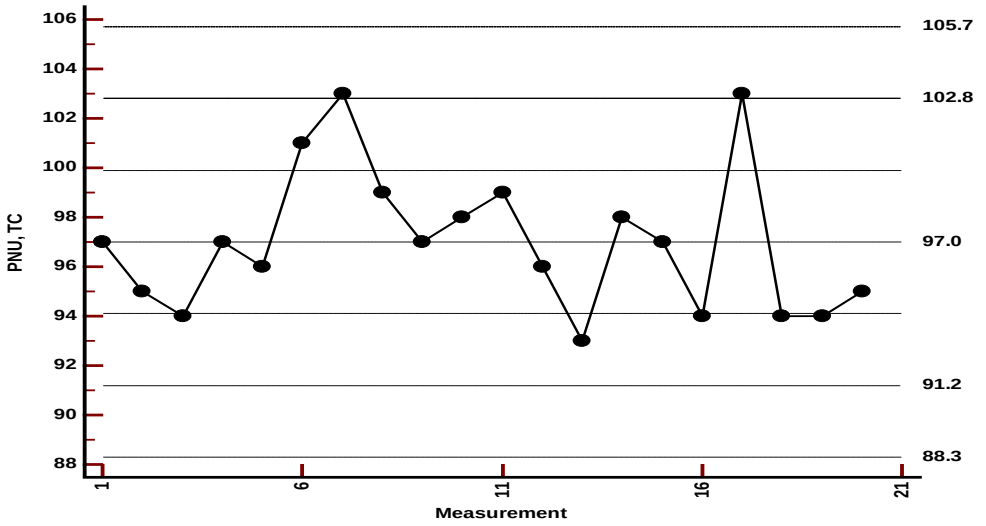
Glucose	**PNU**	94	93	90	86	85	85	93	93	97	87	96	99	96	94	92	90	94	94	85	84
	PPU	265	259	254	244	238	242	259	258	271	251	264	273	261	258	254	248	272	265	256	256
TG	**PNU**	99	100	106	101	92	103	95	94	104	104	91	96	97	93	98	103	102	102	102	100
	PPU	215	217	230	211	208	229	208	205	211	213	203	214	212	206	217	224	227	232	222	217
TC	**PNU**	97	95	94	97	96	101	103	99	97	98	99	96	93	98	97	94	103	94	94	95
	PPU	197	188	190	197	191	200	204	195	186	190	192	191	192	201	192	190	194	198	193	192
HDL-C	**PPHL**	26	28	27	28	26	25	27	26	28	27	-	-	-	-	-	-	-	-	-	-
LDL-C	**PPHL**	203	205	209	208	210	205	202	203	209	207	202	208	203	-	-	-	-	-	-	-
No. of Tests		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

