ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCINECES

DEPARTMENT OF BIOCHEMISTRY

COMPARATIVE ASSESSMENT OF SERUM LIPID PROFILE, COMPLETE BLOOD COUNT AND VIRAL LOAD LEVELS BETWEEN HIV POSITIVE PATIENTS TAKING DOLUTEGRAVIR AND EFAVIRENZ BASED ANTIRETROVIRAL THERAPY AT WOLKITE HEALTH CENTER, SOUTHERN ETHIOPIA

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APPROVAL SHEET

This is to certify that this master's thesis entitled: Comparative assessment of serum lipid profile, complete blood count and viral load levels between HIV positive patients taking dolutegravir and efavirenz based antiretroviral therapy at wolkite health center, southern Ethiopia, a comparative cross sectional study was conducted by Bedilu Sahilu H/ Mariam, and Submitted to the department of Biochemistry for partial fulfillment of the requirements for the degree of Master of science in Medical Biochemistry complies with the regulation of the university and meet acceptance standards with respect to originality and quality.

Signed by Examination Committee:

Examiner:

Name: _____ Signature _____ Date_____

Advisors:

Name: _____ Signature _____ Date_____

Name: _____ Signature _____ Date_____

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ABBREVIATIONS AND ACRONYMS

3TC: Lamivudine

ART: Antiretroviral therapy

CCR5: Chemokine Receptor 5

CRABP1: cytoplasmic retinoic acid-binding protein type 1

CYP2B6: Cytochrome P450 isoenzyme

DTG: Dolutegravir

EDTA: Ethylene Diamine Tetra acetic Acid

EFZ: Efavirenz

FDC: Fixed Dose Combination

HAART: Highly active antiretroviral therapy

ISI: Integrase strand Inhibitor

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean cell Hemoglobin Concentration

MCV: Mean cell volume

NNRTIs: Non-nucleoside Reverse transcriptase inhibitors

NRTIs: Nucleoside Reverse transcriptase inhibitors

PLHIV: People Living With HIV

TASH: Tikur Anbesa Specialized Hospital

TDF: Tenofovir Disoproxil Fumarate

TLD: Tenofovir Disoproxil Fumarate / Lamivudine/Dolutegravir

TLE: Tenofovir Disoproxil Fumarate / Lamivudine/Efavirenz

VCT: voluntary HIV counseling and testing

ABSTRACT

Background: Highly active antiretroviral therapy can achieve rapid HIV suppression and allows restoration of the immune system. However, their long-term use has been associated with various immunological, biochemical and haematological abnormalities such as dyslipidemia, insulin resistance, lactic acidosis, anemia, leukocytopenia, neutropenia and thrombocytopenia

Objective: The main aim of this study was to compare lipid profile, complete blood count, and viral load levels between HIV positive patients taking dolutegravir and efavirenz based ART at Wolkite Health Center, Southern Ethiopia.

Methods: Institution based comparative cross-sectional study was employed to include 75 efavirenz and 75 dolutegravir based ART treated patients, during their routine appointment using consecutive probabilistic sampling technique from May 2020 to July 2021 at Wolkite Health Center, Southern Ethiopia.

Results: This study revealed patients on efavirenz based showed statistically significant ($p = 0.030$) decrease in HDL-C concentration as compared to dolutegravir based antiretroviral therapy users. The mean values of neutrophil was statistically significantly lower ($p= 0.009$) among dolutegravir based ART users. Moreover, the mean values of platelet count was statistically significantly higher ($p = 0.033$) in the efavirenz than the dolutegravir based antiretroviral groups. Viral load suppression rate among dolutegravir and efavirenz based antiretroviral therapy users were 94.6% and 86.7% respectively.

Conclusion: Compared with efavirenz, dolutegravir based ART showed a better lipid profile and significant improvements in erythroid and lymphoid cell lines with lower prevalence of anaemia. Whereas, efavirenz significantly improved ameliorated platelet indices compared with dolutegravir based ART. Both of treatment regimens showed comparable efficacy in viral load suppression.

Key words: dolutegravir, efavirenz, lipid, complete blood count, viral load

1. INTRODUCTION

1.1. Background

On June 5th, 1981, the United States Center for Disease Control and Prevention report showed the occurrence of five atypical cases of pneumonia called pneumocystis carinii pneumonia in young men (CDC, 1982), which was the first report of Acquired Immune Deficiency Syndrome (AIDS) epidemic (CDC, 1981). Since then, HIV continued to be a common global public health issue killing almost 33 million lives so far (CDC, 2018). Although HIV/AIDS causes substantial morbidity and mortality, highly active antiretroviral therapies (HAART) have changed the natural history of the disease and improve the overall health status of people living with HIV/AIDS (PLHIV) (Gulik et al., 2000).

The history for the advent of antiretroviral drugs took more than three decades (Connor & Sperling, 1994). The first decade after the initial report of HIV was the approval of Zidovudine in March 1987, which was followed in the 1990s by many more options with single and triple drug therapies (CDC, 1982). Then, drug preparations like ritonavir tablets and capsules made simplification of the case management and showed improvement in bioavailability (Vella et al., 2012). Early innovations, such as sequential and alternating drugs like nucleoside reverse transcriptase inhibitors (NRTIs) decreased the possibilities of mother to child HIV transition (WHO, 2012). Later, various fixed dose combination (FDC) antiretroviral drugs were added to the management protocol of HIV/AIDS to be given based on CD4 count (WHO, 2014).

Since 2016, WHO recommends lifelong ART for all PLHIV regardless of their CD4 count and the clinical stage of the disease (WHO, 2016). On June 2016, Ethiopia launched the “Treat All policy” to provide HAART to all PLHIV (CSA, 2018). Currently, WHO recommends the use of two NRTIs plus an integrase inhibitor (INSI) or NNRTIs (USAID, 2019). As per WHO, Dolutegravir and Efavirenz based ART have been used as first-line therapy for the treatment of HIV/AIDS globally (WHO, 2018). In Ethiopia, TLD (TDF + 3TC + DTG) or TLE (TDF + 3TC + EFZ) have been used for first-line therapeutic regimens since 2019 (FMOH, 2018).

Dolutegravir sodium is monocarboxylic acid amide and an organic heterocyclic compound having molecular formula of $C_{20}H_{18}F_2N_3NaO_5$ with an exact mass of 441.36 g/mole. It is light yellow to white coloured powder and moderately soluble in water (dolutegravir biotechnology, 2019). However, efavirenz is a NNRTI having a molecular formula of $C_{14}H_9ClF_3NO_2$ with exact mass of 315.67gram/mole (Podany et al., 2017).

World Health Organization planned to end AIDS epidemic by 2030, prevent nearly 28 million new HIV infections and 21 million AIDS-related deaths (USAID, 2017). To achieve this target, it has developed a global 95-95-95 target Catch-Up-Campaign in 2017 (WHO, 2020). This target aims 95% of all people living with HIV to know their HIV status, 95% of those diagnosed with HIV infection to take sustained combination ART, and 95% of all people on ART to be virally suppressed by 2030 (USAID, 2020). Accordingly, in 2019, an estimated 81% of people living with HIV knew their status, 67% were receiving ART and 59% had achieved suppression of the HIV virus globally (USAID, 2019), contributing to a 37% global decline in HIV-related deaths and an 18% global decline in new HIV infections between 2010 and 2017 (WHO, 2017). As of January 2019, in Ethiopia, 79 % of PLHIV knew their HIV status; 97.1 % of eligible people living with HIV were on ART and 87.6% of ART users attained viral suppression (CSA, 2019).

Despite high prevalence of ART associated hematological and biochemical abnormalities, (Miller et al., 2013), no study has yet been reported in Ethiopia. Therefore, assessment of hematological and biochemical changes among ART users is of paramount importance. Thus, this study aimed to assess serum lipid profile, complete blood count and viral load levels among HIV positive patients taking dolutegravir and low dose efavirenz based ART.

1.2. Statement of the Problem

According to WHO, there were an estimated 38 million PLHIV at the end of 2019, of which over two third were living in Africa (WHO, 2019). About 1.7 million people were newly infected and 690, 000 people died from HIV in 2019 globally (USAID, 2019). Although Ethiopia had made a significant improvement in HIV service delivery, the burden of the disease in the country is still high (Assefa et al., 2015). Currently in Ethiopia, there are 665,723 PLHIV, and about 30 people died every day associated with the infection (FMOH, 2019). The UNAIDS Spectrum estimate for PLHIV report showed that 15,898 people were newly infected and 15439 lost their life in 2019 in Ethiopia due to virological failure, hematologic & biochemical complications directly or indirectly associated with the use of HAART (USAID, 2019).

Although HAART has dramatically reduced HIV associated morbidity and mortality, their long-term use lead to various immunological, biochemical and haematological disorders such as dyslipidemia, insulin resistance, osteopenia, lactic acidosis, anemia, leukocytopenia, neutropenia and thrombocytopenia (Lake & Currier, 2013). As per a study conducted by Roberts et al. (1999), dyslipidemia was detected in 77.5% of patients who were on ART. Another study by Squires et al. (2014) showed exposure to Efavirenz and Dolutegravir was associated with an 18% increase in fasting LDL and TG levels, which in turn, leads to metabolic and cardiovascular complications. It was also evidenced that prolonged use of HAART is associated with renal disorders, hepatotoxicity and cardiovascular abnormalities which are the leading causes of mortality in the HIV infected population (Ogunghaonsi et al., 2012).

A study by Walmasly et al. (2012) showed the increased risk for coronary heart disease among HIV positive individuals is due to chronic inflammation and immune activation by long term exposure to HAART. Other studies by Cooper et al. (2009) and Tebas et al. (2018) revealed that ART supplements have been associated with increased markers of endothelial dysfunction and inflammation, which later contributes to chronic diseases and insulin resistance (Eboni et al., 2017).

Hematological abnormalities are among the most common pathological findings among ART users (Tsiakalos et al., 2018). These hematologic abnormalities can lead to heart failure, acute and chronic kidney injury, stroke and pulmonary dysfunctions (Eboni et al., 2017). Although

efavirenz and dolutegravir based FDCs affect more than one haematopoietic line, the degree at which the particular cytopenia happen varies with the type of the regimen (Jacobson et al., 2017; Apostolova et al., 2011). Many studies showed that efavirenz and dolutegravir based ART have been shown to induce apoptosis (Polo et al., 2015; Abdulla et al., 2016). Virological failure and toxicity of antiretroviral treatment are feared complications for clients taking long-term HAART (Abdulla et al., 2016). An observational cohort study in South Africa showed that 9.91% of HIV seropositive patients on either of dolutegravir and efavirenz based ART encountered virological failure at 16 months of ART initiation (Fox et al., 2012). It was also showed that the prevalence of HIV drug resistance for various antiretroviral drugs increased by 29% per year in East Africa (Gupta et al., 2012).

