PREVALENCE AND PREDICTORS OF DYSLIPIDEMIA ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY NAIVE HIV POSITIVE PERSONS IN DEFENCE HOSPITAL, ADDIS ABABA –ETHIOPIA.

BY

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A research paper submitted to Jimma University College of Public Health and Medical Sciences, Department of Medical Laboratory and Pathology for partial fulfillment of the requirement of Master of Science Degree in Clinical Laboratory Science, Specialty in Clinical Chemistry.

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JIMMA UNIVERSITY

COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF MEDICAL LABORATORY AND PATHOLOGY

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ABSTRACT

Background: The introduction of highly active antiretroviral therapy (HAART) has led to a marked reduction in AIDS-related morbidity and mortality. However HAART, has been reported to be associated with a number of side effects in HIV positive persons among which dyslipidaemia and lipodystrophy are common metabolic disorders.

Objective: To assess prevalence and predictors of dyslipidemia on HAART and HAART naïve HIV-positive persons in Defense Hospital, Addis Ababa-Ethiopia from September 20 to October 23, 2013.

Methodology: A facility based comparative cross-sectional study on 228 HIV positive persons on HAART and HAART naïve was conducted from September 20 to October 23, 2013. Ethical approval and clearance was obtained from college of public Health and Medical Sciences Jimma University. Socio-demographic, clinical and laboratory data were collected by a structured questionnaire. A fasting blood sample of 5 milliliter was taken and from this 2.5 milliliter was used for lipid profile and the rest for CD4 analysis. Dyslipidemia was defined as the presence of any of TC ≥ 200 mg/dl, HDL-c < 40 mg/dl, LDL-c ≥ 130 mg/dl, TG ≥ 150mg/dl. For statistical analysis student's t-test and logistic regression were done using Statistical Package for Social Sciences (SPSS) Version 16.00.

Result: Total number of participants enrolled in the study were 228, in which 103 (45%) males and 125 (55%) females. From the participants 114 HIV-positive persons who were on HAART in one group & 114 patients were HAART naïve in the second group were included. The mean value of TC was(202.4 & 186.7), HDL-c(40.5 & 43),LDL-c(126 & 111.4),TG (176 &155) for HAART and HAART naïve respectively. Among the participants involved in the study 84 (73.7%) on HAART and 61 (53.5%) HAART naive persons had at least one lipid abnormality. The prevalence of TC≥ 200mg/dl was 50% & 30%; HDL< 40mg/dl was 43.8% & 36%; LDL-c≥130mg/dl was 48.3% & 28.1%; TG ≥150mg/dl 59.6% & 39% among those on HAART and HAART naïve respectively.

Duration of therapy was significantly and positively associated with TC, LDL-c & TG in which the adjusted odds ratio (95% CI) of duration of therapy was 0.54(0.02-0.36) for TC

≥200 mg/dl; 0.03(0.001-0.70) for LDL-cholesterol ≥130mg/dl and 0.04(0.003-0.84) for TG ≥150mg/dl.

Conclusion and Recommendation:

The prevalence of dyslipidemia at Defense Hospital was high. The mean value of each lipid profile was significantly higher in persons on HAART as compared to HAART naïve HIV positive persons. Participants who receive HAART regimens have high value of dyslipidemia in HIV positive persons as compared to HAART naive HIV-positive persons. Duration of therapy was the risk factor for raised TC, LDL-c & TG which was significantly and positively associated.

The findings of our study indicate that the need to assess lipid profiles at baseline and on HAART to monitor any rising trends. Health professionals who are working in ART clinic should place their effort in lowering of dyslipidemia for those persons on HAART. It also requires Additionally, the results also recommend implementation of well controlled cohort studies for the evaluation of long-term effects of HAART on lipid profiles.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABREVIATIONS

AIDS-Acquired Immunodeficiency Syndrome

AOR-Adjusted Odds Ratio

ART-Anti Retroviral Treatment

BP-Blood Pressure

CDC-Center of Disease Control & prevention

CD4-Cluster of Differentiation

COR-Crude Odds Ratio

D4T- Stavudine

DBP-Dyastolic Blood Pressure

EDTA-Ethylene Diamine Tetra-Acetate

EFV-Efavirenz

FACS count-Fluorescent Activated Cell Sorter

HAART-Highly Active Anti Retroviral Therapy

HDL-c-High Density Lipoprotein cholesterol

HIV-Human Immunodeficiency Virus

LDL-c-Low Density Lipoprotein cholesterol

NA-Not Applicable

NCEP-National Cholesterol Education Program

NRTI-Nucleoside Reverse Transcriptase Inhibitor

NNRTI-Non Nucleoside Reverse Transcriptase Inhibitor

NVP-Nevirapine

PI- Proreus Inhibitor

SSA-Sub Saharan Africa

TC-Total Cholesterol

3TC-Lamivudine

TDF-Tenofovir disoproxil fumarate

TG-Triglyceride

WHO-World Health Organization

ZDV-Zidovudine

OPERATIONAL DEFINITIONS

Body Mass Index (BMI): An index that expresses adult weight in relation to height.It is calculated as weight in kilograms to devided by height in meters squared. BMI of less than 25 is considered as normal, 25-30 over weight & 30-35 Obese for adults.

Cardiovascular disease: Disorders of circulatory system include any injury or disease that damage the heart ,blood and blood vessels

Duration of HAART: The time between starting of the drug to assessment of lipid profile for this study.

Dyslipidaemia: Abnormal lipid profile consists of the following abnormalities either singly or in combination. These include total cholesterol ≥ 200mg/dl triglyceride (TG) levels ≥ 150 mg/dl, high density lipoprotein cholesterol (HDL-C) < 40mg/dl, low density lipoprotein cholesterol (LDL-C) ≥ 130mg/dl.

First Line Regimen: HAART drugs given for HIV positive persons throughout their life when their CD4 count bellow 350 cells/mm^3 to enhance the immune status of the person.

Highly Active Antiretroviral therapy (HAART): Drug regimens either 1^{st} or 2^{nd} line given for HIV positive persons to reduce the mortality and morbidity due to HIV and enhance the immune status of those patients.

Lipodystrophy: Fat metabolism disorder:A disoreder involving the breakdown of fats that causes weight loss and excessively high blood fat levels sometimes diposits of displaced fat around the buttucks and thighs.

Second Line Regimen: HAART drugs given for HIV positive persons when first line regimens fail(cannot increase the CD4 count of the HIV positive individual)

Type of HAART: The alternative drugs of nucleoside and non nucleoside or protease inhibitors in combination given for HIV positive persons for elongation of their life.

CHAPTER ONE: INTRODUCTION

1.1. Background

Acquired Immune Deficiency Syndrome (AIDS) has become the focus of much global concern and that is reaching epidemic proportions in some parts of the world (1). In 2011, an estimated 34 million people were living with human immunodeficiency virus /acquired immunodeficiency syndrome (HIV/AIDS) worldwide; of them 22.9 million were living in Sub-Saharan Africa. About 1.2 million people were estimated to be living with HIV in Ethiopia (2). Different HAART regimens currently being in used and in development have been found in different countries like Abacavir, stavudine, Nevirapine, Atazanavir for increasing life expectancy and immune status of HIV positive persons (3).

The introduction of these HAART has led to a marked reduction in AIDS-related morbidity and mortality (4). These HAART regimens typically include a combination of at least three drugs, such as different association of protease inhibitors, non-nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors (5).

In sub-Saharan Africa (SSA), the epicenter of HIV pandemic, the widely used first-line antiretroviral regimen, inspired by the WHO, combines two nucleoside reverse-transcriptase inhibitors (NRTI) with a NNRTI (5). PI are the compulsory components of the second-line treatment subsequent to the failure of the first-line one in which a change from TDF/3TC/EFV preferred first line combination to TDF/3TC/LPV/r second line combination (6). This HAART however, has been reported to be associated with a number of side effects in HIV/AIDS subjects among which dyslipidaemia and lipodystrophy are common metabolic disorders with increased risk of cardiovascular disease (7) and diabetes in the HIV-positive persons (8-10) and have become an important cause of morbidity and mortality (11).