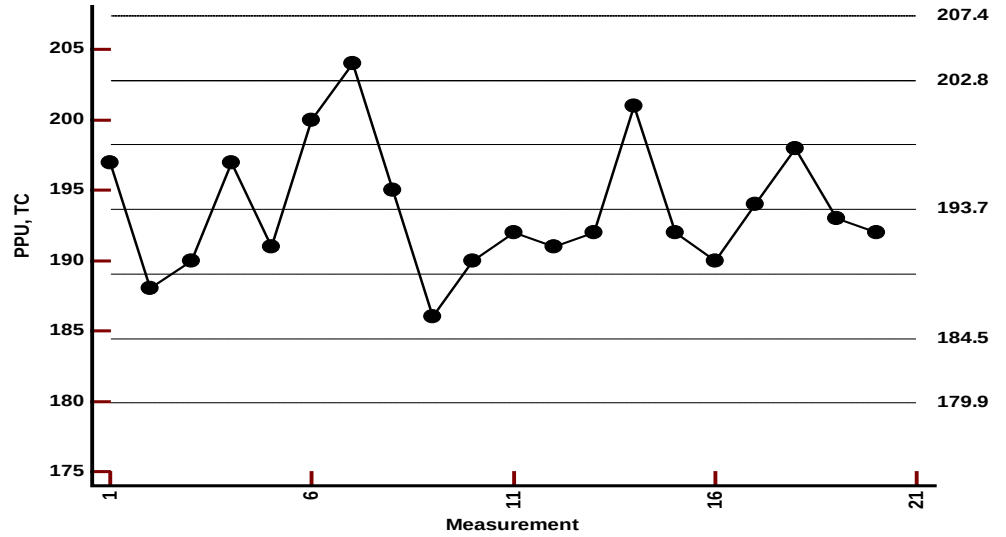


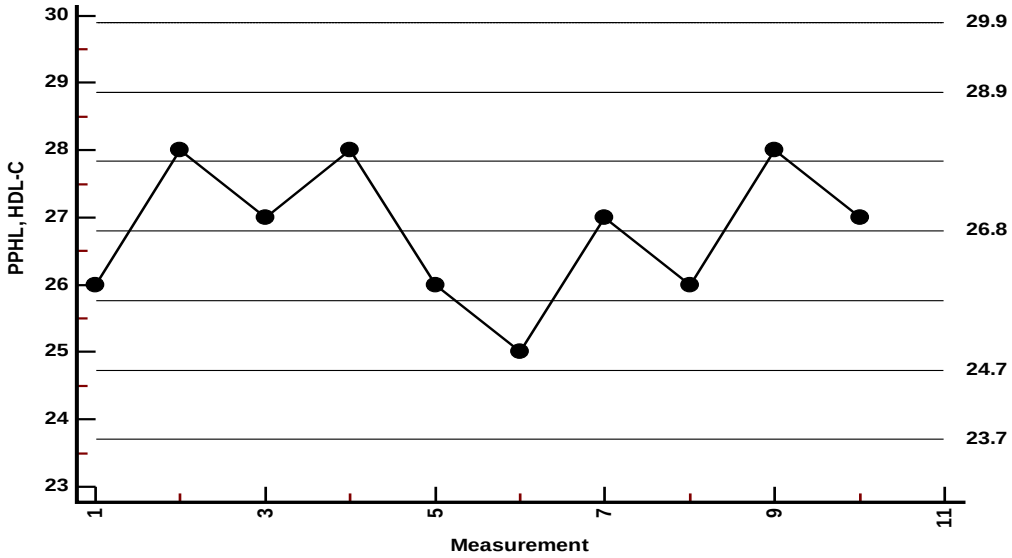


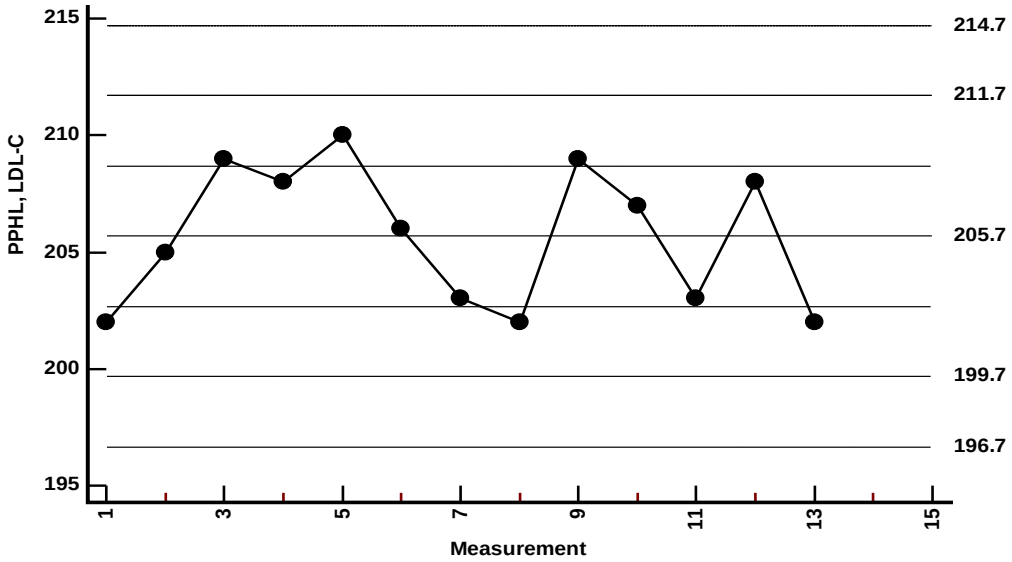

PNU, TG
110
105
100
95
90
85
112.3
107.9
99.1
90.3
85.9
1
6
11
16
21
Measurement


PPU, TG
240
230
220
210
200
190
180
242.2
233.5
216.1
198.6
189.9
1
6
11
16
21
Measurement


PNU, TC
106
104
102
100
98
96
94
92
90
88
105.7
102.8
97.0
91.2
88.3
1
6
11
16
21
Measurement


207.4
202.8
193.7
184.5
179.9
205
200
195
190
185
180
175
PPU, TC
1
6
11
16
21
Measurement


29.9
28.9
26.8
24.7
23.7
30
29
28
27
26
25
24
23
PPHL, HDL-C
1
3
5
7
9
11
Measurement


214.7
211.7
205.7
199.7
196.7
215
210
205
200
195
PPHL, LDL-C
1
3
5
7
9
11
13
15
Measurement

II. Laboratory Investigation (52)

1. Glucose

Method: Hexokinase (HK)

Principle

Enzymatic reference method with hexokinase, HK catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH.

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-phosphate + ADP

D-glucose-6-phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ D-6-phosphogluconate + NADPH + H^+

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340nm.

Reagents-working solutions

R1 Cofactor in vial A and B (liquid)

R2=SR enzymes in vial C (liquid)

Active ingredients

Components	Concentrations			
	R1	R2	Test	
TRIS	100		74	mmol/L
ATP	1.7		1.3	mmol/L
Mg^{++}	4	4	3.5	mmol/L
NADP	1		0.7	mmol/L
HEPES		30	4.5	mmol/L
HK (yeast)		$\geq$ 130	$\geq$19	µkat/L ($\geq$ 1.2kU/L)
G6PDH (microbial)		$\geq$ 250	$\geq$37	µkat/L ($\geq$ 2.2kU/L)
pH	7.8	7.0	7.8	

Reagent handling: Ready for use

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes
Plasma: Li-heparin, EDTA or fluoride plasma

Serum, plasma

Collect blood by venipuncture from fasting individuals using an evacuated tube system. The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~7% in 1 hour (5-10mg/dl). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in sodium fluoride tubes.

Pipetting parameters

		Diluent (H_2O)
R1	150µL	
Sample	2 µL	20 µL
SR	30 µL	
Total volume	202 µL	

Expected values

Serum, plasma	
Fasting	55-115 mg/dl
Adults	70-105 mg/dl
>60 years	80-115 mg/dl
>70 years	83-110 mg/dl

2. Triglycerides

Method: Enzymatic, colorimetric

Principle

Enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone, triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK). The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2).