1.3. Significance of the Study

Antiretroviral drugs are known to achieve rapid HIV suppression and allow restoration of the immune system by suppressing the activity of the virus at various stages (Troll, 2011; Kramer et al., 2009). Despite their applications, several studies revealed long-term utilization of HAART was associated with various immunological, biochemical and haematological disorders (Tarr et al., 2015; Carr et al., 2017). However, to our knowledge, no study has been documented yet in Ethiopia regarding the effects of dolutegravir and efavirenz based ART. Thus, this study aimed to assess on lipid profile, hematologic changes and viral load levels among HIV seropositive clients taking dolutegravir and efavirenz based ART, which in turn put a baseline data for the country.

Besides, this study may help clinicians for early detection, diagnosis and management of abnormalities associated with the use of HAART, which enables them to consider HAART associated problems and incorporated them into routine clinical practice. This study also would have an important input for researchers in identifying the root cause of HAART associated metabolic and biochemical abnormalities and design appropriate therapeutic regimens which can serve as a stepping stone on which further studies could be planned.

The health service organizations may also use the output of this research to plan, monitor, evaluate and predict management outcome and their possible complications, which helps them to improve health care services for PLHIV. In addition, this research may serve as a base line data for other governmental and non- governmental organization for further investigation, treatment guideline preparation and policy making. Furthermore, the findings of this study may play a crucial role for patients to take appropriate care based on their need, save their time and money which could be caused by complications HAART use.

2. LITERATURE REVIEW

2.1. Classification of antiretroviral drugs

According to WHO updated consolidated guidelines, antiretroviral drugs are classified in to six classes (Figure 1): protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, entry inhibitors, integrase strand transfer inhibitors and CCR5 antagonists (WHO, 2018).

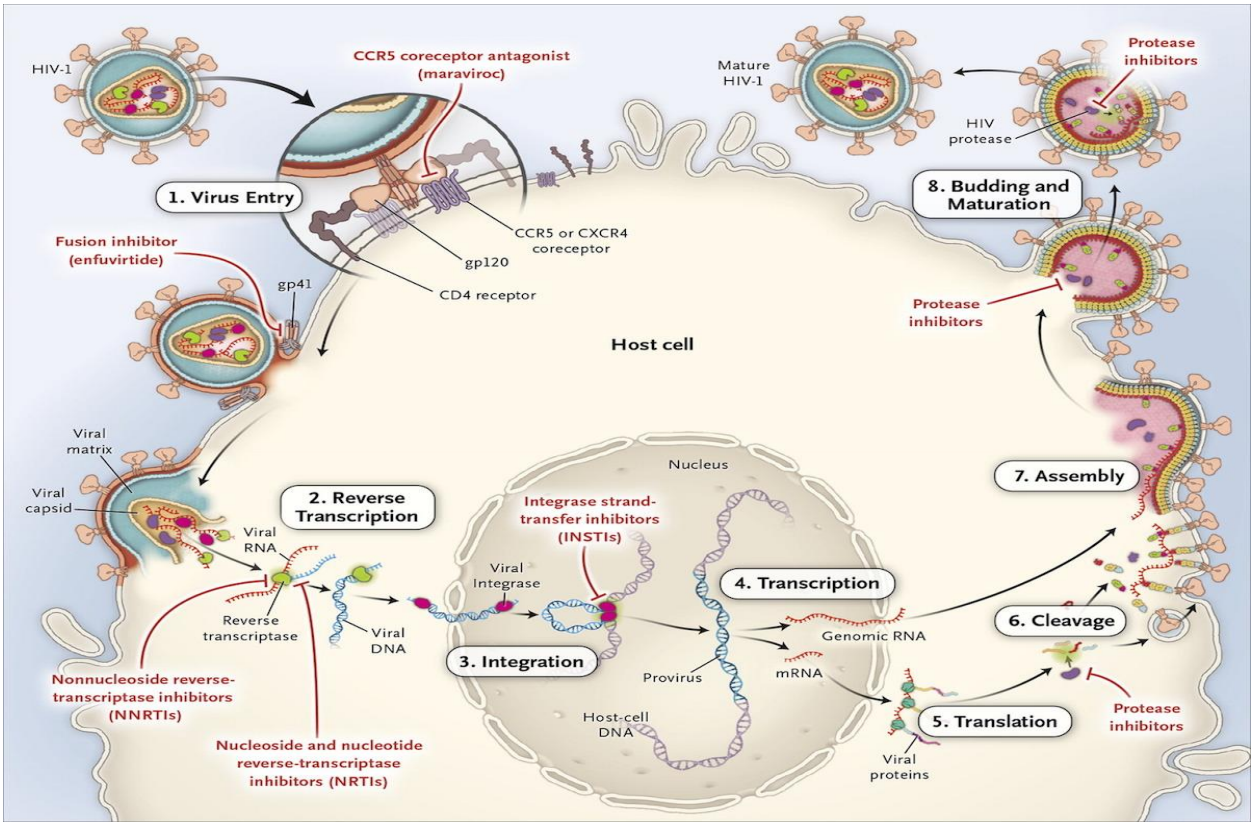


Figure 1: HIV cycle with potential sites of inhibition by ART (Adopted from Sellmeyer, 2013).

The current first-line ART recommended by WHO consists of two NRTIs plus INSIs or NNRTs (USAID, 2018). Accordingly, in Ethiopia, TLD (TDF + 3TC + DTG) and TLE (TDF + 3TC + EFZ) FDCs are used as first line therapy in the treatment of HIV-1 (FMOH, 2018).

Efavirenz is a NNRTI, which binds to a non-catalytic site of the HIV reverse transcriptase enzyme and inhibits its activity (Figure 2). It acts by binding to human plasma proteins, mainly albumin. Up on its action, efavirenz converted to inactive hydroxylated metabolites by the action of the CYP3A4 enzyme. The drug is commonly associated with impaired concentration,

abnormal dreams, insomnia, nausea, vomiting and other metabolic and biochemical abnormalities (Podany et al., 2017).

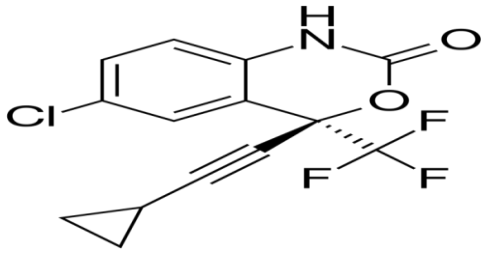


Figure 2: Chemical structure of efavirenz (Adopted by Pondany et al., 2017)

Dolutegravir sodium is an excellent integrase inhibitor that allows divalent cations (Mg^{2+}) to couple with the enzymatic active site of the viral integrase enzyme. Its half-life is about 14 hrs that makes it to be taken once-daily. The absorption of dolutegravir sodium is significantly altered by simultaneous administration with divalent or trivalent cations like iron, calcium, aluminum, and magnesium (dolutegravir biotechnology, 2019).

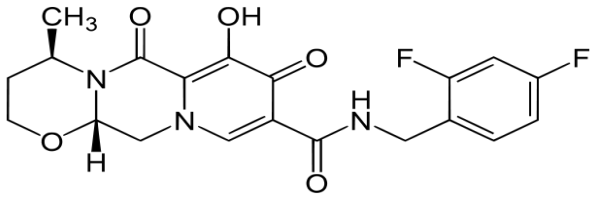


Figure 3: Chemical structure of Dolutegravir (Adopted from Dolutegravir Biotech.)

2.2. Impacts of Dolutegravir and Efavirenz based ART on serum lipid profile

Lipids are organic compounds represented by phospholipids, cholesterol, triglycerides and fatty acids (USAID, 2019). PLHIV frequently present with alterations in lipid parameters due to infection with HIV itself, or the use of HAART (Sellmeyer & Grunfeld, 2013). Mujawar et al. (2016) on their cohort study revealed HIV-associated dyslipidemia is similar to that observed in other chronic infections (Figure 2) as results of inflammation and immune activation.

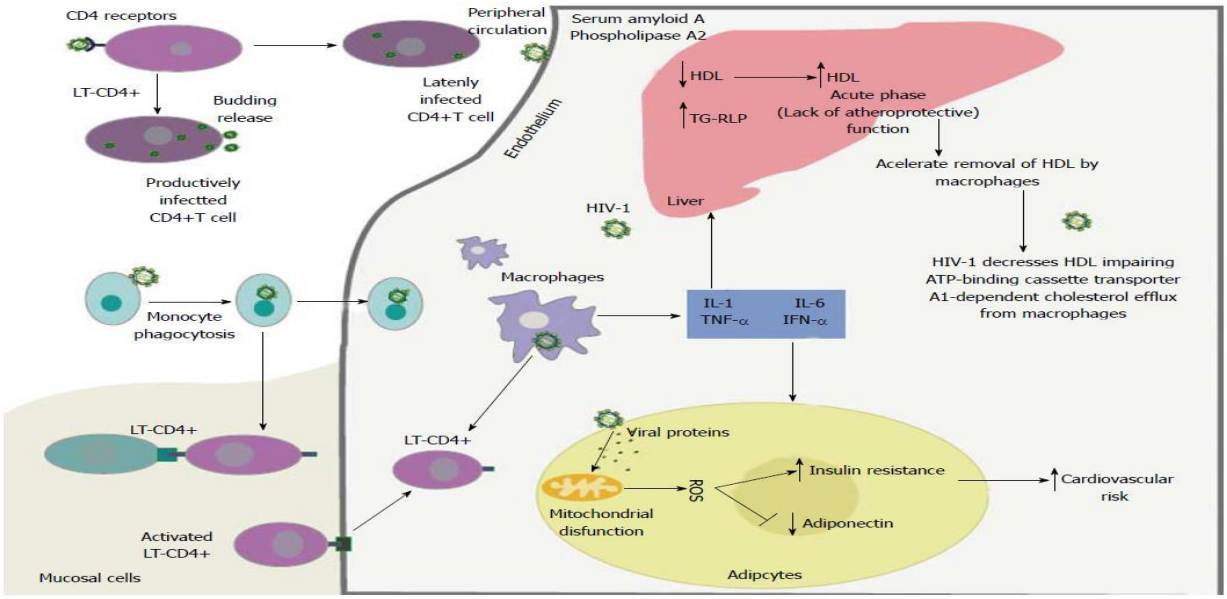


Figure 4: Effects of HIV on lipid metabolism (Adopted from Sherer R, 2013).