Antiretroviral treatments are associated with abnormal changes in the lipid profile in people with HIV infection (12-14). Among the effects of HAART on lipid profile include elevated level of total cholesterol (TC), LDL-cholesterol (LDL-c), triglyceride (TG), and decreased HDL-cholesterol (HDL-c), include with severe triglyceridemia in some HIV positive

persons (15). Some antiretroviral drugs, such as stavudine (d4T) (16), and protease inhibitors (PIs) (17), increase the blood levels of TC, LDL-c, and TGs with variable effects on levels of HDL-c. Nevirapine (NVP) use is associated with increased in LDL-c (18), whereas increases in TC and TG are observed with use of efavirenz (EFV), particularly with longer duration of therapy.

The exact mechanism for the abnormality of lipid is still not clear and the cause could be multi factorial (19). The individual contributions of HIV infection, specific antiretroviral agents, host genetics (20) and changes in body composition, all should be considered (21). A study has shown that HIV replication alone without any influence of antiretroviral drugs enhances production of free fatty acids, lipoproteins and many key proteins associated in lipid synthesis, transport and metabolism (22).

The differential contribution of highly active antiretroviral agents to lipid abnormalities suggests that hypothetically switching patients from first-line to second-line treatment increase dyslipidemia. These high lipids, increase the risk of blood clots, heart disease and heart attack, stroke, and pancreatitis (22) which have an impact on mortality rates (23).

The aim of the study is to assess the prevalence of dyslipidemia among persons receiving WHO recommended HAART and HAART naive HIV positive.

1.2. Statement of the problem

Since the introduction of HAART, morbidity and mortality has decreased greatly in HIV-positive persons."Even though there is no research conducted on rationale of dyslipidemia on HAART by WHO in worldwide, sub Saharan Africa and Ethiopia, there are different researches conducted in different areas." In patients who receive a PI-containing antiretroviral regimen, the prevalence of hyperlipidaemia ranges from 28% to 80% (24), and it includes hypertriglyceridaemia in the majority of cases (40–80%), hypercholesterolemia (10–50%) (25). In a cohort of 212 HIV-positive patients who started a new PI-based antiretroviral regimen, the incidence of hypertriglyceridaemia and hypercholesterolemia was 38.2% and 25%, respectively, after a 12month follow-up (26). Raised cholesterol was seen in 23% receiving NNRTI, Raised TGs were seen in 32% of NNRTI use compared to 15% of NRTI use only and low HDL-C was 25% in NRTI use (27, 28).

The use of HAART is now associated with a series of serious side effects such as dyslipidemia and long-term complications that may have an impact on mortality rates. More deaths occurring from liver disease, kidney disease, and cardiovascular complications are being observed in those individuals with factors related to the virus, the host, and antiretroviral treatment factors (23).

Although dyslipidaemia in HIV-positive persons with HAART is common, not all patients with dyslipidemia require lipid lowering therapy. The goal of therapy, as in the HIV negative population, is to reduce an individual's cardiovascular risk. Therefore treatment of HIV-associated dyslipidemia should be an integral part of a general attempt to improve cardiovascular health, with advice on diet and exercise, smoking cessation, management of hypertension and diabetes where present, and the use of anti-platelet agents are crucial interventions in managing dyslipidemia (29).

Despite these great upcoming challenges there is limited laboratory monitoring to access all the HAART individuals, even WHO ART guidelines do not include a recommendation that lipid monitoring should be conducted in patients receiving HAART (30).The prevalence of dyslipidemia on HAART individuals in resource-limited settings also has not been well characterized (31).

So, the main rationale behind this study is that to determine whether individuals on HAART have high value of dyslipidemia compared to HAART naïve and to assess whether persons on HAART and HAART naive lipid profile assessment is preferential one.

The finding will be useful in helping health care providers to improve the safety of HAART regimens, programmers, policy makers and persons who want to study and introduce measures that will improve the suitability of HAART for users.

1.3. Significance of the study

In resource-poor settings like Ethiopia, little is known about the effect of treatment on patient survival, quality of life and safety of drug regimens. Treatment guidelines are from the developed world (32). This highlights the need for generating regionally suitable data.

Lipid profile assessment like toxicity test is one component which may contribute in its part for assessing the side effect of those drug regimens on users; identifying the magnitude and factors that contribute to the abnormal lipid parameters might have beneficiary effect for those users.

As a result the finding will be useful in helping health care providers in selecting the safety HAART regimens, programmers, policy makers and persons who want to study and introduce measures that will improve the suitability of HAART for users.

The study showed the prevalence of dyslipidemia for HIV positive individuals in antiretroviral treatment initiated and naïve individuals and it also contributed in recommending for the assessment lipid profile of HAART and HAART naïve persons.

There was no study conducted at this site, and this study will provide information or serve as base line to similar studies that are going to be conducted in the future.

CHAPTER TWO: LITERATURE REVIEW

In the early 1990s a number of investigators described the lipid abnormalities associated with HIV infection. A consistent finding from these studies was that patients with advanced HIV infection or AIDS on HAART had high levels of circulating lipids and low levels of HDL cholesterol using than data from HIV treatment naive patients (15). Antiretroviral medications more likely play a causative or permissive role in the pathogenesis of hyperlipidemia in HIV-infected patients. Both the NRTI and the PI component of HAART contribute to the lipid abnormalities and body fat distribution. The NNRTI component of HAART may also contribute to the dyslipidaemia. Lipid disorders are more common with antiretrovirals especially with PI use. Treatment with ritonavir for very short periods causes hypertriglyceridaemia. And also the prevalence of dyslipidemia amongst HIV patients can vary from 11- 80% (33).

A Prospective study was conducted at Australia at St.Vincents Hospital to the effect of NVP & EFV. As the report show the increase of HDL-c was 8.9% (95%CI) larger in the NVP treatment group (42.5%) than in the EFV treatment group (33.7%). In contrast, the increase in TC was smaller in the NVP group (26.9%) than in the EFV group (31.1%). These changes resulted in a decrease of the TC: HDL-c ratio in the NVP group (−4.1%) compared to an increase in the EFV group (34).

Another prospective study which was conducted at India for the effect of NNRIs taking Once-daily. According to the report, the TC, LDL-c, and HDL-c levels were significantly higher at 12 months than at baseline. As the report forwarded, the proportion of patients with TC levels greater than 200mg/dL had increased from 1% to 26% after 12 months of treatment. However, the proportion of patients with HDL-c levels greater than 40 mg/ dL decreased from 91% to 23%. The proportion of patients with TG levels greater than 150 mg/dL did not change significantly (31% vs 32%) (35).

Adescriptive, transversal study was conducted at seven outpatient facilities in the city of Sao Paulo, Brazil 319 persons. Of the 319 evaluated AIDS patients, 243 were receiving HAART (group 1), and 76 were HAART-naïve (group 2). The mean age was 39.5 years and 60.9% of the patients were male. values of total cholesterol (205 vs. 180 mg/dL [$p < 0.001$], HDL-c

(43 vs. 51 mg/dL [p < 0.001]), triglycerides (219 vs. 164 mg/dL [p = 0.004]) and glucose (101 vs. 93 mg/dL [p < 0.001) respectively. No significant difference was found between the two groups regarding LDL-c (p = 0.073) (36).

A cross-sectional study was conducted at Cameron, Yaounde Jamot Hospital. A total of 204 patients were included & according to the result the prevalence of lipid abnormalities (NNRTI-based vs PI-based regimens) was 38% versus 44% for TC≥200 mg/dl (p=0.39). Among patients on NNRTI, median (IQR) levels (mg/dl) of lipid variables, d4T-treated versus ZDV-treated patients were 180.3 (149.2–227.1) and 227.3 (187.7–270.7) for total cholesterol, 133.1 (96.4–167.7) and 136.0 (136.0–101.5–189.3) for TG, 46.6 (27–67.2) and 45.2 (26.3–59.8) for HDL-c, 116.8 (87.7–163.7) and 134.9 (90.6–172.7) for LDL-c and 4.1 (2.5–5.9) and 4.6(3.3–6.9) for total cholesterol/HDL-c ratio (37).

Another cross-sectional study on 276 patients at Cameron, 138 participants on HAART and 138 HAART-naïve participants was conducted. 68 (49.3%) patients in the HAART-naïve group had active tuberculosis and 55 (39.6%) in the HAART group had had tuberculosis prior to been started on HAART. Meanwhile, no patient in the HAART group had active tuberculosis at inclusion criteria. The prevalence of TC ≥ 200 mg/dl was 37.7% among HAART group and 24.6% in the HAART-naïve group. The equivalents for LDL-c ≥ 130 mg/dl were 46.4% and 21%, and those for TC/HDL-c ratio ≥ 5 were 35.5% among patients on HAART and 18.6% among HAART naïve patients. The prevalence of TC≥200 was 42.2%, 35.5% for ZDV& d4T respectively and 37% & 38% for EFV&NVP respectively; the prevalence of LDL-c ≥130 was 57.8% & 41.9% for ZDV& d4T respectively and 37% & 51.1% EFV&NVP respectively while TG≥150 was 48.9% & 40.9 % for ZDV& d4T respectively 43.5% & 43.5% for EFV&NVP respectively (38).