Triglycerides $\xrightarrow{LPL}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{GK}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{GPO}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminophenaszone + 4-chlorophenol $\xrightarrow{POD}$ quinoneimine dye + $4H_2O$

In the presence of peroxidase (POD), hydrogen peroxide effects the oxidative coupling of 4-chlorophenol and 4-aminophenaszone to form a red-colored quinoneimine dye, which is measured at 512 nm. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample.

Reagent-working solution

R mono reagent in vial A and B (liquid)

Active ingredients

Components	Concentrations		
	R	Test	
PIPES	50	40	mmol/L
LPL (microbial)	≥83	≥66	µkat/L (≥4kU/L)
GK (microbial)	≥3	≥2.4	µkat/L (≥0.14kU/L)
GPO (microbial)	≥41	≥ 33	µkat/L (≥2kU/L)
POD (horseradish)	≥1.6	≥1.3	µkat/L (≥0.08kU/L)

ATP	1.4	1.1	mmol/L
Mg^{++}	40	32	mmol/L
4-aminophenaszone	0.13	0.1	mmol/L
4-chlorophenol	4.2	3.4	mmol/L
Sodiumcholate	0.2	0.16	mmol/L
pH	6.8	6.8	

The contains non-reactive surfactants and stabilizers

Reagent handling: ready for use

Specimen collection and preparation

Only the specimen listed below were tested and found acceptable.

Serum (from fasting patients): collect serum using standard sampling tubes.

Plasma (from fasting patients): Li-heparin or EDTA-plasma. EDTA tubes that are less than ½ full may cause a negative bias for triglycerides results.

Patients should refrain from eating for 10 to 14 hours before blood is drawn. Samples must be drawn in a soap and glycerol free device.

Pipetting parameters

		Diluent (H_2O)
R	120 µL	
Sample	2 µL	28 µL
Total volume	150 µL	

Expected value: <200 mg/dl

3. Total Cholesterol

Method: Enzymatic, colorimetric

Principle

Enzymatic, colorimetric method (CHOD/PAP) with cholesterol esterase, cholesterol oxidase and 4-aminoantipyrine, cholesterol esterase (CE) hydrolyze cholesterol esters form free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of cholesterol to form cholest-4-ene-3one and H_2O_2. In presence of peroxidase (POD), the hydrogen peroxide formed

effects the oxidative coupling of phenol and 4-amino-antipyrine (4-AAP) to form a red-colored quinoneimine dye.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-ene-3one} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{POD} \text{quinoneimine dye} + 4H_2O$$

The color intensity of the red quinoneimine dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 520 nm.

Reagent-working solution

R Mono reagent in vial A (liquid)

Active ingredients

Components	Concentrations		
	R	Test	
Phosphate	70	17	mmol/L
Sodium cholate	13	3.1	mmol/L
Phenol	97	23	mmol/L
4-aminoantipyrine	1.7	0.4	mmol/L
CE (microbial)	≥8	≥1.9	µkat/L (≥0.1kU/L)
CHOD (microbial)	≥5	≥1.2	µkat/L (≥0.07 kU/L)
POD (horseradish)	≥50	≥12	µkat/L (≥0.7 kU/L)
pH	6.8	6.8	

The reagent contains non-reactive surfactant and stabilizer.

Reagent handling: ready for use

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable

Serum: collect serum using standard sampling tubes

Plasma: Li-heparin plasma

Fasting and non-fasting samples can be used. Collect blood by using an evacuated tube or syringe. Specimens should be preferably be analyzed on the day of collection.

Pipetting parameters

		Diluent (H_2O)
R	34µL	86 µL
Sample	2 µL	20 µL
Total	142 µL	

Expected values

Recommendations of the NCEP adult treatment panel for the following risk-cutoff thresholds for the US American population.

Risk classification	Total cholesterol
Desirable	<200 mg/dl
Borderline high	200-239 mg/dl
High	≥ 240mg/dl

Clinical interpretation according to the recommendations of the European atherosclerosis society:

Type	mmol/l	mg/dl	Lipid metabolism disorder
Cholesterol	<5.2	<200	
Triglycerides	<2.3	<200	No
Cholesterol	5.2-7.8	200-300	Yes, if HDL-C <0.9 mmol/L (<35mg/dl)
Cholesterol	>7.8	>300	
Triglycerides	>2.3	>200	Yes

4. HDL- Cholesterol

Method: Homogenous enzymatic colorimetric assay

Principle

In the presence of magnesium sulfate and dextran sulfate, water soluble complexes with LDL, VLDL and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL – cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase. Coupled with PEG to the amino groups (approx. 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by

cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

HDL- cholesterol esterase + H_2O $\xrightarrow{\text{PEG-cholesterol esterase}}$ cholesterol + RCOOH

HDL-cholesterol + O2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ Δ4-cholestenone + H_2O_2

$2H_2O_2$ + 4-aminiantipyrine + HSDA + H^+ + H_2O $\xrightarrow{\text{peroxidase}}$ purple blue pigment + $5H_2O$

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

Reagent-working solution

R1 Buffer in vial A (liquid) and vial B (liquid)

R2=SR Enzymes in vial C (liquid)