Antiretroviral therapy associated dyslipidemia are complex conditions involving immunological, hormonal and genetic predisposition (Sherer, 2013). Of all the lipid parameters, high levels of triglycerides and low serum concentrations of HDL can be used as markers of chronic inflammatory activity (Sellmeyer, 2013). The changes in lipid parameters occur within three months of initiating ART, and reach peak after six to nine months (Sherer, 2013).

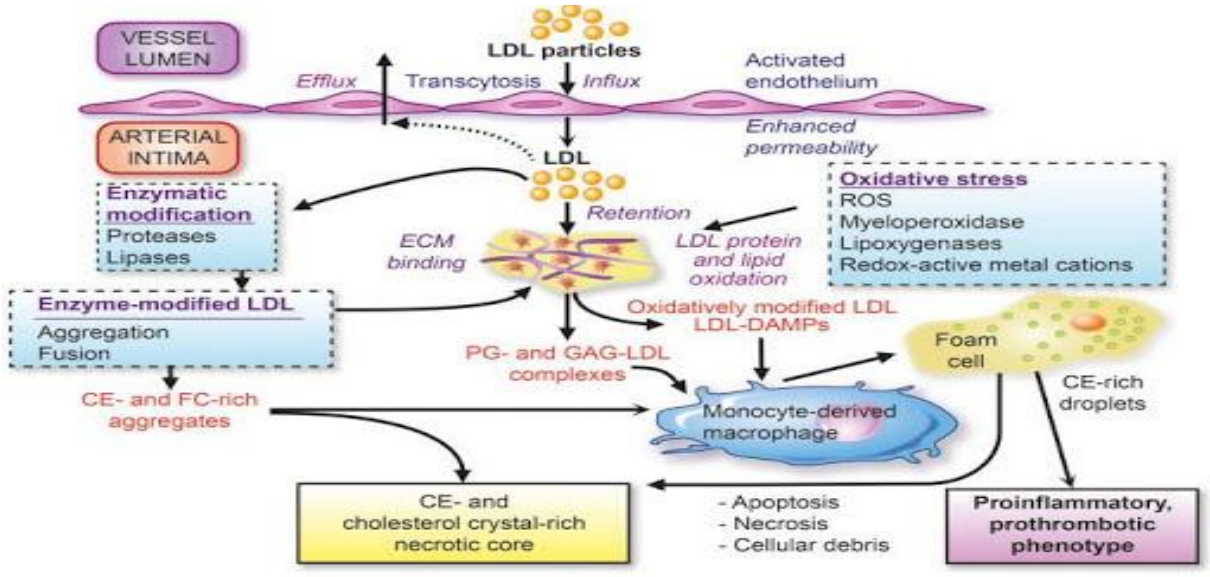


Figure 5: Effects of HIV on lipoprotein metabolism (Adopted from Pinti et al., 2016).

There are various mechanisms by which efavirenz and dolutegravir based HAART can affect serum lipid levels (Zha et al., 2013). These possible mechanisms include: Suppression of the breakdown of the SREBP-1 nuclear form in hepatocytes (Miseres et al., 2012), protection of degradation of nascent ApoB by intracellular proteasomes (Torriano et al., 2016), inhibition of expression of LDL receptors (Pettit et al., 2012), up regulation of liver expression of some key triglyceride biosynthetic enzymes, increasing of the hepatic synthesis of triglycerides (Oh et al., 2017), and inhibition of plasma and tissue lipoprotein lipases (Vanwijk et al., 2015) .

Dyslipidemias associated with the use of efavirenz and dolutegravir might also be due to mitochondrial damage, which inhibits mitochondrial DNA polymerase gamma, leading to mitochondrial DNA depletion, respiratory chain dysfunction and decreased energy production (Pinti et al., 2016). These dysregulation of lipid metabolism in adipocytes and peripheral lipoatrophy are the final effects of ART-induced activation of endoplasmic reticulum stress and metabolic disorders (Shere, 2013).

A study conducted among 110 HIV positive patients in western Africa by Corota et al. (2018) revealed higher prevalence of hyperlipidemia in subjects on HAART. As per the study, the mean values for LDL, TC, and TG concentrations among clients taking dolutegravir were 85.7mg/dl ± 42.2, 158.3mg/dl ± 38.6, and 123.6 mg/dl ± 24.5 respectively, whereas, lower values were observed among patients on efavirenz based HAART with respective values of 89.1mg/dl ± 34.4, 160.6 mg/dl ± 34.8, 130.5mg/dl ± 17. The mean concentration of HDL (38.3 mg/dl ± 13) in the efavirenz treated subjects was significantly higher (p= 0.0043) than those of dolutegravir (27.9mg/dl ± 2.3) treated groups.

Romina et al. (2016) on their randomized trial stated those receiving efavirenz (+24.1mg/dl) showed statistically significantly (p=0.001) higher mean TC increase compared with dolutegravir (+17.1 mg/dl) recipients. Significant differences (p = 0.032) were also seen in LDL, with the highest changes from baseline in patients with efavirenz arm (+8.5 mg/dl to +13.1 mg/dl). The mean HDL levels moderately increased from baseline to week 48 in the dolutegravir and efavirenz arms (+5.2 mg/dl to +8 mg/dl). Regarding TC: HDL, there were a minor decline from mean baseline in both treatment groups.

In a comparative study conducted by Clotet et al. (2014) recruiting 312 study subjects showed efavirenz based ART had smaller effects on serum lipid profiles. The analysis identified the mean increase in TC in the Dolutegravir (from 162.5 to 185 mg/dl) arm from baseline to 48 weeks was clinically significantly greater in the efavirenz (157.6 to 159.9mg/dl) arm; a greater mean increase in LDL-C (92.5 to 94.6mg/dl) was also observed in the dolutegravir compared with efavirenz (89.1 to 92.2mg/dl) arm. It was also shown that, the mean increase in HDL-C levels from baseline to week 48 was small and comparable between dolutegravir and efavirenz groups (43.5 and 43.9mg/dl respectively). Regarding TC: HDL ratio, the efavirenz group showed an increment by 0.4 while in the dolutegravir arm by 0.3 from baseline to week 48, with average TC: HDL ratio of 4.14 and 3.89 for efavirenz and dolutegravir based ART users respectively.

A randomised, double-blinded, trial on HIV positive patients done by Raffi et al. (2013) showed significant changes in mean lipid serum concentrations at 48 weeks of therapy. The study revealed minor increase in mean TC in both the dolutegravir (143.8 to 160.7mg/dl) and efavirenz (140.3 to 159.3mg/dl) arms. The mean LDL levels showed no significant variation, with mean values within a normal range for both the dolutegravir (96.8 to 99.73mg/dl) and efavirenz (93.4 to 96.7mg/dl) groups. Similar minor changes were seen in mean HDL plasma levels in both the dolutegravir (44.4 to 47.1mg/dl) and efavirenz (44.3 to 47mg/dl) arms. The mean TC: HDL ratio moderately decreased in both treatment arms.

On a cohort study conducted by Lucia et al. (2017) on 490 patients found clients on efavirenz group had significant ($p = 0.0013$) TC decrease (196.5mg/dl) at 12 months of treatment than dolutegravir (198.5mg/dl) groups. Mean HDL changed differently in the two groups ($p = 0.0012$), increased in efavirenz (53.5 mg/dl), while decreasing in dolutegravir (49.3mg/dl) group. The decline (120 to 117mg/dl) in LDL cholesterol was significant during therapeutic change from TLE to TLD based groups ($p < 0.0005$). The mean TG decrease (126 to 114mg/dl) from TLE to TLD was statistically significant ($p = 0.0016$). The mean TC/HDL showed a significant ($p = 0.0009$) decrease during switch to DTG (4.4 to 3.93) which was associated with the difference in sex, anti-cholesterol drug use and age.

In a multicenter, randomized, open-label clinical trial in Spain conducted by Martinz et al. (2016) showed integrase inhibitors cause only a minor increase in lipid profiles. As per the study, the proportion of patients with increased triglycerides (> 4.7 mg/ dL) and cholesterol values (>

6.3 mg/ dL) was higher in the NNRTI group. On 12-month of follow-up, efavirenz based ART group showed significant increase in lipid parameters compared with the dolutegravir group. A study conducted at ART centre, Bellary, Karnataka, India, over a period of 12 months in comparing blood levels of lipid between HIV patients taking Efavirenz and Dolutegravir based ART recruiting 200 patients revealed a significant increase ($p < 0.005$) in LDL-C, VLDL, TC, TG & TC/HDL-C ratio in ART patients taking TLE compared to those on TLD. The study indicated the abnormal lipid profile was higher in ART patients on TLE compared to ART patients on TLD (Indumati et al., 2018).

In a cross-sectional study by Friis et al. (2013) showed the prevalence of hypercholesterolemia, hypertriglyceridemia, and low HDL was 11–28%, 21–34%, and 18–26% respectively depending on the antiretroviral regimen. According to lipid profile cut-off values of TC and LDL, Aurpibul (2017) showed the prevalence of hypertriglyceridemia in HIV-infected clients who began dolutegravir (7%) was lower than those on efavirenz (10.7%). Regarding TG and HDL levels, the study revealed higher prevalence of hypercholesterolemia among efavirenz (16.7%) than dolutegravir (13.7%) based ART users.

A study conducted by Buchacz et al. (2018) on changes in lipid profile among 342 adults on first-line HAART in rural Uganda revealed increased proportions of dyslipidemia among NNRTI and INSI users. As per the study, the proportions of dyslipidemia among NNRTI & INSI users respectively were 38.9% & 23.1% for low HDL, 18.1% & 13.7% for hypercholesterolemia, 21.8% & 13.9% for hypertriglyceridemia, 16.8% & 20.1% for high levels of LDL and 24.7% & 13.3% for high TC/HDL levels. In another cross-sectional study conducted by Pefura et al. (2011) on first-line antiretroviral therapy and dyslipidemia in Cameroon showed no change in HDL-C levels between TLD and TLE drug regimen. According to this study, the prevalence rate of LDL-C associated dyslipidemia is higher for TLE (46.4%) than TLD (33.8%), and the TC and TG associated dyslipidemia among TLD (13%, 16.1%) than TLE (7%, 9.2%) users. After 18 months of treatment the prevalence of low concentration of HDL-C was 38.5% and 22.9% for clients on efavirenz and dolutegravir based antiretroviral therapy respectively.