A cross sectional study was conducted in Nigeria on 50 first line HAART & HAART naive persons and on 25 sero negative individuals .The results of this study showed a significant increase in the serum TC ($p<0.05$) of the males and females HIV subjects on HAART compared to the HIV subject HAART naïve and the seronegative control. TC & TG levels were significantly higher in the males HIV-positive persons compared to the females. There was no significant difference ($p<0.05$) in the means serum HDL-c levels of the HIV

infected subjects on HAART compared to those HAART naïve and the sero negative. Compared to the normal HIV sero negative female subjects and HIV infected female subject of HAART naive, HIV-infected female subjects on HAART had significantly higher LDL-c levels (39).

A cross-sectional study was recruited at ART clinic of Hawassa-Ethiopia on 226 individuals. The prevalence of TC ≥ 200 mg/dl (43.4%), LDL-c ≥130 mg/dl (33.6%) and TG ≥150mg/dl (55.8%) among HAART persons. The prevalence of TC≥200 was 43.1%,43.6% for ZDV& d4T respectively and 39.1% & 46.3% for EFV&NVP respectively; the prevalence of LDL-c ≥130 was 34.5% & 32.7% for ZDV& d4T respectively and 30.4% & 35.8 % EFV&NVP respectively while TG≥150 was 55.2% & 56.2 % for ZDV& d4T respectively 54.3% & 56.7% for EFV&NVP respectively (40).

Another cross-sectional study using sequential sampling technique was done for the effect of dyslipidemia and metabolic syndrome at Jimma-Ethiopia on 313 HAART subjects for the assessment of lipodystrophy and found low HDL cholesterol in male 40(36.7%), low HDL cholesterol in female 109(53.4%). The Prevalence of dyslipidemia from the study population of any type with one type or more was 48.2%. The most common types of dyslipidemia was low HDL-c level (32.6%), followed by high ratio of total cholesterol to high density lipoprotein cholesterol (≥4.5) in (25.6%), elevated triglycerides cholesterol level in (18.2%), high LDL-c level in (6.9%), high total cholesterol level in (6.7%) (41).

Dyslipidemia stands out as one of the most prevalent metabolic changes in patients with HIV, what makes it essential to feasibility of research in therapeutic care to clarifying of the clinical management. It is noted that nutritional guideline and/or hypolipidemic use, when have there been acceptance to treatment, takes place improvements of the lipid profile, can also there be normalization of those levels, in particular of the triglyceride levels. However, the adherence neither always takes place, what difficult the management of those patients.

CHAPTER THREE: OBJECTIVES

3.1. General Objective

To assess the prevalence and predictors of dyslipidemia for HIV positive persons on HAART and HAART naïve who attend the ART clinic at Armed Force Referral Hospital, Addis Ababa-Ethiopia, from September 20 to October 23,2013.

3.2. Specific objectives

- To evaluate dyslipidemia among HIV-positive persons on HAART individuals from September 20 to October 23,2013.
- To evaluate dyslipidemia among HIV positive persons of HAART naïve from September 20 to October 23, 2013.
- To assess factors affecting dyslipidemia for HIV positive persons on HAART and HAART naïve individuals from September 20 to October 23, 2013.

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Our null hypothesis is there will be no difference in lipid profile between HIV positive persons on HAART and HAART naïve and the alternative hypothesis is there will be difference.

CHAPTER FOUR: MATERIALS & METHODS

4.1. Study area and period

The study was conducted in Addis Ababa defense referral hospital. Addis Ababa is the capital city of Ethiopia, Small Mountains and a rugged terrain make up the Addis Ababa geography. The geographical coordinates of Addis Ababa are 9.03° North 38.74° East latitude and longitude, Located at the foothills of the Entoto Mountains and an elevation of around 2,400 meters above sea level. The city is spread over an area of about 222 square kilometers. And this referral hospital delivering health services to the Defense forces, civilians in the Defense Ministry and their dependants, as well as public patients referred by other specialty hospitals. The total number of subjects who ever enrolled for HIV care are 4892, of them 3750 are adults of them 1627 are on HAART and 1423 are HAART naïve and active during the study period. It was conducted from September 20 to October 23, 2013.

4.2. Study design

Facility based comparative cross-sectional study was used.

4.3. Source population

All adult HIV positive persons who were on HAART and those HAART naive during the study period in army hospital ART clinic.

4.4. Study population

All clients sampled from the source population who fulfill eligibility criteria were included in the study.

4.5. Inclusion criteria

The following criteria were used as inclusion criteria for those who included into the study:

- Age ≥18 years

4.6. Exclusion criteria

Exclusion criteria were collected by using Chart review checklist from their log book because participants having any of these criteria are recorded and attached with ART log book. These were used as exclusion criteria in our study:

- Known diabetes mellitus patients:- LDL & Triglycerides level is increase due to (1) an increase in release of free fatty acid from the adipose tissue, (2) an increase in fatty acid synthesis in the liver, and (4) an increase in hepatic very low density lipoprotein production
- Renal failures patient: - LDL and Triglycerides are elevated due to a combination of increased hepatic production and decreased clearance of very low density lipoprotein, with increased LDL production.
- Patients taking anti TB drugs :- altering lipid metabolism
- Corticosteroids:-Concomitant uses with corticosteroids:glucocorticoid use is associated with increased synthesis hypertriglyceridemia
- Participants who change their regimen in less than a year of the study period.
- Those who took HAART for at less than a year before the study period & those who have not good adherence. One year was taken as a criterion because the Changes in lipid parameters significantly occur after approximately 12 to 24 months of combination antiretroviral therapy (42).This exclusion criteria was used only for who were on HAART.

4.7. Sample size and Sampling technique

4.7.1.Sample size

Assuming WHO disease stage as main predictor of mortality rates at the end of the study,
P1=proportion of HIV positive persons on HAART with LDL-c ≥130, taken as 40.8% (38).
P2=proportion of HIV positive persons naive for HAART with LDL-c ≥130 taken as 21% (43).
Level of significance (α/2) = 5%
Power (1- β) = 90%
r = (Ratio of exposed: non exposed)=1:1

$$\mathbf{n = [Z\,\alpha/2\,(\sqrt{2pq})/(p1 - p2)\; + Z\beta\sqrt{(P1q1 + \; p2q2)/(p1 - p2)}]^2}$$

Where:-

n: Number of exposed /unexposed persons

Z α/2: z-score for two tailed test based on α level p= (p+p2)/2

Zβ: z-score based on β level q= (q1+q2)/2

P1: Proportion of exposed with disease q1:1-p1

P2: Proportion of unexposed with disease q2: 1-p2

Based on the above assumptions the calculated sample size is 112, since it is equal proportion formula N=2n=2x112=224.

By assuming estimated non respondent rate = 10%

$224 \times 10/100 = 22$

22+224=246,

Since 246/3050 >0.05, using the adjusted sampling formula the final sample size is 228 i.e.114 for HAART and 114 for HAART naïve HIV positive persons.

4.7.2. Sampling technique

Stratified sampling was used as those who were on HAART and HAART naïve and consecutive sampling technique was used from each stratum until the required sample size was achieved.

4.8. Data collection procedure

The questionnaire was originally prepared in English and then translated to Amharic. Data collectors & supervisor were selected from the Hospital (one BSc-nurse for socio-demographic collection, one phlebotomist, two laboratory technologists for laboratory analysis were assigned throughout the data collection period). The questionnaire was pre

tested from 5% of the sample size by trained data collectors prior to the original data collection. For each participant, data was collected on the socio-demographic background by using structured questionnaire and checklists were used for chart review and observation. Chart review checklist was used to collect data for past medical history and exclusion criteria which were used in the study. Observational checklist was used for recording laboratory measure about lipid profile and CD4 count.

Five milliliters of blood was collected (after an overnight fast) from the antecubital fossa. 2.5 milliliters of the blood was discharged into EDTA tube and the remaining 2.5 milliliters was discharged into a chemically clean plastic tube, allowed to clot, centrifuged for five minutes at 3000 rpm. The serum was separated and the serum obtained was then used for lipids determination. The TC/HDL-c ratio was also calculated. Lymphocytes count for all participants used flow cytometry methods implemented with BD FACSCount (BD USA).