Active ingredients

Components	Concentrations			
	R1	R2	Test	
MOPS[a]	19.1		14.1	mmol/L
Dextran sulfate	0.001		0.0007	mmol/L
Magnesium sulfate.7H2O	≥ 8.1		≥ 6.0	mmol/L
HSDA[b]	0.958		0.709	mmol/L
AOD (recombinant)	≥50		≥37	µkat/L (≥2.2 kU/L)
POD (horseradish)	≥ 167	≥334	≥206	µkat/L (≥12 kU/L)
PIPES[c]		9.9	2.44	mmol/L
CE (microbial)		≥3.3	≥ 0.8	µkat/L (≥0.05 kU/L)
CHOD (microbial)		≥ 127	≥ 31	µkat/L (≥1.9 kU/L)
4-aminoantipyrine		2.46	0.6	mmol/L
pH	7.0	7.0	7.1	

a) 3-morpholino-propanesunfonic acid

b) Sodium N-(2-hydroxy-3-sylfopropyl)-3,5-dimethoxyaniline

c) Piperazine-1,4-bis(2-ethanesulfonic)acid

Both reagents contain stabilizers and a preservative.

Reagent handling: ready for use

Specimen collection and preparation

Only the specimen listed below were tested and found acceptable.

Serum: collect serum using standard sampling tubes.

Plasma: heparin (Li-, Na-, NH_4-) or EDTA (K_3-) plasma.

Store plasma ate 4^oC prior to analysis. EDTA plasma has the advantage that lipoproteins have enhanced stability during storage at 4^oC. Fasting and nonfasting samples can be used. Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

Pipetting parameters

	Diluent (H_2O)
R1	150 µL
Sample	2.5 µL
SR	50 µL
Total volume	202.5 µL

Expected values

Sex	No risk	Moderate risk	High risk
Female	>1.68mmol/L (>65mg/dl)	1.15-1.68 mmol/L (45-65 mg/dl)	<1.15 mmol/L (<45 mg/dl)
Male	>1.45 mmol/L (>55mg/dl)	090-1.45 mmol/L (35-55 mg/dl)	<0.90 mmol/L (<35 mg/dl)

National cholesterol education program (NCEP) guidelines: <40mg/dl: Low HDL- cholesterol (major risk factor for CHD), ≥ 60 mg/dl: High HDL- cholesterol (negative risk factor for CHD)

11.LDL-Cholesterol

Method: Homogenous enzymatic colorimetric assay

Principle

This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included

in the enzymatic method for cholesterol determination (cholesterol esterase-cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. This direct assay meets the 1995 NCEP goals of <4% Total CV, bias ≤4% versus reference method, and ≤12% total analytical error.

LDL-cholesterol ester + H_2O → (detergent cholesterol esterase, selective micellary solubilization) cholesterol + free fatty acid

LDL-cholesterol + O_2 → (cholesterol oxidase) Δ4-cholestenone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + HSDA + H^+ + H_2O → (Peroxidase) purple blue pigment + 5 H_2O

The color intensity of the blue quinoneimine dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

Reagents - working solutions

R1 Buffer in vial A (liquid) and in vial B (liquid).

R2 = SR Enzymes in vial C (liquid).

Active ingredients

Components	Concentrations			
	R1	R2	Test	
MOPS[a]	20.1	20.1	19.2	mmol/L
HSDA[b]	0.958		0.688	mmol/L
AOD (recombinant)	≥50		≥36	µkat/L (≥2.2 kU/L)
POD (horseradish)	≥167	≥334	≥200	µkat/L (≥12 kU/L)
Magnesium sulfate · 7 H2O		8.11	1.94	mmol/L
4-Aminoantipyrine		2.46	0.58	mmol/L
CE (microbial)		≥50	≥12	µkat/L (≥0.7 kU/L)
CHOD (microbial)		≥33	≥8	µkat/L (≥0.5 kU/L)
pH	6.5	6.8	6.4	

a) 3-morpholino-propanesulfonic acid

b) Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

Both reagents contain stabilizers and a preservative.

Reagent handling

Ready for use

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum: Collect serum using standard sampling tubes. Plasma: Heparin (Li-, Na-, NH4-) or K3-EDTA plasma. Store plasma before analysis. EDTA plasma has the advantage that lipoproteins have enhanced stability during storage at 4°C. Comparable non-fasting results were observed with the beta quantification method. Specimens should preferably be analyzed on the day of collection.

Stability: 7 days at 2-8°C and 30 days at -70°C

Pipetting parameters

Diluent		(H_2O)
R1	150 µl	
Sample	2 µl	7 µl
SR	50 µl	
Total volume	209 µl	

Expected values

Optimal	< 100 mg/dl
Near optimal /above optimal	100-129 mg/dl
Borderline high	130-159 mg/dl
High	160-189 mg/dl
Very high	>190

III. Questionnaire

Date: _____
Format Number: _____

Patient Identification Number: _____

1. Sex: ☐ Male ☐ Female
2. Age: _____ years
3. Marital Status: ☐ Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated
4. Educational level: ☐ No Education ☐ Primary ☐ Secondary ☐ Tertiary
5. Occupation: ☐ Gov't employee ☐ Merchant ☐ House wife ☐ Daily labor ☐ Other
6. Current weight: _____Kg
7. Current height: _____m
8. BMI: _____ Kg/m2
9. Date of HIV positive confirmed: _____/_____/_____dd/mm/yy.
10. Did you start HAART? ☐ Yes ☐ No

10.1. If the above question is yes, duration on HAART is _____months.

11. Which combination of antiretroviral therapy currently you are using?

☐ D4T-3TC-NVP ☐ AZT-3TC-EFV ☐ D4T-3TC-EFV ☐ Other, _____.