In a study on comparative changes of lipid levels in HIV infected adults treated with dolutegravir and efavirenz by Walmsley et al. (2016), dolutegravir showed broadly a better effect on lipid profile versus efavirenz. The study revealed, the overall prevalence of hypertriglyceridemia was

37% and 60% in HIV patients taking dolutegravir and efavirenz based ART respectively. Besides, the proportions hypercholesterolemia, high levels of LDL concentration, low levels of HDL concentration and high levels of TC: HDL ratio were 17.6% vs. 14.1%, 22.5% vs. 15.1%, 37.9% vs. 20.9%, 25.2% vs. 12.8% respectively in patients taking efavirenz and dolutegravir based ART users.

2.3. Impacts of dolutegravir and efavirenz based ART on complete blood count

A complete blood count is a set of laboratory tests that provide information about white blood cells, red blood cells, platelets, hemoglobin, hematocrit and other indices (Watkins et al., 2009). HIV has been reported to cause diverse degree of immunopathogenesis and leads to different haematologic and biochemical consequences (Asgeir et al., 2011). The dominant hematologic consequences among PLHIV are peripheral cytopenias which have become more common with the use of HAART (Watkins et al., 2009). The most common cytopenias observed among clients taking ART include thrombocytopenia, anemia, neutropenia, leucopenia and lymphopenia (Ibeh, 2013).

In a study conducted at Umudike, Abia State, Nigeria by Omadamiro & Jimoh (2017) on hematological and biochemical changes in patents on HAART revealed administration of ART showed an improvement in immune system with significant ($p < 0.001$) increase in mean Hgb concentration, Hematocrit and RBC indices. As per this study, the average haemoglobin concentration among patients taking dolutegravir (12.6 ± 0.85) had higher than those taking efavirenz (12.40 ± 0.57) based HAART.

A study conducted by Stein and his colleagues (2012) on blood cell count reported that there was a significant decline in mean absolute leucocyte count among HIV positive patients taking efavirenz (7.92 x 10^{3}/dL ± 4.25) than dolutegravir (7.55 x 10^{3}/dL ± 4.18). Regarding the differential count, the study revealed mean neutrophil count increased in both TLE (70 ± 12.53%) and in TLD (72.4 ± 9.05 %) based ART users. The average lymphocyte count was higher in dolutegravir (20.57 ± 6.57) than efavirenz (19.97± 4.11) based ART with mean hemoglobin lower in TLE (13.51 ± 1.36) than TLD (13.94 ± 2.19) users. The platelet count was 195.21 ± 45.32 in efavirenz and 176.01 ± 43.02 in dolutegravir based ART.

A study on antiretroviral treatment and associated anemia in rural Tanzania on a pooled sample of 25 has shown that most complete blood count parameters were increased significantly in patients who received ART. Accordingly, patients who were on TLD (13.9 ± 2.05 g/dL) had higher Hgb than those on TLE (13.7 ± 3.17 g/dL). In addition, the study showed an increased mean WBC count from 8.5 x 10^3 to 7.2 x 10^3/dL, and absolute lymphocyte count from 19.1 to 20.2%, platelet count from 200.5 x 10^3/dL ± 1.23 to 169.32 x 10^3/dL ± 1.07, and neutrophil count from 73.1 to 70.9 in TLE than TLD patients respectively (Asgeir et al., 2011).

A study conducted in Tamale, Ghana on 300 HIV infected clients, administered with efavirenz and dolutegravir based HAART showed average white blood cells count was 7.446 ±5.066 vs. 6.733 ± 2.008, neutrophil (%) 51.52 ± 18.37 vs. 44.26±14.46, basophils (%) 4.0 ± 0.2 vs. 3.9±0.06 were significantly higher in efavirenz, while lymphocyte (%) 33.11 ± 14.05 vs. 43.94 ± 12.87 increased significantly ($p < 0.005$) in dolutegravir based HAART administration. As per the study, hemoglobin (g/dL) 10.88 ± 2.255 vs. 11.63 ± 2.40, hematocrit (%) 32.75±6.239 vs. 34.85 ± 5.91, mean cell volume 81.96±10.77 vs. 88.0 ± 12.96, mean cell hemoglobin concentration 33.54±1.651 vs. 34.50±5.368, red cell distribution width standard deviation 44.26±11.78 vs. 68.70 ± 42.64 were significantly higher in TLD groups (Simon et al., 2017).

Amegor et al. (2015) in Benue State of Nigeria conducted a study on hematological changes of efavirenz and dolutegravir administered with tenofovir and lamivudine. As per the study, haemoglobin level (10.833 ± 1.328 vs. 12.87± 2.59) was significantly higher in the later compared with the former group. However, PCT (0.263 ± 0.124 vs. 0.158 ± 0.062), MPV (8.667 ± 1.290 vs. 6.479 ± 1.502), P-LCR (34.70±9.930 vs. 17.32 ± 8.894), P-LCC (113.0 ± 68.29 vs. 41.64 ± 21.54) and PDW-S (12.97 ± 3.083 vs. 7.567 ± 2.255) were significantly higher in the efavirenz compared with the dolutegravir group.

A cross-sectional study on determination of hematological and immunological parameters involving 114 HIV positive participants in southern Brazil revealed significant difference in CBC parameters among patients taking variety of ART. Accordingly, the mean Hgb concentration of participants on efavirenz (12.1 g/dL) was significantly ($p < 0.05$) lower than dolutegravir groups (13.41g/dL); The mean MCV in the efavirenz group (89.9 fL) was significantly lower than dolutegravir based subjects (91.0 fL); while the mean RDW-SD was significantly higher in efavirenz based subjects (45.8 fL) than dolutegravir group (43.5 fL). With

respect to WBC count, the study revealed TLD (7.21 x 10^3/dL) patients had significantly lower WBC than TLE (8.31 x 10^3/dL). Although neutrophil count is higher in TLE (72.9%) than TLD (70.5%), the mean lymphocyte count is significantly lower in patients taking TLE (18.3%) than TLD (20.11%) patients. The prevalence of anemia decreased significantly from 10.6 to 9.6 % upon shift from TLD to TLE patients, with higher proportion of thrombocytopenia in TLE (15%) than TLD (13.4%) patients (Castro & Goldani, 2016).

Emelda & Gokenda (2014) on their study on determination of hematological parameters among 138 HIV positive patients taking HAART at Mbagathi District Hospital, Nairobi, Kenya showed a statistically significant ($\rho = 0.011$) difference in Hgb between TLE & TLD groups. The study revealed mean Hgb concentration in patients taking TLE (13.1 g/L) were clinically significantly lower than the mean Hgb concentration in those taking TLD (13.7 g/L). There was a statistically significant difference between the two treatment groups in red blood cell count ($\rho = 0.0001$), white blood cell ($\rho = 0.027$) and platelet count ($\rho = 0.00878$).

Biochemical and haematological changes among 203 HIV positive subjects in Nigeria done by Scadden et al. (2015) showed reduction of ESR, eosinophil, absolute and differential lymphocytes, granulocytes and total WBC in patients taking INSIs and NNRTIs throughout the assessment period. At the 12th months, thrombocytopenia (15%) and anemia (12%) in NNRTIs were reduced to 11% and 9% in INSIs respectively, while neutrophilia (45 to 46%), leucopenia (10.8 to 17.7%) and lymphopenia (1 to 10%) increased in INSIs groups than NNRTIs groups.

A cross-sectional study done in Ghana on 200 HIV seropositive individuals showed that the prevalence of cytopenias varies with the type of HAART used. Accordingly, anaemia (10% to 8%), leucopoenia (6% to 4%) and lymphopenia (1.4% to 1.2%) showed significant ($p = 0.002$) decrement in Dolutegravir than Efavirenz users while there were a significant ($p< 0.005$) increase in thrombocytopenia (16.7% to 32.1%) and neutrophilia (59% to 61%) in patients taking dolutegravir than efavirenz based ART (Afari & Blay, 2018).

A study on prevalence of HIV related thrombocytopenia among clients at Mbarara regional referral hospital, Mbarara, southwestern Uganda reported that the prevalence of anemia was 37.8% among HAART-naive and was 21.7% for clients who were on efavirenz and 9.8% who were on dolutegravir based ART for up to 12 months. The study also revealed that the

prevalence of thrombocytopenia was 25% at baseline and 5.7% for efavirenz and 21% for dolutegravir based ART (Taremwa et al., 2015).

2.4. Impacts of dolutegravir and efavirenz based ART on viral load level

As the major goal of ART is to achieve long-term and durable suppression of HIV replication, WHO recommends the use of routine HIV viral load testing as the best option to monitor treatment response and identify treatment failure among HIV seropositive subjects taking ART (WHO, 2018). Thus, monitoring viral loads of HIV seropositive clients guides the use of successful treatment, identify adherence problems and determine therapeutic switches to second line therapy (USAID, 2019). According to WHO, viral load should be monitored routinely at 0, 6 months, at 12 months, and then every 12 months to track treatment failure and drug resistance (WHO 2019). Viral load among ART clients vary because of genetic variation, poor medication adherence, development of drug resistance and type interactions between drugs (USAID, 2018). According to a randomised clinical trial conducted by Van Lunzen et al. (2012) treatment with daily doses of dolutegravir 50mg had superior (89.1%) virological control compared with efavirenz 400 mg (78.1%) given twice daily. Another study by Rockstroh et al. (2013) revealed clients on INSI class of ART have demonstrated high efficacy, tolerability and more rapid virologic suppression rates as compared to NNRTI groups.

In a retrospective single centered study at Yale-New Haven Hospital by Jacobson & Karen (2014), 87.1% (18.3% undetected) of clients on efavirenz and 92.3% (30.1% undetected) of those on dolutegravir based ART achieved virological suppression within 1 year of initiating ART. Besides, patients on INSI based regimens were more likely to achieve virological suppression at a lower median time (61 days) to suppression compared to NNRTI (138 days) regimens. Moreover, a study by Jielin et al. (2019) on potential impact of INSIs in comparison with NNRTIs in British and Canada revealed viral load suppression in 98% of clients on INSIs and 94% of those on NNRTIs at six months of treatment.

In a study conducted by Cohn et al. (2018) in Italy, 613 patients were randomized to efavirenz or dolutegravir both in combination with tenofovir and lamivudine. After 48 weeks of follow up, 74.5% of the dolutegravir arm and 69% of the efavirenz arm have viral load suppression. According to a study conducted by Hoenigl et al. (2016) randomizing 214 adults, patients taking

TLD and TLE showed a significant ($p = 0.0015$) difference in viral load suppression (87.5%) in the dolutegravir arm and 76% in the efavirenz arm). But, Han yeninzn et al. (2014) in their clinical trial of 120 HIV positive adults, demonstrated comparable number of patients on dolutegravir (78%, of which 28.7% had undetected viral load) and efavirenz (77.5%, 25.1% had undetected viral load) based ART achieve virologic suppression at 12 months of administration.