Lipid profile was assessed through enzymatic Methods using Clinical chemistry Analyzer (HumStar80, USA) for all persons and includes total cholesterol (TC), HDL-cholesterol (HDL-c) and triglycerides (TG) but Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald method (LDL = CHOL - ({TG/5} + HDL), except in patients with TG levels higher than 400 mg/dL. The left over specimen was burned by using incineration.

In accordance with the US National Cholesterol Education Program, Adult Treatment Panel III (NCEP-ATP III) guidelines, abnormal lipid profile was be defined as TC $\geq$ 200 mg/dl, HDL-c < 40 mg/dl, LDL-c $\geq$ 130 mg/dl, TG $\geq$ 150mg/dl, TC/HDL-c ratio $\geq$ 5 (44). For the purposes of the study, an elevation of any one of the lipid parameters to a level above these limits were considered as dyslipidemia.

4.9. Method of enrollment

The selected study participants were requested to participate in the survey up on coming to the ART clinic. They were first informed about the purpose of the study and then screened for eligibility, if they are volunteer to participate. Once eligibility is established, informed consent was obtained from each participant.

4.10. Data Analysis Procedure

Data cleaning was made manually by removing missing/conflicting ideas and responses to questions about relevant information. All responses to the survey questions were coded against the original English version. Data was entered in to SPSS version 16.0 for analysis. Recoding and re-categorizing was also made for relevant variables. Descriptive statistics using frequency distribution was performed for socio-demographic, clinical, and laboratory values. The association between the independent and dependent variables were assessed using binary logistic regression model. Multivariate analysis using logistic regression was performed to control effect of confounding variables. Differences in means and proportions for participants' characteristics were assessed using Student t-test.

The magnitude of the association between the different variables in relation to lipid profile status were measured through Adjusted odds ratio(AOR) and their 95% confidence interval (CI). A probability threshold of $P < 0.05$ was set as the threshold of statistical significance.

4.11. Data quality control

Different persons were participated for retranslation of the questionnaire for checking consistency & the Amharic version was used for the actual interview. Two days training regarding the objective of the conduct of interview have been provided for data collectors and supervisor. Study participants were informed clearly about the objective of the study. Standard operational procedure (SOPs) was followed for CD4 and clinical chemistry tests from pre analytic to post analytic stages. Chemistry analyzer instruments tests were checked for validity using normal (Humatrol N) and abnormal (Humatrol P) controls and FACS scan also by low, medium & high controls according to the manufacturer's manual. Data quality was assured by prior training of Data collectors and daily checking of results.

4.12. Study variables

4.12.1. Dependent variables

Presence of dyslipidemia

4.12.2. Independent variables

Age, sex, CD4 count, type of HAART regimen, duration on HAART regimen, body mass index, blood pressure.

4.13. Materials and Equipments

For the study the following materials were used

- ✓ Laboratory equipment; (Humastar 80, FACScount, Micropipettes, Vacutainer tube, Centrifuge, syringe & needle, cotton)
- ✓ Reagents (HDL reagents ,Triglycerides liquid reagents, Cholesterol reagents, ,BD FACSCount™ CD4 reagents)
- ✓ Stationary materials

4.14. Ethical consideration

The research protocol together with the consent form was submitted to Jimma University College of public health sciences ethical review Board to get approval. Letter of permission was obtained from Jimma University, Medical Laboratory and Pathology department to Defense Hospital. Prior to interview and discussions; study participants were requested their consent in written form. Full informed consent was obtained from all participants in each data collection after explaining about the purpose, confidentiality, protection and anonymity of data. The consent form was written in Amharic and read to those who are illiterate or passed to them who need it.

In this study, blood collection for lipid profile assessment and CD4 count was used and the collected blood specimen only be used for the study but not for other purpose. The participants are free to with draw from the study at any time without losing any of the benefits they are supposed to obtain from the hospitals under study. The abnormal results were communicated with their physician for better management of the patients.

4.15. Limitations

The cross-sectional nature of the study so that causal relationship is not possible.

4.16. Dissemination plan:

The result of this study will be presented and after approval disseminated in hard and soft copy to Jimma University, Efforts will be made to publish the paper on journal and reserve in the library.

CHAPTER FIVE: RESULTS

5.1. General characteristics of study participants

This cross-sectional study was conducted between September 20 and October 23, 2013 at Addis Ababa Defense Hospital. In this study the response rate was 100%. Total number of participants enrolled in the study were 228, with 103 (45%) males and 125 (55%) females. Two groups were included: the first group 114 HIV-positive persons who were on HAART and the second group 114 were HAART naive. First-line HAART regimens were a combinations of 2NRTI and 1NNRTI and all regimens included 3TC. The number of patients on ZDV/3TC/NVP and ZDV/3TC/EFV regimens were 36 (31.6%) and 30 (26.3%) respectively, while those on TDF/3TC/NVP and TDF/3TC/EFV regimens were 26(22.8%) and 17 (14.9%) respectively and the rest 5 were on secondline (Table 1).

Table 1:general Characteristics of HAART related variables in Defense Hospital Addis Ababa-Ethiopia starting from September 20 to October 23, 2013.

Variable		On HAART(n=114)	HAART naïve(n=114)
Gender: n(%)	Male	53(46.5)	50(43.8)
	Female	61(53.5)	64(56.2)
Age, (years): n (%):	18–30 years	24(21)	32 (28)
	31–40 years	67(58.7)	62(54.5)
	41–50 years	15 (13.3)	15(13.2)
	>50 years	8(7)	5(4.3)
BMI (Kg/m2): n (%)	<18	5(4.4)	9(7.9)
	18–25	78(68.4)	87(76.3)
	>25	31(27.2)	18(15.8)
Blood pressure :n(%)	Bp<140 &/90	61(53.5)	68(59.6)
	BP ≥ 140 & / 90	53(46.5)	46(40.4)
CD4+ count (cells/mm3): n(%)			
	< 350	19(16.7)	49(43)
	350-550	52(45.6)	51(44.7)
	>550	43(37.7)	14(12.3)
Type of HAART: n(%)			
Firstline:	ZDV,3TC,EFV	30 (26.3)	_____
	ZDV,3TC,NVP	36 (31.6)	_____
	TDF,3TC,EFV	17 (14.9)	_____
	TDF,3TC,NVP	26(22.8)	_____
Secondline:	TDF,3TC,LPV/r	5(4.4)	_____
Duration of HAART (in months)			_____
	12–36	51(44.7)	_____
	36-60	24(21)	_____
	60-84	14(12.4)	_____
	84-108	13(11.4)	
	>108	12(10.5)	_____

5.2: Dyslipidemia and characteristics of lipid profiles

The Mean value of TC, LDL-c, TG were significantly higher in persons on HAART than in HAART-naïve where as HDL-c was significantly higher HAART-naïve than on HAART persons, ($p<0.05$) (Table 3). Among the participants involved in the study 84 (73.7%) on HAART and 61 (53.5%) of HAART naive persons had at least one lipid abnormality. The prevalence of dyslipidemia on HAART for TC, LDL-c & TG was greater than HAART naïve which was significantly different (Table 3).

Table 2: Comparison of the mean of catagorical variables by HAART status at Defense Hospital,Addis Ababa-Ethiopia starting from September 20 to October 23, 2013.

Variable	Mean(SD)	t(95% CI)]	P-value
Age : HAART naive	34.4(6.5)		
On HAART	36.3(7.8)	1.98(0.01, 3.74)	0.32
TC : HAART naive	186.7(24)		
On HAART	202.4(24)	4.9(9.4, 21.9)	<0.001
HDL-c :HAART naive	43.8(8)		
On HAART	40.5(6)	-3.5(-5.2, -1.5)	<0.001
LDL-c :HAART naive	111.4(24.7)		
On HAART	126(22.8)	4.6(8.8, 20)	<0.001
TG : HAART naive	155(23)		
On HAART	176(36.8)	5(12, 28)	<0.001
CD4 :HAART naive	339(76)		
On HAART	500(157)	9.89(128, 193)	<0.001
BMI :HAART naive	22.1(2.6)		
On HAART	23.3(2.7)	3.4(0.5, 1.9)	0.86
TC/HDL ratio: HAART naive	4.4(1.2)		
On HAART	5.2(1.3)	4.5(0.4, 1.1)	0.53

Table 3: Serum lipid profiles of study population by HAART status in Defense Hospital Addis Ababa-Ethiopia starting from September 20 to October 23, 2013.