12. Is there any ARV change within the last three months? ☐ Yes ☐ No

12.1. If the above question is yes, the previous drug was _____ and the changed drug is _____.

12.2. The reason of drug change was _____.

13. Have you ever used drugs other than HAART? ☐ Yes,_____ ☐ No

13.1 If yes; _____.

End of interview, Dear participant, thank you very much for taking your valuable time.

IV. Questionnaire, Amharic Version

ቀን _____

የቅፅ ቁጥር _____

የተሳታፊዉ መለያ ቁጥር ----------

1. ፆታ ☐ወንድ ☐ሴት
2. እድሜ --------- ዓመት
3. የጋብቻ ሁኔታ፡ ☐ያላገባ ☐ ያገባ ☐ የፈታ ☐ የሞተበት ☐ ተለያይተዉ የሚኖሩ
4. የትምህርት ደረጃ፡ ☐ ያልተማረ ☐ የመጀመሪያ ደረጃ ☐ ሁለተኛ ደረጃ ☐ ሦስተኛ ደረጃ
5. የሥራ ሁኔታ፡ ☐የመንግስት ሰራተኛ ☐ ኔጋዴ ☐የቤት እመቤት ☐የቀን ሰራተኛ ☐ ሌላ
6. ክብደት---------- ኪግ
7. ቁመት ---------- ሜ
8. BMI ----------ኪግ/ሜ^2
9. የኤች አይ ቪ በሽታ በደምዎ መኖሩን ያረጋገጡበት --------/------/------ቀን/ወር/ዓመት
10. የኤች አይ ቪ በሽታ የእድሜ ማራዘሚያ መድሃኒት ጀምረሃል/ሻል? ☐አዎ ☐አልጀመርኩም

10.1. መልሱ አዎ ከሆነ; ከጀመርህ/ሽ ---------- ወር ሆነ?

11. የትኛዉን የኤች አይ ቪ በሽታ የእድሜ ማራዘሚያ መድሃኒት ነዉ በመዉሰድ ላይ የምትገኘዉ/ኝዉ?

☐ D4T-3TC-NVP ☐ AZT-3TC-EFV ☐ D4T-3TC-EFV ☐ Others ----------

12. ባለፉት ሥስት ወራት ዉስጥ የእድሜ ማራዘሚያ መድሃኒት ለዉጥ አድርገህ/ሽ ነበር? ☐አዎ ☐አይደለም

12.1. መልሱ አዎ ከሆነ; በፊት የነበረዉ መድሃኒት ---------- ሲሆን የአሁኑ ደግሞ ----------ነዉ::

13. ከእድሜ ማራዘሚያ መድሃኒት ዉጭ ሌላ መድሃኒት ተጠቅዉ ያዉቃሉ? ☐አዎ ☐አይደለም

13.1. መልሱ አዎ ከሆነ; _____

ቃለመጠይቁ ተጠናቋል::

ዉድ ተሳታፊየ; ጊዜዎን ሠዉተዉ ለሰጡኝ ቃለመጠይ በጣም አመሰግናለሁ::

V. Information Sheet for Participants

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY

Title: Antiretroviral Treatment Associated Hyperglycemia and Dyslipidemia among HIV Infected Patients at Burayu Health Center, Addis Ababa, Ethiopia.

Principal Investigator: Molla Abebe (BSc, SMLS, AAU)

Advisors:

Samuel Kinde (MSc, Lecturer, SMLS, AAU)

Belete Tegbaru (PhD, Researcher, EHNRI)

Sponsor: Addis Ababa University

Information Sheet to Study Participants

1. Introduction

We would like to conduct a medical research with the title "Antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients at Burayu Health Center, Addis Ababa, Ethiopia". We are going to inform you about the purpose, responsibility of investigators or data collectors to keep confidentiality and how we are going to use the data. Before you decided to give your written consent, you should be well informed about the study and feel free to ask any question which is not clear for you. It is entirely your choice. If you decided to take part, you can change your mind later on and withdraw from the study**.** The decision not to join the study will not cause you to lose any medical benefits. If you decide not to take part in this study, your doctor will continue to treat you.

Before making your decision:

- Please carefully read this form or have it read to you
- Please listen to the study investigator or study staff explain the study to you
- Please ask questions about anything that is not clear
- Feel free to take home an unsigned copy of this form and take your time to think about it and talk it over with family or friends

2. Description and Purpose of the Study

HIV/AIDS is one of the top diseases that affect the health of many people in Ethiopia. To decrease morbidity, mortality and prolong life, HAART is initiated science 2003 and have been used by many patients in our country. However, there is shortage of information on the possible metabolic complications of HAART like, diabetes mellitus and cardiovascular diseases.

The purpose of this study is to assess antiretroviral treatment associated hyperglycemia and dyslipidemia among HIV infected patients. We want to assess the exact prevalence and magnitude of hyperglycemia and dyslipidemia. We hope this information will help to improve diagnosis, treatment and management of hyperglycemia and dyslipidemia related metabolic disorders caused by combination antiretroviral treatment.

3. Risk and Discomfort

There is no more discomfort associated with this study. But, there could be minor pain following the blood drawing and which would disappear in short duration. The amount of blood that will be taken from each participant will be 5-6 ml which will not affect your health. This is what you usually give for your follow up and additional blood will not be collected. There is no major risk in participating in this research, as the whole procedure is carried out by experienced health professionals following the standard good clinical practice. If you have any discomfort, you can contact any of the investigators in this project.