In a cross-sectional study carried out over 12 months at HIV clinic of the University of Port Harcourt Teaching Hospital, Nigeria, clients on dolutegravir had comparable viral load suppression rate (85.25 %) when compared with efavirenz based FDC (84.97%). Besides, 23.6% of the former and 19.9% of the later had undetectable viral load (Paul et al., 2020). In another study by Coumil (2013) in Cameroon, there was no significant difference in viral suppression between dolutegravir (85.4%) and efavirenz (85.9%) users. The study also found, among clients with baseline viral loads above 100,000 copies, only 66.2% of the dolutegravir and 61.5% of the efavirenz group had viral load suppression

In a study conducted in South Africa by Dugdale and colleagues, 1053 patients were randomized to TLE or TLD with different formulations. After 48 weeks of administration, a viral suppression was seen in 79% in TLE, 84% in DTG/TAF/FTC, and 85% in TLD users (Dugdale et al., 2019). However, in a study by Wamsley et al. (2018), efavirenz based ART showed better virological suppression (87%) than dolutegravir based ART (85.3%). Furthermore, Rosen and his colleagues showed statistically significant ($p < 0.05$) improvements in viral suppression for dolutegravir taken once-daily than efavirenz twice-daily (Rosen et al., 2016).

3. OBJECTIVES

3.1. General objective

The general objective of this study was to compare lipid profile, complete blood count and viral load levels between HIV patients taking dolutegravir and efavirenz based ART at wolkite Health Center, Southern Ethiopia.

3.2. Specific objectives

- ✓ To compare serum lipid profiles of people living with HIV using dolutegravir with efavirenz based ART.
- ✓ To compare complete blood cell counts of people living with HIV using dolutegravir with efavirenz based ART.
- ✓ To compare viral loads of people living with HIV using dolutegravir with efavirenz based ART.

4. MATERIALS AND METHODS

4.1. Study area and period

The study was conducted from May 2020 to June 2021 at Wolkite Health Center, Southern Ethiopia. Based on the primary health care unit organization classification (FMOH, 2012), Wolkite health center is a level one health facility with a catchment area of over 120,000 inhabitants, most of whom are merchants. It provides preventive, curative and rehabilitative health care for the people of Wolkite town, Nono, Kebena and Abeshighe woredas. The health center provides voluntary HIV counseling and testing (VCT), Provider initiated HIV testing and counseling (PIHTC) and ART services. It also offers different ambulatory HIV care and treatment on every weekday services for PLHIV. Currently, at the ART clinic, there are 476 adult people living with HIV taking ART. Of them, 48 % received efavirenz-based regimen and 51% dolutegravir-based ART (Gurage Zone Health Department, 2019).

4.2. Study design

Institution based comparative cross-sectional study design was carried out to compare lipid profile, Complete blood count, and viral load levels between HIV positive patients taking dolutegravir and efavirenz based ART at wolkite Health Center, Southern Ethiopia.

4.3. Population

4.3.1. Source population

All HIV positive clients on antiretroviral therapy at Wolkite Health center

4.3.2. Study population

HIV positive clients taking dolutegravir and efavirenz based ART which were older than 18 years old at Wolkite Health Center, Southern Ethiopia.

4.4. Sample size determination and sampling technique

4.4.1. Sample size determination

The total sample size is calculated using StatCalc-Epi-info data for cross sectional study by double proportion formula taking in to account the following assumptions: 2-sided confidence

level at 95%, standard margin of error (power of 80%) and 1:1 ratio of dolutegravir and efavirenz group adults. Hypothetical proportion of triglyceridemia among clients taking Dolutegravir and Efavirenz based ART as 37% and 60% respectively (Walmsley et al, 2016).

The statistical formula used to calculate the sample size was:

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

Where $\bar{P}$ is the average between the proportions of triglyceridemia among clients taking dolutegravir and efavirenz based ART

Thus, a total of 150 Study participants, 75 of which efavirenz and 75 dolutegravir based ART treated patients at least for six months and sign consent during their routine appointment were included in the study.

4.4.2. Sampling technique

Consecutive probabilistic sampling technique was employed in which, all ART patients who visited Wolkite Health Center during the study period and fulfilled the inclusion criteria were included until the required sample size was fulfilled.

4.5. Data collection tools and procedures

Pre-tested structured questionnaire was used for data collection, which was composed of socio demographic and base line medical characteristics. Every client who visited the ART clinic during the study period was evaluated by the data collectors for eligibility criteria. The selection and inclusion of patients continued until the required number of patients was fulfilled. After the client signed a written consent, socio demographic, clinical, laboratory and treatment related variables were collected by interviews and medical records using a structured questionnaire. Vital signs like blood pressure, pulse rate, respiratory rate, and weight, waist circumference, and body mass index were recorded and fasting blood sugar was measured using glucometer. Finally, 6ml of blood was collected by venipuncture.

4.6. Study variables

4.6.1. Dependent variables

- ✓ Lipid profile
- ✓ Complete blood count
- ✓ Viral load level

4.6.2. Independent variable

- ✓ HAART regimen

4.7. Definitions of terms and operational definitions

According to the criteria established by the US National Cholesterol Education (NCEP, 2010), dyslipidemia and associated factors is defined as the presence of any of the following:

- ✓ Hypertriglyceridemia: Fasting triglyceride level > 150mg/dL
- ✓ Hypercholesterolemia: Fasting total cholesterol level > 200mg/dL
- ✓ Low level of HDL-C: Serum HDL-C level < 40 mg/dL
- ✓ High level of LDL-C: Serum LDL-C level > 130 mg/dL
- ✓ High level of TC/HDL: Serum TC/HDL-C > 5
- ✓ Hypertension: Systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg or previous history of hypertension
- ✓ Diabetes mellitus: Serum Fasting glucose level >126 mg/dL or previous history of DM
- ✓ Obesity: Body Mass Index > 30kg per meter square or waist circumference > 102cm for males and > 88cm for females

Hematologic abnormalities are considered when there is change in the number and morphology of blood cells. Cytopenias are defined as the presence of any of the following (WHO, 2014)

- ✓ Leucopenia: total WBC count < 4000 cells/ dL

- ✓ Lymphocytopenia: lymphocyte count of < 800 cells/ dL
- ✓ Anemia: hemoglobin <13 g/dL (men) and <12 g/dL (women)
- ✓ Thrombocytopenia: platelet count $<150 \times 10^3$/ dL
- ✓ Neutropenia: neutrophils count <1000 cells/ dL

Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents (DHHSP, 2017) categorized viral load levels as:

- ✓ Undetected viral load: viral load level < 20 copies/mL
- ✓ Viral load suppression: viral load level < 1,000 copies/mL
- ✓ High viral load: viral load 1,000 – 100,000copies/mL
- ✓ Very high viral load: viral load > 100,000copies/mL

4.8. Eligibility criteria

4.8.1. Inclusion criteria

Adult and volunteer HIV positive patients who have been taking Dolutegravir or Efavirenz based ART for at least six months.

4.8.2. Exclusion criteria

The following HIV patients on ART were excluded from the study:

- ✓ HIV positive patients taking ART for less than 6 months and more than 12 months.
- ✓ ART patients taking anti- tuberculosis treatment
- ✓ ART patients with diabetes
- ✓ ART patients with hypertension
- ✓ ART patients with known history of liver diseases
- ✓ ART patients with bleeding disorders

- ✓ Pregnant or menstruating women
- ✓ ART patients with an active AIDS-defining condition or an active cancer
- ✓ ART Patients with current or previous history drug use such as antibiotics, birth control pills and estrogens, anti-diabetics or anti-hypertensive, cholesterol-lowering drugs, or anti-gout medications (Brittenham et al., 2013).

4.9. Quality assurance

All chemicals and reagents used in this study were analytical grade, and each biochemical and hematologic tests were performed by experienced laboratory technologist following standard operational procedures. The data collection was done by the principal investigator and ART focal persons. Collected data and blood sample was checked every day by the principal investigator for its completeness. Challenges faced were discussed with data collectors and laboratory personnel overnight. Data were checked again for its completeness and quality before run.

4.10. Laboratory procedures

4.10.1. Sample collection

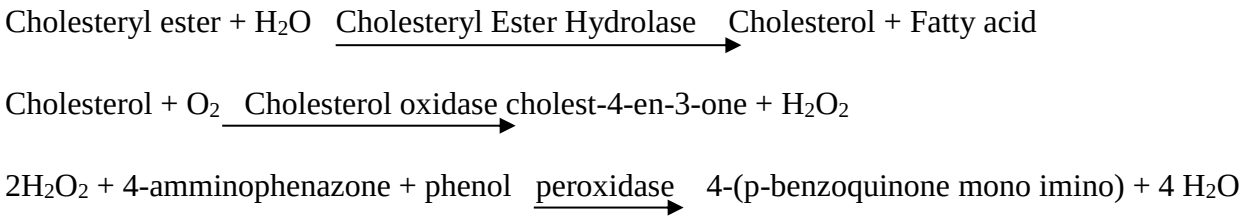
About 6 ml of whole blood was collected from median antecubital vein, with study participants comfortably seated after an overnight fasting. 4ml was collected into plain tubes for chemistry, while 2ml was collected into anti-coagulated vacutainer tubes with EDTA for complete blood cell count. The sample for lipid profile and viral load was centrifuged at 3000 revolution per minute for 30 minutes. The serum obtained was stored at 4°C, then 2ml sent to TASH laboratory for lipid assays, and the remaining 2ml transported to Ethiopian public Health institute for viral load determination using NUNC tubes.