Parameter	On HAART(n=114)	HAART naive(n=114)	P-value
Total Dyslipidemia: absent	30(26.3%)	53(46.5%)	
present	84(73.7%)	61(53.5%)	0.005
Total cholesterol: < 200mg/dl	57(50%)	80(70%)	
≥ 200mg/dl	57(50%)	34(30%)	0.002
HDL-cholesterol: < 40mg/dl	50(43.8%)	41(36%)	0.18
≥ 40mg/dl	64(56.2%)	73(64%)	
LDL-cholesterol: < 130mg/dl	59(51.7%)	82(71.9%)	
≥ 130mg/dl	55(48.3%)	32(28.1%)	0.001
Triglycerides < 150mg/dl	46(40.4%)	70(61%)	
≥150mg/dl	68(59.6%)	44(39%)	0.001
TC/HDL-c ratio: < 5	57(50%)	77(67.5%)	
≥ 5	57(50%)	37(32.5%	0.003

5.3. Dyslipidemia and HAART

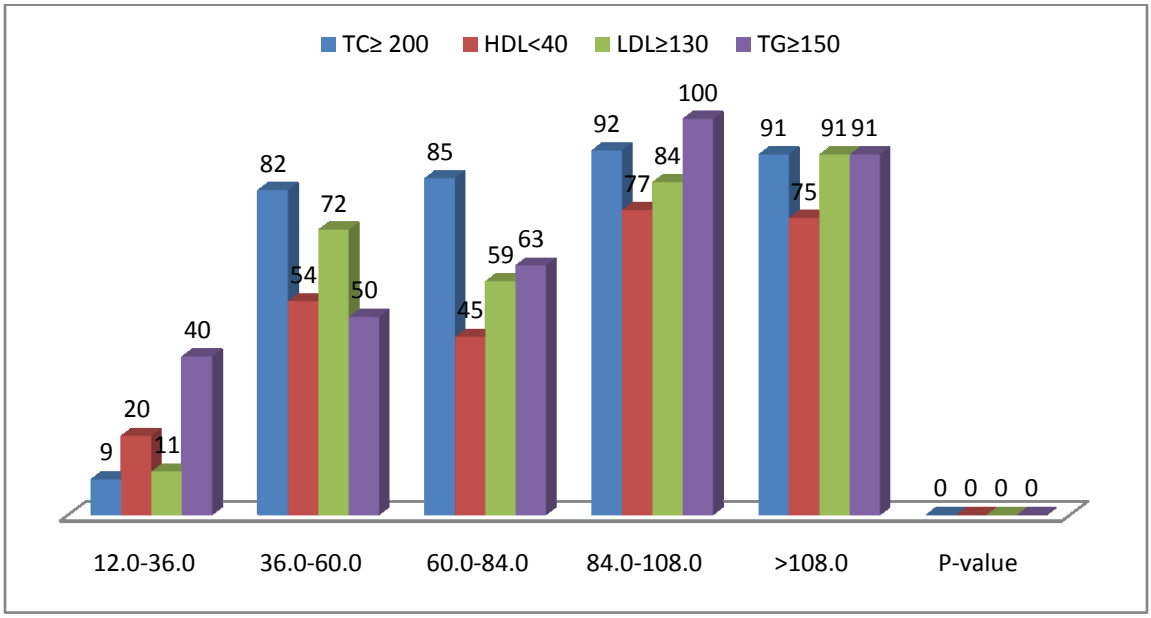

Fig 1. Dyslipidemia among persons on HAART by duration of therapy in Defence Hospital,Addis Ababa-Ethiopia from(n=53,22,14,13,12 for 12-36,36-60,60-84,84-108 & >108)periods of therapy respectvely.(Y-axis dyslipidemia in percent , X-axis duration of therapy in months)

No significant difference observed in lipid profile derangements between patients receiving ZDV when compared to those on TDF; and patients treated with EFV when compared to those treated with NVP. In addition, no significant difference observed in lipid derangements between persons on HAART males and females (Table 4).

Table 4: Prevalence of dyslipidemia (in mg/dl) among persons by sex and HAART among HAART persons in Defense Hospital, Addis Ababa-Ethiopia starting from September 20 to October 23, 2013.

Parameter	**TC ≥ 200**	**HDL-c < 40**	**LDL-c ≥ 130**	**TG ≥ 150**	**TC/HDL-c ratio ≥5**
Sex :Male(n=53)	26 (49%)	28(51%)	27(47%)	34(62%)	30(56%)
Female(n=61)	31(51%)	22(38%)	28(49%)	34(57%)	29(41%)
p-value	0.84	0.25	0.87	0.34	0.78
Firstline HAART					
ZDV-based (n=66)	29(43.9 %)	31(47%)	31(47%)	39(59%)	34(51.5%)
TDF-based (n=43)	24(55%)	16(37.2%)	21(48.8%)	26(60.5%)	20(46.5%)
p-value	0.48	0.60	0.98	0.97	0.87
NVP-based (n=62)	33(53.2%)	29(46.8%)	34(54.8%)	36(58%)	33(53.2%)
EFV-based (n=47)	20(42.5%)	18(38.3%)	18(38.3%)	29(61.7%)	21(44.7%)
p-value	0.54	0.67	0.23	0.92	0.46

5.4: Dyslipidemia and risk factors

Binary logistic regression analysis was applied between each independent variable for each dyslipidemia and Multiple logistic analysis which was adjusted for variables having $p< 0.025$ in binary logistic regression analysis for potential confounding factors. Age, BMI, CD4 count, type of HAART, blood pressure, duration of therapy and being on HAART were analyzed through this method. Duration of therapy was the risk factor for raised TC, LDL-c & TG which was significantly and positively associated. Those who were exposed for HAART greater than three years were 0.5 times more likely having TC ≥ 200mg/dl than who were exposed for less than or equal to three years [AOR(95% CI)=0.54(0.02-0.36)]. Regarding LDL-c those who were exposed for HAART for greater than three years were 0.03 times more likely having LDL-c ≥ 130mg/dl than who were exposed for less than or equal to three years [AOR(95% CI)=0.03(0.001-0.70)]. For TG those who were exposed for HAART for greater than three years were 0.04 times more likely having TG ≥ 150mg/dl than who were exposed for less than or equal to three years [AOR(95% CI)=0.04(0.003-0.84)]. On the other hand there was positive association between TC and blood pressure in which [AOR(95% CI)=0.13(0.02-0.68).

Table 6: Associations of variables with dyslipidemia among persons on HAART7 HAART naive in Defense Hospital Addis Ababa-Ethiopia starting from September 20 to October 23, 2013.

Explanatory variable		**Outcome variables (in mg/dl & 95%CI)**			
		TC ≥ 200	**HDL< 40**	**LDL ≥ 130**	**TG ≥ 150**
Male	COR	1.00(0.58-1.72)	0 .96(0.57-1.63)	1.00(0.65-1.92)	1.23(0.71-2.01)
	P	0.990	0.880	0.720	0 .510
	AOR	NA	NA	NA	NA
Age>40	COR	0.13(0.03-0.60)	4.32(1.12-16.54)	0.12(0.03-0.58)	0.56(0.15-1.73)
	P	**0 .009**	**0.030**	**0.008**	0.280
	AOR	2.83(0.14-56)		3.8(0.21-69)	
	P	0.5	NA	0.36	NA
CD4≥350	COR	0.32(0.14-0.66)	2.20(0.96-4.42)	0.25(0.12-0.54)	0.32(0.13-0.65)
	P	**0 .003**	0.060	**0 .001**	**0.003**
	AOR	4.72(0.37-49.53)		1.5(0.24-9.34)	2(0.40-8.04)
	P	0.24	NA	0.66	0.38
BMI>25	COR	0.08(0.03-0.18)	5.22(2.63-10.72)	0.09(0.04,0.22)	0.24(0.12-0.50)
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	AOR	0.25(0.03-2.05)	1.24(0.36-4.07)	0.20(0.04-1.34)	2.72(0.4-17)
	P	0.213	0.704	0.090	0.270
On HAART	COR	0 .43(0.25-0.74)	1.44(0.85-2.41)	0.43(0.24-0.70)	0.40(0.23-0.67)
	P	**0 .002**	0 .180	**0 .001**	**0.001**
	AOR	1.2(0.75-11.52)		0.47(0.65-2.88)	0.85(0.73-9.46)
	P	0.930	NA	0.360	0.240
Duration of HAART					
	COR	0.09(0.001-0.09)	11.54(2.64-49.61)	0.01(0.001-0.11)	0.06(0.007-0.50)
	P	**<0.001**	**0.001**	**<0.001**	0.009
	AOR	0.54(0.02-0.36)	3.7(0.50-28)	0.03(0.001-0.70)	0.04(0.003-0.84)
	P	**0.030**	0.200	**0.030**	**0.020**
Blood pressure ≥140 &/90					
	COR	0.04(0.02-0.08)	8.12(4.43-14.52)	0.06(0.03-0.11)	0.11(0.06-0.20)
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	AOR	0.13(0.02-0.68)	2.34(0.60-8.32)	0.24(0.06-1.04)	0.46(0.12-1.82)
	P	**0.012**	0.221	0.062	0.243