4. Incentives and Compensation

Your participation will not have any cash payment. It is not ethical to pay you because of participation in this study. But the cost for general medical examination will be covered by the project. The result of the laboratory finding will be communicated to your physician for use in the management of the disease. You will have the chance to know your general health status from the medical examination. And if the medical examination reveals any abnormalities that need immediate treatment, your doctor will be notified about the result.

5. Participant Role

If you are voluntary and decide to participate in this project, you will be requested to give blood sample for glucose and lipid profile determination. On volunteer base you will be asked some demographic questions.

6. Participant's Right and Withdrawal from the Study

Your participation is completely voluntary and you have the right to refuse to be in this study. You can stop at any time after giving your consent. This decision will not affect in any way your current or future medical care or any other benefits to which you are otherwise entitled. The investigator and/or sponsor may stop you from taking part in this study at any time if they decide it is in your best interest, or if you do not follow study instructions.

7. Confidentiality

We respect your privacy and we will keep all your information private. Your study records will identify you by a subject identification number and not by your name. Any information that identifies you will not be shared with anyone else without your written permission. All information obtained about you for this study will be kept confidential to the extent allowed by law. Your information will be used only for above mentioned purpose. The results of the study may be published for scientific purposes. However, your identity will not be given out.

8. Agreement

You will be asked for signature of agreement. This is to make sure that your agreement to participate in the mentioned study is on volunteer and informed basis. Otherwise there is no other reason for signing. The study is approved by ethical committee of school of medical laboratory sciences at Addis Ababa University. Getting signatures of agreement from participant is the one criteria of committee for the indication of no one can participate in the study without participant consent and agreement. Participants will make agreements on their volunteer bases. You have full right to get full information about study procedures and other related issues with languages of your choice.

9. Communication

In case you might have any questions, unclear ideas and doubt about the project use the following contact addresses:

Molla Abebe (PI, AAU)

Email: mollish77@gmail.com Mobile: +2519-13-32-97-07

Samuel Kinde (MSc, lecturer, AAU)

Email: samuelkinde@yahoo.com Mobile: +2519-11-85-46-86

Belete Tegbaru (PhD, Researcher, EHNRI)

Email: beletegbaru@gmail.com Mobile: +251911-69-70-10

Thanks in advance for your patience!

VI. Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Molla Abebe (PI)

Signature: _____ Date: _____

VII. Informed consent for study participants, Consent declaration

I have been informed about the study, which plans to assess the occurrence of hyperglycemia and dyslipidemia in adult patients receiving highly active antiretroviral therapy. The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and health center access.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my blood for the mentioned study. I was agreed that the specimen would be tested for cholesterol, triglycerides, HDL-C and glucose. I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction in a language I understand.

I was also told that results for the lipid profiles and glucose would be reported timely to my ART counselor and that I may ask there for information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for the purpose of assessing the occurrence of hyperglycemia and dyslipidemia in individuals on long-term highly active antiretroviral therapy.

Signature: Date:

Participant: _____ _____

Person discussed with _____ _____

VIII. Amharic Version of Information Sheet for Participants

ቅጥያ VIII. <u>የተሳታፊወች መረጃ ቅጽ</u>

አዲስ አበባ ዩኒቨርሲቲ

ጤና ሳይንስ ኮላጅ

አላይድ ጤና ሳይንስ ት/ቤት፣ የህክምና ላቦራቶሪ ት/ክፍል

አርስት፡ በደም ላይ የስከኳርና ቅባት መጨመርን የኤድስ መድሃኒት በመውሰድ ላይ ያሉ በቡራዩ ጤና ጣቢያ ክትትል እያደለጉ በሚገኙ ህሙማን ላይ ማጥናት

<u>መሪ ተመራማሪ፡</u> ሞላ አበበ (አዲስ አበባ ዩኒቨርሲቲ)

<u>አማካሪ፡</u>

ሳሙኤል ክንዴ (አዲስ አበባ ዩኒቨርስቲ)

በለጠ ተግባሩ (ዶ/ር ፣ ኢጤስምኢ)

ወጭዉን የሚሸፍንዉ፡ አዲስ አበባ ዩኒቨርስቲ

የተሳታፊዎች የመረጃ ቅጽ

1. መግቢያ

እኛ በደም ላይ የስኳርና ቅባት መጨመርን የኤድስ መድሃኒት በመውሰድ ላይ ያሉ በቡራዩ ጤና ጣቢያ ክትትል እያደረጉ በሚገኙ ህሙማን የህክምና ጥናት ለማካሄድ ፈልገናል ። ስለዚህ የጥናቱን አላማ ፣የመሪ ተመራማሪውንና መረጃ ሰብሳቢውን የስራ ድርሻ እንዲሁም የናንተን ምስጢር እንዴት እንደምንጠብቅና መረጃውን እንዴት እንደምንጠቀምበት እንገልፅላችኋለን። የፅሁፍ ማረጋገጫ ከመስጠታችሁ በፊት ፣ስለ ጥናቱ በቂ መረጃ እንዲኖራችሁ ያስፈልጋል። በተጨማሪም ያልገባችሁን ማንኛውንም አይነት ጥያቄ በነፃነት መጠየቅ ትችላላችሁ። በዚህ ጥናት መሳተፍ ሙሉ በሙሉ የናንተ ምርጫ ነው። በጥናቱ መሳተፍ ፈልጋችሁ ከጀመራችሁ በኃላም ማቋረጥ ትችላላችሁ። በጥናቱ መሳተፍም ሆነ አለመሳተፍ በህክምናዉ የምታገኙትን ጥቅም አያስቀርባችሁም። በጥናቱ ባትሳተፉም የናንተ የህክምና ባለሙያ እናንተን ማከሙን ይቀጥላል።