4.10.2. Lipid profile analysis

4.10.2.1. Determination of total cholesterol

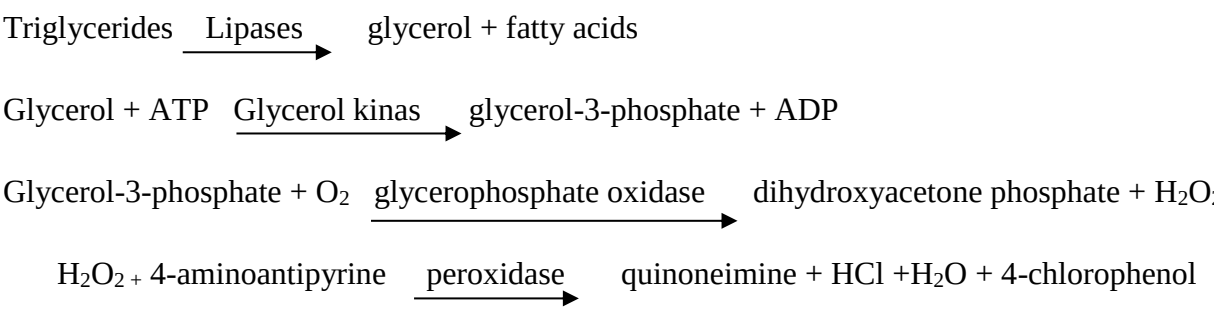
Test principle: Cholesterol was measured enzymatically in serum in a series of reactions that hydrolyzed cholesterol esters and oxidized the 3-OH group of cholesterol. Esterified cholesterol was hydrolyzed to free cholesterols by cholesterol esterase. The free cholesterol then oxidized to form hydrogen peroxide, which later reacts with phenol and 4 -amino antipyrine in the presence

of a catalyst called peroxidase to produce a red coloured quinoneimine dye complex. The absorbance was measured at 500nm wave length and proportional to cholesterol concentration present in the sample. The color intensity is proportional to the concentration of cholesterol. Acceptable cholesterol levels were considered to be less than 200mg/dL (DHHSP, 2000). The reaction steps are is as follows:



4.10.2.2. Determination serum triglycerides

Test principle: Triglycerides are measured enzymatically in serum using a series of reactions in which triglycerides are hydrolyzed to produce glycerol using lipoprotein lipases. The glycerol is then phosphorylated by adenosine triphosphate (ATP) by glycerol kinases (GK) to form glycerol-3 - phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is then oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). A red colored product will be formed by the action of peroxidase (POD) catalyzed reaction of 4-amnioantipyrine and phenol with hydrogen peroxide (H_2O_2). The optical density will be read at 540 nm wave length, which will be proportional to the concentration of triglyceride present in the sample. Desirable level will be considered when TG level is less than 150 mg/dL (DHHSP, 2000): The reaction sequence is as follows:



4.10.2.3. Determination of serum High density lipoprotein cholesterol

Test principle: Serum high density lipoprotein cholesterol is measured directly in serum with the following basic principle: The apoB containing lipoproteins in the specimen are reacted with

a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-cholesterol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of $Mg^{2+,}$ which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The absorbance is then measured at 600 nm. HDL level greater or equal to 40mg/dL considered to be acceptable level (DHHSP, 2000).

The reaction sequences are summarized as follows:

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ⟶ soluble non-reactive complexes with apoB-containing lipoproteins

HDL-cholesteryl esters PEG-cholesteryl esterase ⟶ HDL-unesterified cholesterol + fatty acid

Unesterified cholesterol + O_2 PEG-cholesterol oxidase ⟶ cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H_2O + H^+ peroxidase ⟶ qunoneimine dye + H_2O

4.10.2.4. Determination of serum Low density lipoprotein cholesterol

The Friedewald formula was used to calculate LDL concentration, which uses measured values of fasting cholesterol, triglycerides and HDL. The formula is given as: LDL-cholesterol = total cholesterol - HDL– (TG/5); Where TG/5 can be taken as an estimate of VLDL and all values were expressed in mg/dL (Friedewald et al., 1972). Desirable levels of LDL-chol are those below 130 mg/dL in adults (DHHSP, 2000).

4.10.3. Complete blood count

Test principle: Complete blood count was performed by Hemax 330 hematology analyzer, which is an advanced fully automated hematology analyzer which was first, calibrated using three controls low, normal and high levels from Humatrol then patients samples were run to obtain the full haemogram report. The non-coagulated blood collected in EDTA tubes were placed on a roller which facilitates mixing and each sample was then fed into the open system machine and sucked by a probe. Complete blood cell counts report was obtained and printed out**.**

The hemoglobin concentration is measured photometric and hematocrit value, red cell indices mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) calculated automatically. It used electrical impedance method to determine WBC, RBC, PLT and differential counts. As the cell pass through small aperture, a change in electrical resistance occurs generating an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of WBC, RBC, and PLT counted and the intensity of each pulse is essentially proportional to the cell volume (Brittenham et al., 2013).

4.10.4. Viral load determination

Test principle: The COBAS® TaqMan® instrument for specimen processing amplification, detection, and Quantification of HIV-1 RNA over 20copies/ml was used to determine HIV viral load by using the manufacturer's instructions with the use of the AmpliPrep version 2.0. The COBAS® TaqMan® HIV-1 test is a nucleic acid amplification test for the quantitation of HIV-1 RNA in human EDTA plasma or from a PSC dried plasma spot. The test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target. A master mix (containing buffer, dNTP mix, $MgCl_2$, Taq polymerase and nuclease-free water) was added to each PCR tube, and finally appropriate primer was added. PCR tubes were placed in thermal cycler for 30 cycles of the amplification program, which included three steps: (1) denaturation, (2) annealing, (3) elongation giving real time viral load in copies per ml (Gunthard et al., 2016).

4.10.5. Blood glucose level determination

Test principle: The Ascensia® CONTOUR® Blood Glucose Meter was used to monitor blood glucose levels. A whole blood of sample volume 0.6μL was used. When the blood sample is applied to the test strip, the glucose in the blood reacts with the chemicals on the test strip, producing a small electrical current. This current is measured and then a result is displayed by the monitor. The size of the current depends of the amount of glucose in the blood sample. Finally, the meter will beep and the fifteen second countdown will commence when sufficient sample has been applied to the test strip (Brittenham et al., 2013).

4.11. Data management and statistical analysis

Data analysis was carried out using SPSS version 25.0 statistical software package after it has been exported from epidata. Categorical and discrete variables were described as frequency and percentage, and comparisons of the categorical variables were made using Chi-Square tests. Continuous variables were described as Means ± Standard deviation and their comparisons were done using the Student's t-test. Differences in lipid, CBC and viral load between the two study groups were considered to be of statistical significant at an error probability of less than 0.05 ($p < 0.05$) with 95% confidence interval.

4.12. Ethics statement

The research was conducted in accordance with institutional review board of Addis Ababa University, college of health sciences, department of Medical Biochemistry. Ethical clearance was obtained from department of Biochemistry as well as from College IRB. Official letter was written to Gurage zone health department, Wolkite town health office and Wolkite health center. All the principles of ethics like confidentiality were kept. The obtained data was used only for the purpose of this research and all study participants agreed and signed a written consent.

4.13. Utilization and dissemination of the study findings

The findings of the study were presented to Addis Ababa University College of health science & medicine. Publishing of the study findings in a peer reviewed journals will be considered.

5. RESULTS

5.1. Demographic and clinical characteristics of study participants

As shown below (table 1), from a total of 150 HIV seropositive clients who were on HAART and enrolled in the study, 75 of which were efavirenz and 75 of them dolutegravir based ART users. The mean ages of the study subjects were 32.69 ± 9.558 and 33.36 ± 10.107 years for TLE and TLD respectively with no statistically significant different (p = 0.679) between them. There was no significant difference (p = 0.662) in sex distribution between the two group (M: F = 1: 1.27 for efavirenz and 1: 1.33 for dolutegravir). The average duration of clients on efavirenz and dolutegravir based ART were 10.09 ± 1.757 and 9.88 ± 1.945 months respectively with no significant difference (p = 0.403) between them. There was no statistically significant difference between clients on the two drug regimens regarding their average body weight (p= 0.291), BMI (p= 0.172), Waist circumference (p= 0.57), systolic (p= 0.817) and diastolic (p= 0.413) BP and blood sugar levels (p=0.936).

Table 1: Demographic and clinical characteristics of HIV seropositive patients taking efavirenz and dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Characteristics	TLE (n = 75; mean ± SD)	TLD (n = 75; mean ± SD)	p- value
1	Patient age (Mean)	32.69 ± 9.558	33.36 ± 10.107	0.679
2	Body weight (Kg)	47.2 ± 9.76	44.23 ±7.10	0.291
3	Body mass index (kg/m^2)	20.43 ± 5.28	19.33 ± 4.49	0.172
4	Waist circumference (cm)	86.62 ± 1.07	85.19 ± 42	0.57
5	Diastolic blood pressure (mmHg)	72.11 ± 9.43	79.08 ± 13.85	0.413
6	Systolic blood pressure (mmHg)	117.34 ± 31.09	110. 57 ± 27.41	0.817
7	Fasting blood sugar (mg/dL)	91.57 ± 8.80	89.41 ± 6.43	0.936
8	Mean ART duration (Months)	10.09 ± 1.757	9.88 ± 1.945	0.482

5.2. Baseline hematological parameters of study participants

The baseline levels of all the components of complete blood count showed no significant difference between the two groups (table 2). The p-values of clients on efavirenz and dolutegravir based ART were 0.187, 0.272, 0.304, 0.228, 0.872, 0.078 and 0.290 for total WBC, neutrophil, lymphocyte, RBC counts, Hemoglobin, RDW CV and Platelete respectively.