CHAPTER SIX: DISCUSSIONS

This cross sectional study conducted in a resource-poor setting was to assess the prevalence of lipid abnormalities associated with the use of HAART regimens. The majority of the study participants were females. This difference in part indicated that more females were seeking care and treatment services than does the men. This may be the very reason that females visit health institutions for maternal services like family planning, antenatal care and delivery.

The finding shows that CD4 cell count in the HAART group was significantly different from the HAART naive group, thus showing the effects of HAART in improving the immunological properties of the subjects.The mean value of each lipid profile in our study were significantly higher on HAART persons as compared to HAART naïve persons which was similar with the study conducted in Sao Paulo, Brazil (36).

We found that the proportions of raised TC, LDL-c, TG and TC/HDL-c were significantly higher in HAART group when compared to HAART naive group which indicates the atherogenic(15,23) effect of treatment for the development of cardiovascular diseases. The association between HAART and adverse lipid profile has been largely described for regimens when hypothetically switching patients from first-line to second-line treatment. These high lipids, increase the risk of blood clots, heart disease and heart attack, stroke, and pancreatitis which have an impact on mortality rates (22).

The proportion of patients with TC≥ 200mg/dl among our persons on HAART (50%) was higher than that reported in Cameron (37.7%), Hawassa-Ethiopia(43.4%) & Jimma-Ethiopia(6.4%) (38,40,41), the difference might be due to sampling technique, the cut of value they used. In Jimma the cut of value was used as TC≥240 mg/dl. A prospective study conducted in India for the effect of NNRTIs. As the report forwarded, the proportion of patients with TC≥ 200mg/dL had increased from 1% to 26% after 12 months of treatment (35).

The prevalence of HDL-c<40mg/dl in our HAART group was 43.8%. This is comparable with the prevalence rate reported from Hawasa-Ethiopia which was 43.4% (40). However, it is higher than the prevalence reported from Jimma-Ethiopia (32.6%) (41), it may be due to life style & physical activity.

The prevalence of LDL-c≥ 150mg/dl in our HAART group was 48.3%. It is comparable with the prevalence reported from Cameron which was 46.4% (38). We found that the prevalence of raised TG in HAART group was 59.6%, this is comparable with the report from Hawasa-Ethiopia(55.8%) (40) but different from India, Cameron, Jimma-Ethiopia (35,38,41).The prevalence of among these study areas were 32%, 39%, 18.2% respectively. However, there are suggestions that the magnitude of lipid profile derangements induced by HAART showed variation with duration of treatment, across populations and setting.

The reports from rural Australia, India & Cameron indicated that the proportion of lipid profile derangements were different for each HAART regimens(34,35,38). The two NNRTIs included in the WHO first-line regimens are NVP and EFV. Nevirapine use is associated with increased in LDL-c (18), whereas increases in TG are observed with use of efavirenz, particularly with longer duration of therapy. This is true also in our study in which the prevalence of LDL-c≥150mg/dl was 54.8% & 38.3% (p=0.23) for NVP & EFV respectively and prevalence of TG were 58% & 61.7% NVP & EFV respectively. The report of this 2NNRI study indicated that patients on NVP group had improved TG concentration and had relatively low lipid profile derangements when compared to those on EFV (30).

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1.Conclusion

In general the prevalence of dyslipidemia at Defense Referral Hospital was 73.7% & 53.5% persons on HAART & HAART naïve respectively which indicate a high value of dyslipidemia with a highest proportion of triglyceride particularly with longer duration of HAART. Participants who receive HAART regimens have high value of dyslipidemia in HIV positive persons as compared to HAART naive HIV-positive persons in our setting. The mean value of each lipid profile was significantly higher for persons on HAART as compared to HAART naïve HIV positive persons. Duration of therapy was the risk factor for raised TC, LDL-c & TG which was significantly and positively associated.

7.2.Reccomendation

- The findings of our study indicate that the need to assess lipid profiles of HIV positive persons at baseline before initiation of HAART.
- There should be also lipid profile assessment for HIV positive on HAART persons to monitor any rising trends.
- Health professionals who are working in ART clinic should place their effort in lowering of dyslipidemia for those persons on HAART.
- Additionally, the results also recommend implementation of well controlled longtudinal studies for the evaluation of long-term effects of HAART treatment on lipid profiles and their potential impact on cardiovascular health of people living with HIV.

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ANNEXES

Annex-I: Questionnaire & Informed consent

Unique ART Number_____

INFORMED CONCENT

The following questionnaire consists of different questions related to the study with 3 parts. The Questions will be filled from patient interview, ART register & pre-ART log and laboratory results.

My name is _____, I am working temporarily as a data collector with the department of Medical Laboratory and pathology of Jimma university which is conducted among HIV infected patients. The objective of the study is to assess the accumulation of fat(cholesterol) on both HIV positive HAART and HAART naïve patients and to determine the undesired side effect of the drug so that to show for those individuals to be treated with a better drug. A number of people are needed in this study to conduct the study elsewhere. So I am going to ask you some about your background, HIV/AIDS and your responses are completely confidential. We use ART identification number rather than your name so there is no any condition to expose you for any secondary person. You do not enforce to answer any question that you do not want to answer, however your honest answer to this question will help us to understand factors affecting lipid profiles. We would like to thank you in advance for your help. Are you willing to participate? If yes=(1) continue, if No=(2) stop.

Thank you for your participation!!!

Principal investigator Name _____Signature_____

Checklist

Laboratory _____ ID _____ Assessor _____ Date _____

Exclusion criteria (if there is) will be collected from their log book because participants having any of these criteria are recorded and attached with their log book .

General status of individuals	**y**	**N**	**comment**
Diabetic patient			
Renal failure patient			
Taking anti TB drug			
Change their drug regimen			
Taking Corticosteroids			

QUESTIONAIRE

Name of Hospital_____ Unique ID of patient_____

S. No	Variables	Answer	code
001	Sex	Male	1
		female	2
002	Age	/ ..... / Year	
003	Height	/……/meters	
004	Weight	/……/kg	
005	Blood Pressure	…….mmHg	
006	When do you know your HIV status ?	.....months	
007	ART status	Pre- ART	1
		ART initiated	2

FOR ART PATIENTS FROM THE CARD

008	Duration of ART	_____months	Code
009	Adherence level	Good	1
		Fair	2
010	HAART therapy	1. 1st line Regimen	1
		2. 2nd line Regimen	2
011	Name of Regimen(write the codes)	1. ZDV,3TC,NVP	1
		2. ZDV,3TC,EFV	2
		3. TDF,3TC,NVP	3
		4. TDF,3TC,EFV	4
		5. ZDV,3TC, LP/r	5

Laboratory Observational checklist format (in mg/dl)

Ser. No	Unique ID	TC	HDL-c	LDL-c	TG	CD4	Remark

<u>መጠይቆች ና የመወያያ ነጥቦች በአማርኛ</u>

የኤ. አር. ቲ. መለያ ቁጥር_____

ይህ መጠይቅ ለዚህ ጥናት የሚረዱ የተለያዩ ጥያቄዎችን በ 3 ክፍል ይዟል፣ጥያቄዎቹ ተሳታፊዎችን በመጠየቅ፣ ከኤ.አር.ቲ እና ከቅድመ ኤ.አር.ቲ መዝገብ እና ከላቦራቶሪ ዉጤት ነዉ::