ከመወሰናችሁ በፊት፡

ሀ. ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብላችሁ በትክክል አድምጡ

ለ. የጥናቱ ባለሙያዎችን ስለጥናቱ የሚገልፁትን በተክክል አድምጡ

ሐ. ግልፅ ያልሆነላችሁን ነገር በሙሉ በነፃነት ጠይቁ

መ. የዚህን ቅጽ ቅጂ በነጻነት ወደ ቤታችሁ ወስዳችሁ፤ ከቤተሰቦቻችሁ ወይም ከጓደኞቻችሁ ጋር ጊዜ ሰጥታችሁ በመነጋገርና መወሰን ትችላላችሁ።

2. የጥናቱ ገለፃና አላማ

ኤድስ በኢትዮጵያ ዉስጥ የከፋ የጤና ችግር ከሚያመጡ በሽታዎች አንዱ ነዉ። በበሽታዉ የመጠቃት መጠንን፣ሞትን ለመቀነስ እና ህይዎትን ለማራዘም የኤድስ መድሃኒት ከ 1996 ዓ.ም ጀምሮ እስከ አሁን ድረስ በኢትዮጵያ በብዙ ሰዎች በጥቅም ላይ ይገኛል። ነገር ግን ከመድሃኒቱ ጋር ተያያዝ ስለሆኑ እንደ የስኳርና የልብ ህመም ያሉ የጤና ችግሮች የመረጃ እጥረት አለ።

የዚህ ጥናት አላማ በደም ላይ የስከኳርና ቅባት መጨመርን የኤድስ መድሃኒት በመውሰድ ላይ ባሉ ህሙማን ላይ መዳሰስ ነዉ። እኛ ትክክለኛዉን የስኳርና ቅባት መጨመርን ስርጭትና መጠን ማወቅ እንፈልጋለን።የዚህ ጥናት ውጤትም በኤድስ መድሃኒት ሳቢያ በሚከሰተዉ የስከኳርና ቅባት መጨመር ምክንያት ለሚመጣዉ ማንኛዉም በሽታ ምርመራ፣ ህክምናና ቁጥጥር ይጠቅማል።

3. ሊከሰቱ ስለሚችሉ ስጋቶች እና የምቾት መጓል

በዚህ ጥናት ተሳታፊ መሆንዎ እንደተለመደዉ ለኤች አይ ቪ በሽታ ምርመራና እንክንካቤ ከሚያደርጉት የተለየ የምቾት መጓደል አያስከትልብዎትም። ነገር ግን ናሙና በሚወሰድነት ጊዜ በትቂት ደቂቃ ሊጠፉ የሚችል መጠነኛ የሆነ የህመም

ስሜት ሊያስከትል ይችላል፡፡እንዲሁም የናሙና አወሳሰዱና ሌሎች ቅደም ተከተሎች የሚከናወኑት በሰለጠኑ የህክምና ባለሙያወች የህክምና ደንብ በሚፈቅደዉ የንጽህና አጠባበቅ ደረጃ በመሆኑ ይህ ነዉ የሚባል ስጋት አይኖርም፡፡

4. ጥቅማጥቅምና ማካካሻ

የናንተ በዚህ ጥናት መሳተፍ ቀጥተኛ የሆነ ክፍያ የለውም፡፡ በመሳተፋችሁ ምክንያት ክፍያ መክፈል ስነምግባሩ አይፈቅድም፡. ነገር ግን በዚህ ጥናት ምክንያት የምታወጡት ወጭ ካለ ጥናቱ ይሸፍናል፡፡ ከላብራቶሪ ምርመራ የተገኘዉ ዉጤት ከጤና ባለሙያዉ ጋር በመነጋገር በሽታዉን ለመቆጣጠር ጥቅም ላይ ሊዉል ይችላል፡፡አጠቃላይ የጤና ምርመራ ይደረግልዎታል፡፡በዚህ ምርመራ የተለየ ወይንም ያልተጠበቀ ዉጤት ቢታይ አስፈላጊ ህክምና የሚያገኙበት ሁኔታ ይመቻችልዎታል፡፡

5. የተሳታፊዎች ሚና

በዚህ ጥናት ለመሳተፍ ከፈቀዱና ከወሰኑ ለጥናቱ የሚዉል የደም ናሙና እንዲሰጡ ይጠየቃሉ፡፡ የናንተን የግል ህይወት በተመለከተ በፈቃደኝነት ላይ የተመሰረተ ጥያቄና መልስም ይኖረናል፡፡