Table 2: Baseline hematological parameters of HIV seropositive patients taking Efavirenz and Dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Parameters	TLE (n = 75; mean ± SD)	TLD (n = 75; mean ± SD)	p- value
1	Total WBC ($x10^3$/dL)	9.57 ± 3.73	8.88 ± 2.52	0.187
2	Neutrophil count (%)	68.75 ± 10.14	66.77 ± 11.78	0.272
3	Lymphocyte count (%)	22.43 ± 6.58	21.45 ± 4.94	0.304
4	Monocytes (%)	5.65 ±1.61	5.33 ± 0.176	0.241
5	Eosinophils (%)	2.04 ± 2.26	2.67 ± 0.308	0.157
6	Basophils (%)	0.0069 ± 0.003	0.004 ± 0.002	0.521
7	RBC count ($x10^6$/ dL)	3.79 ± 0.78	3.93 ± 0.77	0.228
8	Hemoglobin (g/dL)	12.76 ±2.65	12.84 ± 2.75	0.872
9	Hematocrit (%)	34.42 ± 6.99	34.37 ± 6.92	0.964
10	RDW CV (%)	12.86 ± 1.61	13.45 ± 2.38	0.078
11	MCV (fL)	102.89 ± 14.64	87.62 ± 6.67	0.209
12	MCH (pg)	33.93 ± 2.36	32.68 ± 2.85	0.104
13	MCHC (g/L)	373.32 ±8.01	373.02 ± 9.77	0.841
14	Platelete count ($x10^3$/dL)	199.41±72.00	212.32 ± 76.64	0.290
15	PDW	16.79 ± 3.72	16.37 ± 0.48	0.333

At baseline, the prevalence of cytopenia in HIV patients on both drug regimens was high (Figure 4). The proportion of leukopenia, neutropenia, lymphopenia, anemia and thrombocytopenia were 5.3% vs. 2.77%, 2.7% vs. 6.7%, 18.7% vs. 25.3%, 36% vs. 34.7%, and 26.7 % vs. 18.6% in TLE and TLD patients respectively, all of which showed no significant difference between clients on either of the drug arms**.**

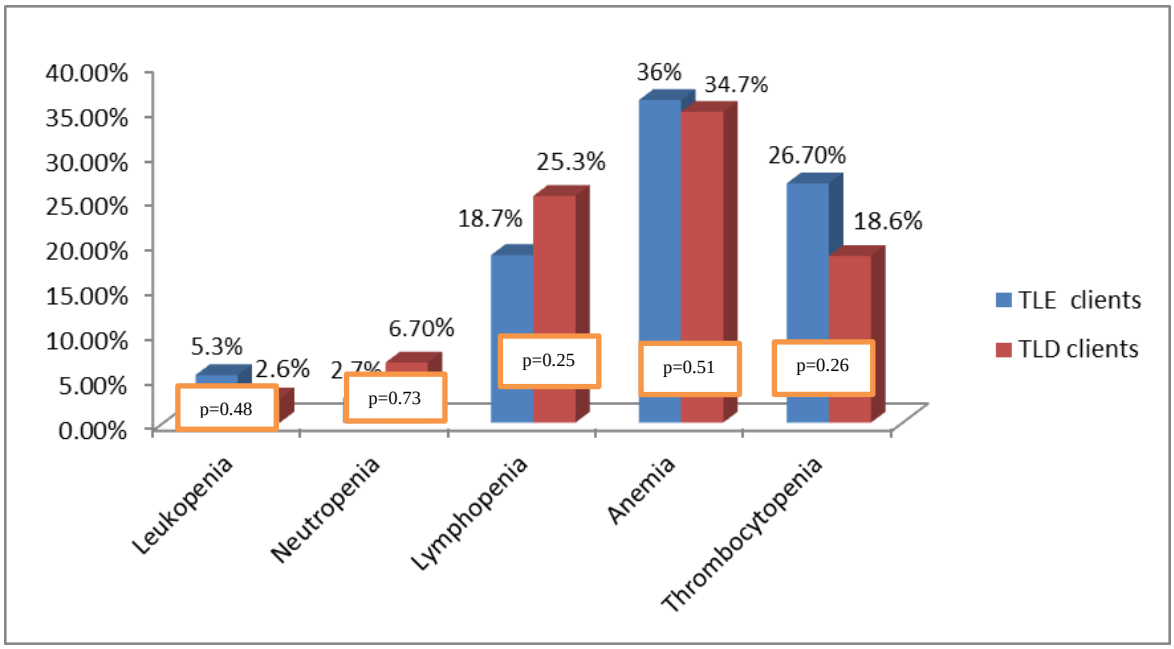


Figure 6: Prevalence of cytopenias among efavirenz and dolutegravir based ART at baseline, SNNPR, Ethiopia, 2021.

5.3. Baseline viral load characteristics of study participants

At base line, it was noted that the mean viral load of clients on TLE (23484.71 ± 3994 copies/ml) was higher than those in TLD (24467 ± 3926 copies/ml) group with no significant difference (p= 0.879) between them (table 3). It was also found that only 13 (17.3%) of TLE & 12 (16%) of TLD clients showed viral suppression with no statistical difference (p = 0.827) between them at base line.

Table 3: Baseline viral load levels of HIV seropositive patients taking efavirenz and dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Viral load classification	TLE (N = 75; %)	TLD (N = 75; %)	p- value
1	Undetected viral load	3 (4%)	4 (5.3%)	0.904
2	Viral load suppression	10 (13.3%)	8 (10.6%)	0.883
3	High viral load	60 (80%)	60 (80%)	0.753
4	Very high viral load	2 (2.67%)	3 (4%)	0.831

5.4. Serum lipid profiles of study participants

Regarding lipid profile (Table 4), clients on dolutegravir had lower mean TC level (159.61 ± 33.21 mg/dL) than efavirenz (160.67 ± 39.56mg/dL) based ART with no statistically significant difference (p = 0.860) between them. This study also revealed clients on efavirenz had statistically significantly (p = 0.030) lower HDL concentration (39.62 ± 7.83mg/dL) than those on dolutegravir (42.05 ± 5.57 mg/dL) based ART. At the same time, TC: HDL was significantly (p = 0.035) higher among efavirenz (4.46 ± 1.62) than dolutegravir (3.99 ± 0.99) based ART users. The mean levels of TG were slightly higher in efavirenz (132.61 ± 49.94 mg/dL) than dolutegravir (131.80 ± 25.90mg/dL) based groups with no significant difference (p= 0.901) between them.

Table 4: Average lipid profiles of HIV seropositive patients taking efavirenz and dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Lipid Parameters	TLE (n = 75; mean ± SD)	TLD (n = 75; mean ± SD)	p- value
1	Total Cholesterol (mg/dL)	160.67 ± 39.56	159.61 ± 33.21	0.860
2	HDL –C (mg/dL)	39.62 ± 7.83	42.05 ± 5.57	0.030
3	TG (mg/dL)	132.61 ± 49.94	131.80 ± 25.90	0.901
4	LDL –C (mg/dL)	93.64 ± 42.09	92.01 ± 28.25	0.781
5	TC : HDL	4.46 ± 1.62	3.99 ± 0.99	0.033

Various scales of dyslipidemias have been observed among clients who were on both treatment regimens (Figure 5). There was a statistically significantly ($\chi 2$ (df = 1, n = 75) = 4.515, ρ = 0.034), higher proportions of low HDL among efavirenz (38.7%) than dolutegravir (22.7%) based ART users. High TC: HDL was also significantly ($\chi 2$ (df = 1, n = 75) = 4.515, ρ = 0.041) higher in efavirenz (26.7%) than dolutegravir (13.3%) based ART users. Otherwise, the difference in the prevalence of hypercholesterolemia ($\chi 2$ (df = 1, n = 75) = 0.852, ρ = 0.356), hypertriglyceridemia ($\chi 2$ (df = 1, n = 75) = 3.175, ρ = 0.075), and high LDL concentration ($\chi 2$ (df = 1, n = 75) = 1.384, ρ = 0.239) showed no significant differences between the two groups.

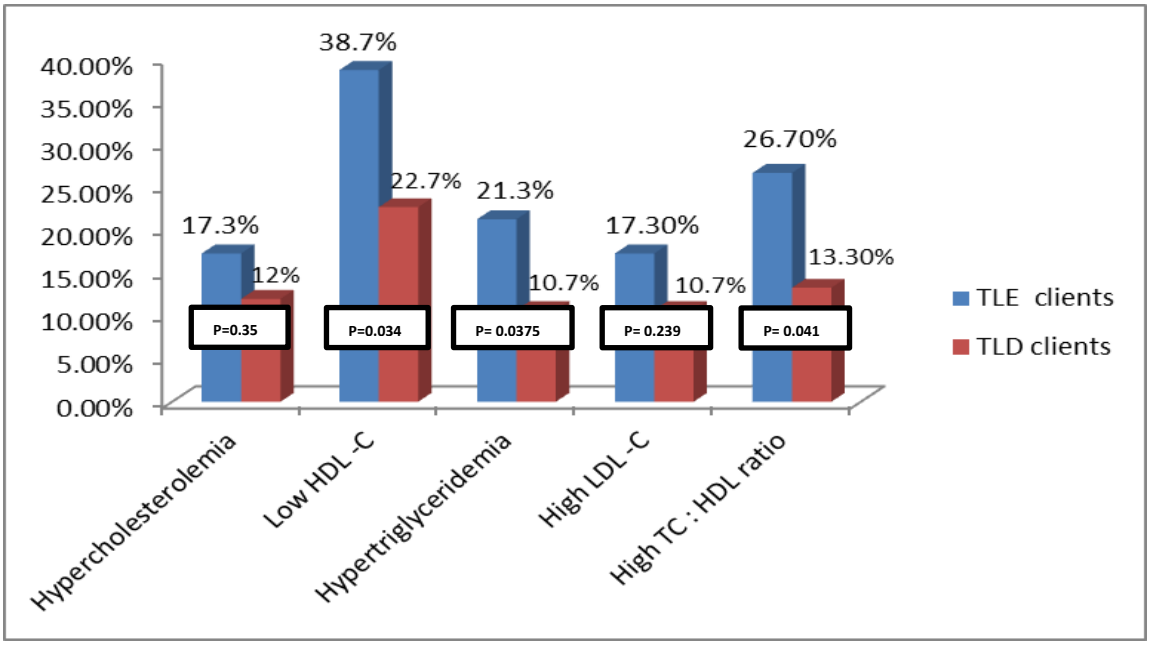


Figure 7: Prevalence of dyslipidemias among efavirenz and dolutegravir based ART users, SNNPR, Ethiopia, 2021.

5.5. Complete blood count profiles of study participants

This study revealed the average white blood cells count ($x10^3$/dL) 7.48 ± 2.52 vs. 6.93 ± 2.41, lymphocyte (%) 27.62 ± 6.58 vs. 29.45 ± 6.98, RBC count ($x10^3$/dL) 3.97 ± 0.70 vs. 4.12 ± 0.83 were not statistically significantly different between efavirenz and dolutegravir based arms respectively (Table 5).