<u>የስምምነት መግለጫ</u>

ጊዜዎን ሰዉተዉ ቃለመጠይቁን ለማካሄድ ስለፈቀዱ በቅድሚያ ማመስገን እፈልጋለሁ፡፡

ስሜ_____ይባላል፡፡በጅማ ዩነቨርሲቲ የህክምና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪና ፓቶሎጅ ትምህርት ክፍል የኤች.አይ.ቪ መድሀኒት የሚያስከትለዉን ያልተፈለገ የስብ ክምችት የሚያደርገዉን ጥናትና ምርምር በጊዜአዊነት መረጃ በመሰብሰብ ላይ ነኝ፡፡ ውድ የጥናቱ ተሳታፊ:-እኛ የምናጠናው የስብ ክምችትን ኤች አይቪ በደማቸው ባለባቸው ፀረ ኤች አይቪ መድሃኒት በሚወስዱና ባልጀመሩ ሰዎች ላይ ነው፣የዚህ ጥናት ዋና አላማም መድሃኒቱ የሚያስከትለዉን ያልተፈለገ የስብ ክምችት በማጥናት ቫይረሱ ያለባቸውን ሰዎች በተሻለ መድሀኒት የሚታከሙበትን መንገድ መጠቆም ነው፡፡ በጥናቱ ውስጥ ከእርስዎ የምናገኛቸው ማንኛውንም መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፣ በጥናቱ ውስጥ የምንጠቀመው የሚስጥር የፀረ ኤች አይቪ መድሃኒት መጠቀሚያ ቁጥርዎን በመሆኑ እርስዎን የሚገልጽ ምንም አይነት መለያ አይኖርም:: እርስዎን ማንነትዎም በጥናቱ ውጤት ላይም ይሁን በማንኛውም ሁኔታ አይገለጽም፡፡የሚሰበሰበዉ መረጃ በዋናዉ አጥኝ በጥንቃቄ የሚቀመጥ ሲሆን ጥናቱ ሲጠናቀቅ ማንም ሰዉ በማያገኘዉ ሁኔታ ይወገዳል፡፡ዉድ የጥናቱ ተሳታፊ መጠይቁ እንደተጠናቀቀ ላቦራቶሪ የደም መቅጃ ክፍል በመሄድ ደም ይሰጣሉ ከደም ምርመራ የሚገኘዉ ዉጤት የእርስዎን ኮሌስትሮል መጠን ማወቅ ያስችላል፡፡መጠይቁ እና ደም መስጠቱ የሚካሄደዉ በፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ከዚህ ጥናት የሚገኘዉ ዉጠጤት ወደፊት ፕሮግራሙን የተሻለ ለማድረግ ይጠቅማል፡፡በአጠቃላይ መጠይቁ ከ5-10 ደቂቃ ይወስዳል፡፡

በትዕግስትና በጥሞና አዳምጠዉ ለመመለስ የሚያደርጉትን ጥረት እያደነቅን በቅድሚያ ከልብ እናመሰግናለን፡፡

በጥናቱ ለመሳተፍ ፈቃደኛ ኖት ? ፈቃደኛ ካልሆነ/ች አመስግነህ አሰናብት/ች፡፡ፈቃደኛ ከሆኑ ቀጥል/ይ፡፡

ፈቃደኝነታቸዉን ያረጋገጠዉ/ችዉ ጠያቂ ስም _____ ፊርማ _____

<u>መጠይቅ</u>

የሆስፒታሉ ስም _____
የመጠይቅ ቁጥር_____ የሚስጥር የፀረ ኤችአይቪ
መድሃኒት መጠቀሚያ ቁጥር _____

ተራ ቁጥር	ጥያቄ	መልስ	ኮድ
001	ፆታ	ወንድ	1
		ሴት	2
002	እድሜ	_____አመት	
003	ቁመት	……ሜትር	
004	ክብደት	……ኪ.ግ	
005	የደም ግፊት መጠን	_____(syastolic)mmHG _____(diastolic)mmHG	
006	ከኤች አይቪ ጋር ስንት አመት ኖሩ	/...../አመት	
007	የፀረ-ኤችአይቪ መድሃኒት ሁኔታ	ቅድመ ፀረ ኤችአይቪ	1
		ፀረ-ኤች.አይ.ቪ.መድሃኒት የጀመረ	2

<u>ኤ.አር.ቲ ለጀመረ ሰው የሚሞላ</u>

ተ.ቁ	ጥያቄ	መልስ	ኮድ
008	ፀረ ኤች. አይ. ቪ መድሃኒት እየወሰዱ የቆዩበት ጊዜ	/..... .. ../ ወራት	
009	የፀረ-ኤችአይቪ መድሃኒቱን በትክክል የመውሰድ ደረጃ	ጥሩ	1
		መካከለኛ	2
010	የሚወስደው የፀረ ኤች. አይ. ቪ መድሃኒት	1. መጀመሪያ ደረጃ	1
		2. ሁለተኛ ደረጃ	2
011	መድሃኒቱ ስም (የመድሃኒቱ ኮድ ይጠቀስ)	_____	

Annex-II: Standard Operating Procedures(SOPs)

1.Standard Operating Procedure for Total cholesterol

PERSONNEL: All appropriately trained Scientific/Technical staff and trainees under supervision.

PRINCIPLE: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol Esterase
Cholesterol Ester + H2O ---------> Cholesterol + RCOOH

Cholesterol Oxidase
Cholesterol + O2 ----------> Cholestene-3-one + H2O2

Peroxidase
2H2O2 + 4-Aminoantipyrine + Phenol ----> Quinoneimine + 4H2O

SAMPLE

A. Sample Type - Serum is recommended although plasma (Lithium heparin, EDTA) is acceptable.

B. Sample Stability - After separation, 7 days at room temperature, 30 days at 4°C. Before separation see data from stability study.

C. Sample Volume - 3µL (+25µL for dead volume).

D. Interferences - There are no reported interferences. Haemolysed (up to 2g/L), icteric and lipaemic samples are not known to interfere.

HEALTH AND SAFETY

Refer to the Departmental Safety Manual Index Code : **SAFETY1.DOC.**

Risk Assessment

ALL REAGENTS SHOULD BE CONSIDERED HARMFUL BY INGESTION

PREPARATION OF STANDARD

Reconstitute one bottle of calibrator with 5mls of de-ionised water using a volumetric pipette. Mix on rotor mixer for 20 minutes.

PREPARATION OF REAGENTS

Reagent supplied in kit, Store at 4C on the reagent shelf in fridge or in a cold room as available. Use reagent as supplied. Stable until date on box.

Each pack contains Working Reagent R1 4 x 22.5ml.It is essential to complete stock control sheets when a new kit is opened.

Reagents Concentration in test

Phosphate buffer (pH 6.5) = 103 mmol/L

4-Aminoantipyrine =0.31 mmol/L

Phenol = 5.2 mmol/L

Cholesterol Esterase > 0.2 kU/L

Cholesterol Oxidase > 0.2 kU/L

Peroxidase > 10.0 kU/L

Preservative

QUALITY CONTROL

FREQUENCY OF CALIBRATION

Done every day and Stable up to 30 days at -20^{o}C

Control preparation:

1. Wait until the sera reach to room temperature
2. Reconstitute the lyophilized sera with 5ml of distle water
3. wait until the solution homogenized(to 30 min)
4. Dispense control sera into neck tubes (sufficient)

Note: check the procedure if there are abnormal control results.

PROCEDURE

Refer to Part 1 of the Standard Operating Procedure.

See Appendix for instrument specific parameters.

CALCULATION AND VERIFICATION OF DATA

The machine calculates the desired calculation

Linearity

The test is linear up to a cholesterol concentration of 750 mg/dl(19.3mmol/l).dilute samples with a higher cholesterol concentration 1+2 with physiological saline and repeat the determination. Multiply the result by 3.

REPORTING OF RESULTS

a.) Report to one decimal place.

b.) Computer :- Results are passed through computer.

Results processing

a) The result is reported in mg/dl

-with normal ,If control with in the limit range

- Not flagged, and result in linearity range

-Not pathologic.

Results processing

If a request is notified to the laboratory as urgent then the results must be telephoned to the appropriate ward. Mark the computer record as telephoned. Refer to the "Protocol for the verbal transmission of results".