6. የተሳታፊዎች መብት

የናንተ በጥናቱ መሳተፍ በነፃ ፈቃደኝነት ላይ የተመሠረተ ሲሆን በጥናቱ ላለመሣተፍ መቃውም ትችላላችሁ፡፡ ለመሳተፍ ከወሰናችሁ በኋላም በማንኛውም ጊዜ ማቋረጥ ትችላላችሁ፡፡ ይህ ዉሳኔያችሁ እናንተ አሁንም ሆነ ወደፊት ለምታገኙት የህክምና ወይም ሌላ ጥቅም ላይ የሚያመጣው ችግር የለም፡፡ ለናንተ ጥቅም ሲባል ወይም እናንተ የጥናቱን ሂደት በትክክል ካለመከተላችሁ የተነሳ አጥኝው የህክምና ባለሙያ፣ መሪ ተማራማረው ወይም የጥናቱን ወጭ ሸፋኝ አካል እናንተን ከጥናቱ እንዳትሳተፉ የማድረግ መብት አላቸው፡፡

7. ምስጢር ጠባቂነት

እኛ የግል ምስጢራችሁንና የግል መረጃችሁን በፍፁም ምስጢረኝነት እንጠብቃለን፡፡ በመመዝገቢያ ደብተሩ ላይ በመለያ ቁጥር እንጅ በስም አንመዘግብም፡፡ እናንተን የሚገልፅ መረጃ ያልእናንተ የፅሁፍ ማረጋገጫ ለሶስተኛ አካል ተላልፎ አይሠጥም፡፡ ሁሉንም አይነት የናንተን መረጃዎች ህግ አስከ ሚፈቅደው ድረስ ምስጢራችሁን አንጠብቃለን፡፡ የናንተ መረጃዎች ከላይ ለተጠቀሰው አላማ ብቻ ይዉላሉ፡፡ የጥናቱ ውጤት ለሳይንሳዊ አላማ ሊታተም ይችላል፡፡ ይሁንና እናንተን የሚገልፅ መረጃ ግን አይሠጥም፡፡

8. ስምምነት

እናንተ መስማማታችሁን በፊርማችሁ እንድትገልፁልን በትህትና እንጠይቃችኋለን፡፡ ይህ ስምምነት እናንተ በፈቃደኝነት እና በመረጃ ላይ የተመሠረተ ተሳትፏችሁን ለማረጋገጥ ነው፡፡ ከዚህ ውጭ እናንተ የምትፈርሙበት ምንም ምክንያት የለም፡፡ ይህ ጥናት በአዲስ አበባ ዩኒቨርስቲ የህክምና ላብራቶሪ ትምህርት ቤት የስነምግባር ኮሚቴ የተረጋገጠ ነዉ፡፡ የስነምግባር ኮሚቴው ከተሳታፊዎች የሚገኘውን የፊርማ ሰምምነት እንደ ዋና መስፈርት በመቁጠር ማንም ያለራሡ ፈቃድና ስምምነት እንዳልተሳተፈ/ች የሚያረጋግጥበት ነው፡፡ ተሳታፊዎች በፈቃደኝነት ላይ የተመሰረተ ሰምምነት ታደርጋለችሁ፡፡ እናንተ ስለጥናቱና ተያያዥ ጉዳዮች ሙሉ የሆነ መረጃ በፈለጋችሁት ቋንቋ የማግኘት ሙሉ መብት አላችሁ፡፡

9. **<u>ግንኙነት</u>**

ጥናቱን በተመለከተ ጥያቄ፣ ግልፅ ያልሆነላችሁ ሀሳብ ወይም የሚያጠራጥራችሁ ነገር ካላ የሚከተሉትን አድራሻዎች መጠቀም ትችላላችሁ::

- ሞላ አበበ (መሪ ተመራማሪ፣ አዲስ አበባ ዩኒቨርሲቲ)
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- ሳሙኤል ክንዴ (አዲስ አበባ ዩኒቨርሲቲ)
  ኢሜል፡ <u>samuelkinde@yahoo.com</u> ሞባይል፡ +2519-11-85-46-86

- በለጠ ተግባሩ (ዶ/ር፣ኢጤስምኢ)
  ኢሜል፡<u>beletegbaru@gmail.com</u> ሞባይል፡ +251911-69-70-10

ስለተሳትፏችሁ በጣም እናመሰግናለን!!!

ቅጥያ IX. <u>የመሪ ተመራማሪው ማረጋገጫ</u>

ይህ ጥናት ስነምግባርን እና ሳይንሳዊ ሂደትን የጠበቀ ምርምር ስለመሆኑና ጥናቱን በተመለከተ ለእያንዳንዱ ለሚመለከተው አካል ያለበትን ወቅታዊ ሁኔቴ ለማሳወቅ ሙሉ ሃላፊነት እንደምወስድ ከዚህ በታች በማስቀመጠው ፊርማ ላረጋግጥ እወዳለው፡፡

ሞላ አበበ (መሪ ተመራማሪ)

ፊርማ_____ ቀን_____

ቅጥያ X. <u>የፈቃደኝነት ማረጋገጫ ቅፅ</u>

የኤች አይ ቪ ቫይረስ በደማቸዉ ዉስጥ በሚገኝና የኤች አይ ቪ ኤዲስ የእድሜ ማራዘሚያ መድሃኒት በሚወስዱ ግለሰቦች ላይ የስከኳርና ቅባት መጨመርን በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑና የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ ሙሉ መረጃ በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና ለ cholesterol, triglycerides, HDL-C, LDL-C እና glucose ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ክትትል ለሚያደርግልኝ የጤና ባለሙያ እንደሚሰጡልኝና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

	<u>ፊርማ</u>	<u>ቀን</u>
ተሳታፊ	----------	----------
መረጃዉን ያስረዳዉ አካል	----------	----------