ADULT REFERENCE RANGE

Total cholesterol (TC) < 200mg/dl

CLINICAL SIGNIFICANCE

Cholesterol is synthesised and utilised by most tissues of the body and is a component of cell membranes. It is catabolised only by the liver and consequently any excess of cholesterol, or cholesterol derived from cell breakdown, must be transported to the liver. Part of the cholesterol is degraded by the liver to bile acids and bile salts while the remainder is excreted as cholesterol. Many hormones particularly the thyroid hormones affect cholesterol metabolism. Certain types of hyperlipidaemia are associated with increased risk of cardiovascular disease. In affluent societies there is a high incidence of ischaemic heart disease. Primary causes may be familial. Secondary causes are diabetes

mellitus, hypothyroidism, nephrotic syndrome, SLE and paraproteinaemia, and alcohol abuse.

Limitation

If working reagent exposed to direct sun light or In appropriate sample(citrated plasma)

2.Standard Operating Procedure for High Density Lipoprotein(HDL)

PERSONNEL: All appropriately trained Scientific/Technical staff and trainees under supervision.

PRINCIPLE

Anti human-β-lipoprotein antibody in Reagent 1 binds to lipoproteins other than HDL-c (LDL, VLDL and chylomicrons). The antigen-antibody complexes formed block enzyme reactions when reagent 2 is added. HDL-cholesterol is quantified by the presence of an enzyme chromogen system.

$$\text{LDL,VLDL and chylomicrons} \xrightarrow[\text{Antibody}]{\text{Anti human-β-lipoprotein}} \text{Antigen-antibody complexes}$$

$$\text{HDL-cholesterol} + H_2O + O_2 \xrightarrow{\text{Cholesterol esterase}} \text{Cholest-4-en-3-one} + \text{Cholesterol oxidase Fatty acids} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \xrightarrow{\text{Peroxidase}} \text{Blue dye} + 2H_2O + \text{N-ethyl-N-(2-hydroxy-3-sulpho-propyl)- 3.5-dimethoxy-4-fluoroanilide(F-DAOS)}$$

SAMPLE

A. Sample Type - Serum is recommended although plasma (EDTA)
is acceptable.

B. Sample Stability - After separation, 3 days at room temperature, 7 days at 4°C.

C. Sample Volume - 2µL (+25µL for dead volume).

D. Interferences - There are no reported interferences. Haemolysed, icteric and lipaemic samples are not known to interfere.

HEALTH AND SAFETY

Refer to the Departmental Safety Manual Index Code : **SAFETY1.DOC**

Risk Assessment

ALL REAGENTS SHOULD BE CONSIDERED HARMFUL BY INGESTION

PREPARATION OF STANDARD

Reconstitute one bottle of HDL calibrator with 3mls of de-ionised water using a volumetric pipette. Mix on rotor mixer for 20 minutes.

Reagent supplied in kit. Store in the fridge at 4°C on the reagent shelf or in a cold room as available. Use reagent as supplied. Stable until date on box.

Each pack contains:- Working Reagent R1 4 x 27ml

-Working Reagent R2 4 x 9ml

Reagents Concentration in test

Anti human-β-lipoprotein antibody

Cholesterol esterase = 0.8 IU/ml

Cholesterol oxidase= 4.4 IU/ml

Peroxidase = 1.7 IU/ml

Ascorbate oxidase= 2.0 IU/ml

Good's buffer (pH 7.0)= 30 mmol/L

N-ethyl-N-(2-hydroxy-3-sulpho-propyl)- 3.5-dimethoxy-4-fluoroaniline(F-DAOS)= 0.20 mmol/L

4-aminoantipyrine 0.67 mmol/L

Preservative

Detergent

QUALITY CONTROL

Internal QC and External QA must be performed as defined for this chemistry in the Quality Control Policy, **QC1.DOC**.

FREQUENCY OF CALIBRATION

Reagent blanks and a single point calibration weekly or change of reagent lot.

PROCEDURE

Refer to Part 1 of the Standard Operating Procedure. See Appendix for instrument specific parameters.

CALCULATION AND VALIDATION OF DATA

Refer to the QC Policy, Index Code : **QC1.DOC** for current ranges and Westgard rules, as appropriate, to determine acceptability of quality control results.

Linearity

0.05 – 4.65 mmol/L Results >4.65 automatic re-run will dilute sample in accordance with instrument specific parameters (see appendix) or dilute sample 1/5, with deionised water.

REPORTING OF RESULTS

A) The result is reported in mg/dl

-with normal ,If control with in the limit range

- Not flagged, and result in linearity range

-Not pathologic.

B.) Report to one decimal place..

C.) Computer :- Results are passed from LIMS.

Results processing

If a request is notified to the laboratory as urgent then the results must be telephoned to the appropriate ward. Mark the computer record as telephoned. Refer to the "Protocol for the verbal transmission of results".

ADULT REFERENCE RANGE

HDL-c > 40 mg/dl

CLINICAL SIGNIFICANCE

Cholesterol is synthesised and utilised by most tissues of the body and is a component of cell membranes. It is catabolised only by the liver and consequently any excess of cholesterol, or cholesterol derived from cell breakdown, must be transported to the liver. Part of the cholesterol is degraded by the liver to bile acids and bile salts while the remainder is excreted as cholesterol. Many hormones particularly the thyroid hormones affect cholesterol metabolism.

Certain types of hyperlipidaemia are associated with increased risk of cardiovascular disease. In affluent societies there is a high incidence of ischaemic heart disease. Primary causes may be familial. Secondary causes are diabetes mellitus, hypothyroidism, nephrotic syndrome, SLE and paraproteinaemia, and alcohol abuse.

3. Standard Operating Procedure for Triglyceride

PERSONNEL: All appropriately trained Scientific/Technical staff and trainees under supervision.

PRINCIPLE

Triglyceride is determined after a reaction between 4-aminoantipyrine and 4-chlorophenol and hydrogen peroxide takes place, forming quinoneimine, which is a coloured product. The amount of coloured compound formed is proportional to the amount of triglycerides in the specimen.

Trigliceride $\xrightarrow{LPL}$ Glycerol + fatty acid

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

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

$2H_2O_2$ + Aminoantipyrine + 4-chlorophenol $\xrightarrow{POD}$ Quinoneimine +HCl + $4H_2O$

SAMPLE

A. Sample type: serum is Recommended but heparinised or EDTA plasma also used.

B. Sample Stability : freeze specimens at −25°C for up to 4 months or refrigerate at 2–8°C for up to 3 days.

C. Sample Volume: 10 µl of serum

D. Interferences: Avoid haemolysed specimens.

HEALTH AND SAFETY

Refer to the Departmental Safety Manual Index Code : **SAFETY1.DOC**

Risk Assessment

ALL REAGENTS SHOULD BE CONSIDERED HARMFUL BY INGESTION

Reagents Concentration in test

PIPES buffer (pH 7.5)= 50 mmol/L

4-chlorophenol= 5 mmol/L

4-aminoantipyrine =0.25 mmol/L

Magnesium ion= 4.5 mmol/L

ATP =2 mmol/L

Lipases ≥1.3 U/ml

Peroxidase ≥0.5 U/ml

Glycerol kinase ≥0.4 U/ml

Glycerol-3-phpsphate oxidase ≥1.5 U/ml

QUALITY CONTROL

Internal QC and External QA must be performed as defined for this chemistry in the Quality Control Policy, **QC1.DOC**

FREQUENCY OF CALIBRATION

Reagent blanks and a single point calibration weekly or change of reagent lot.

PROCEDURE

Refer to Part 1 of the Standard Operating Procedure. See Appendix for instrument specific parameters.

CALCULATION AND VERIFICATION OF DATA

Refer to the QC Policy, Index Code : **QC1.DOC** for current ranges and Westgard rules, as appropriate, to determine acceptability of quality control results.

Linearity range: upto750 mg/dl

Report the result : The result is reported in mg/dl

-with normal ,If control with in the limit range

- Not flagged, and result in linearity range , Not pathologic.

ADULT REFERENCE RANGE

TG< 150 mg/dl

Increased over 200 mg/dl

LIMITATIONS

A.if the the colour of the reagent changed result will be altered

B. Lipemic specimen usually generates turbidity of the sample reagent mixture, which leads to falsely elevated results.

C. Ascorbate gives falsely low values

References

1.Clinical diagnosis and Management by laboratory method17th edition

2.C.R MAITI concise note of medical laboratory

ASSURANCE AND APPROVAL

Assurance of principal investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research paper and provision of the required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this application.

Name of student: Habtamu wondifraw

Date _____ Signature _____

Approval advisors

1. Name of first advisor: Mehidi Kassim

Date _____ Signature_____

2. Name of second advisor: Dr. Tilahun Yemane

Date _____ Signature _____