Table 5: Complete blood count values of HIV seropositive patients taking efavirenz and dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Parameters	TLE (n = 75; mean ± SD)	TLD (n = 75; mean ± SD)	p- value
1	Total WBC ($x10^3$/dL)	7.48 ± 2.52	6.93 ± 2.41	0.170
2	Neutrophil count (%)	60.73 ± 9.88	56.55 ± 9.40	0.009
3	Lymphocyte count (%)	27.62 ± 6.58	29.45 ± 6.98	0.108
4	Monocytes (%)	5.32 ± 1.67	4.95 ±1.33	0.134
5	Eosinophils (%)	1.76 ± 0.85	1.66 ± 0.86	0.728
6	Basophils (%)	0.0043 ± 0.002	0.0035 ± 0.002	0.841
7	RBC count ($x10^6$/ dL)	3.97 ± 0.70	4.12 ± 0.83	0.254
8	Hemoglobin (g/dL)	13.34 ±2.09	13.90 ± 2.56	0.148
9	Hematocrit (%)	35.76 ± 5.42	37.13 ± 6.42	0.159
10	RDW CV (%)	12.63 ± 1.19	12.59 ± 1.23	0.834
11	MCV (fL)	90.83 ± 5.32	89.31 ± 5.47	0.086
12	MCH (pg)	34.02 ± 2.37	37.35 ± 3.43	0.405
13	MCHC (g/L)	374.34 ±8.46	375.15 ± 9.67	0.609
14	Platelete count ($x10^3$/dL)	196.21 ± 55.22	175.92 ± 60.02	0.033
15	PDW	16.42 ± 0.45	16.23 ± 0.55	0.052

The mean values of hemoglobin (g/dL) 13.34 ±2.09 vs. 13.90 ± 2.56, hematocrit (%) 35.76 ± 5.42 vs. 37.13 ± 6.42, mean cell volume (MCV) 90.83 ± 5.32 vs. 89.31 ± 5.47, mean cell hemoglobin concentration **(**MCHC) 374.34 ±8.46 vs. 375.15 ± 9.67 showed no significant difference in TLE vs. TLD Clients respectively. However, the mean values of neutrophil (%) showed statistically significantly (p= 0.009) lower among TLD (56.55 ± 9.40) than TLE (60.73 ± 9.88) users. At the same time, the mean values of platelet count was statistically significantly (p = 0.033) higher in the efavirenz (196.21 ± 55.22 $\times 10^3$/ dL) than the dolutegravir (175 .92 ± 60.02$\times 10^3$/ dL) group.

Various degrees of haematological abnormalities were also observed among the two classes of antiretroviral drug users (Figure 6). There was statistically significantly ($\chi 2$ (df = 1, n = 75) = 4.341, ρ = 0.037) higher proportion of thrombocytopenia (32.0% vs. 17.3%) and lower ($\chi 2$ (df = 1, n = 75) = 3.888, ρ = 0.049) proportion of anaemia (10.7 % vs. 22.6%) among dolutegravir than efavirenz based ART users. However, the proportions of leukopenia ($\chi 2$ (df = 1, n = 75) = 1.500, ρ = 0.221), neutropenia ($\chi 2$ (df = 1, n = 75) = 1.190, ρ = 0.275) and lymphopenia ($\chi 2$ (df = 1, n = 75) = 1.010, ρ = 0.315) showed no significant differences between the two FDC users.

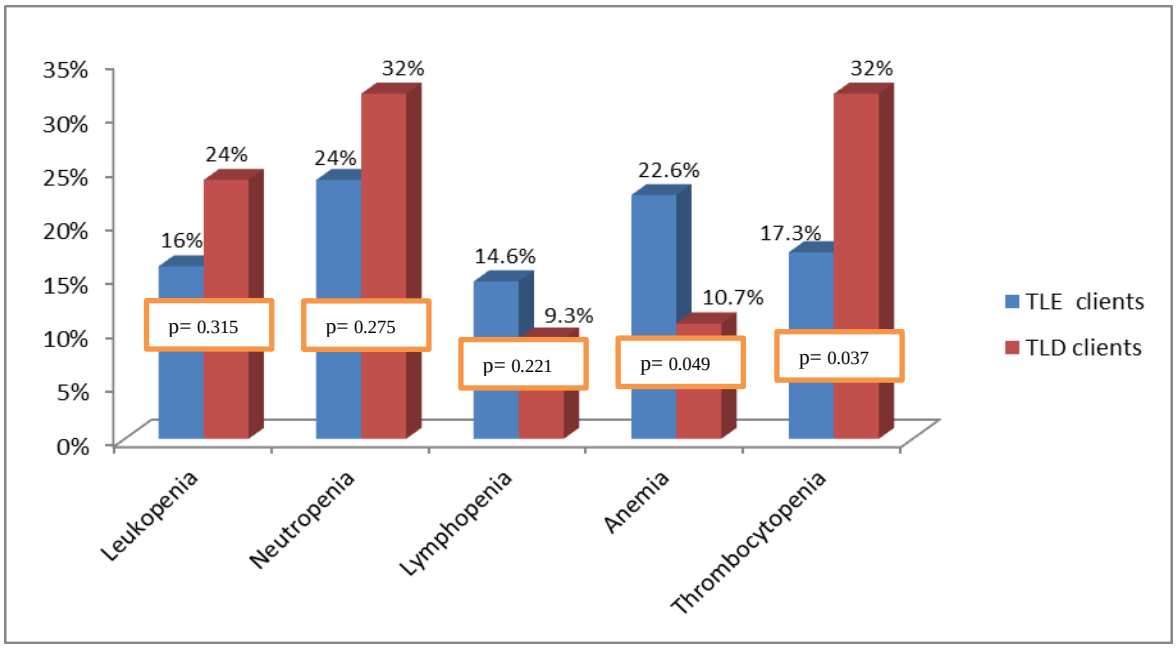


Figure 8: Prevalence of cytopenia among efavirenz and dolutegravir based ART users, SNNPR, Ethiopia, 2021.

5.6. Viral load profile of study participants

Table 6 shows comparative average viral load levels between HIV positive patients taking TLD and TLE FDCs. Accordingly, the mean viral load level among clients on efavirenz (3778.49 ± 163.56 copies/ml) was higher than those on dolutegravir (1816.56 ±116.71) based antiretroviral drugs, with no significance variation (p= 0.399) between them.

Table 6: Viral load values of HIV seropositive patients taking efavirenz and dolutegravir based ART, SNNPR, Ethiopia, 2021.

S.N	Viral load classification	TLE (N = 75; %)	TLD (N = 75; %)	$\chi 2$	p- value
1	Undetected viral load	15 (20%)	22 (29.3%)	4.334	0.228
2	Viral load suppression	50 (66.7%)	49 (65.3%)	3.105	0.178
3	High viral load	9 (12%)	3 (4%)	4.482	0.214
4	Very high viral load	1 (1.3%)	1 (1.3%)	3.511	0.314

The study also revealed a non-significantly ($\chi 2$ (df = 1, n = 75) = 2.836, ρ = 0.092) higher proportion of viral load suppression among TLD (94.6%) than TLE (86.7%) users (Figure 7). 10 (13.3%) clients on efavirenz and 4 (5.3%) on dolutegravir based ART had virological failure with no significance difference (ρ = 0.159) between them.

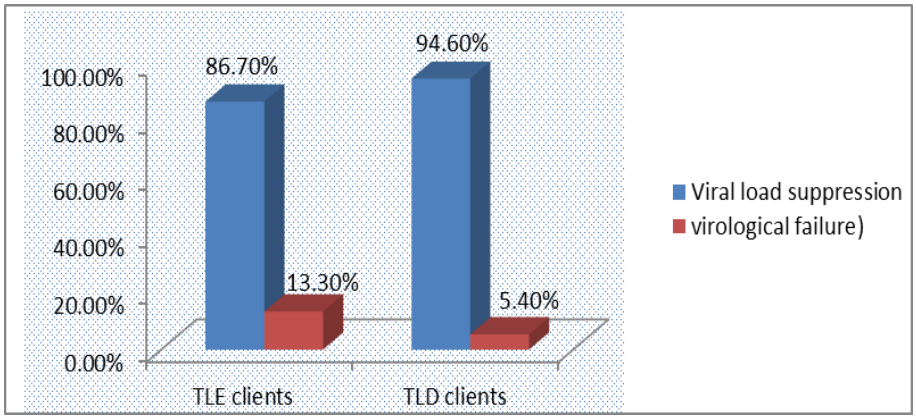


Figure 8: Prevalence of virological suppression and failure among efavirenz and dolutegravir based ART users, SNNPR, Ethiopia, 2021.

6. DISCUSSION

Highly active antiretroviral drugs are effective therapeutic regimens in terms of improving longevity and preventing opportunistic infections among PLHIV (WHO, 2018). However, their prolonged use is associated with metabolic, hematologic and biochemical abnormalities (Eboni et al., 2017). Thus, this comparative cross-sectional study aimed to evaluate serum lipid profile, complete blood count and viral load levels among HIV seropositive clients who were either on dolutegravir or efavirenz based ART.

6.1. Lipid profile

There are various possible mechanisms of lipid alterations associated with the use of HAART. It may be due to the capacity of the drugs to increase hepatic de novo lipogenesis by promoting the hepatic accumulation of sterol regulatory element-binding protein-1 (Miseres et al., 2012). These HAART might also protect nascent ApoB from degradation by intracellular proteasomes, thus increasing hepatic secretion of ApoB-containing lipoproteins (Pettit et al., 2012).

The other possible mechanism for dolutegravir and efavirenz associated high levels of lipid and dyslipidemia in the current study may be due to reduced expression of LDL receptors by the drugs, which leads to increased plasma LDL concentration (Pettit et al., 2012). These therapies might also up regulate liver expression of some core triglyceride biosynthetic enzymes, which can directly stimulate the hepatic synthesis of triglycerides (Oh & Hegele, 2017). Besides, these groups of ART might also alter hydrolysis of TG rich lipoproteins by preventing plasma and tissue lipoprotein lipases (Vanwijk et al., 2015). Furthermore, the drugs may bind to LRP1 and prevent free fatty acid storage in adipose tissue and increases serum triglyceride-rich lipoproteins (Pettit et al., 2012). Accumulation some NNRTIs and INTIs metabolites might trigger mitochondrial damage and inhibit mitochondrial DNA polymerase gamma, which consequently leads to mitochondrial DNA depletion, respiratory chain dysfunction, decreased energy production (Pinti et al., 2016).

In our study**,** we investigated clients on efavirenz based ART showed increased levels of serum TC, TG and LDL compared with clients on dolutegravir based ART. The prevalence of hypercholesterolemia, hypertriglyceridemia and increased LDL level were higher among clients on efavirenz based ART. These findings are in agreement with studies done by Martinz et al.

(2016); Wohl et al. (2016); Buchacz et al. (2018) and Corota et al. (2018). In contrary to the findings of the current study, some other studies such as Spriz et al. (2010); Carr et al. (2017) and Omadamiro et al. (2017) showed higher TC, TG and LDL-C levels and associated dyslipidemia in dolutegravir based ART.

Here we also found that HDL concentration in Efavirenz groups had statistically significantly ($p = 0.030$) lower than those on dolutegravir based antiretroviral treatment with significantly higher prevalence of low level of HDL ($\rho = 0.034$) and high level TC: HDL ($\rho = 0.041$). These findings are in agreement with previously published studies such as Buchacz et al. (2018); Moor (2018); Corota et al. (2018). However, other studies by Iffen et al. (2010); Carr et al. (2017) and Kiangrta et al. (2017) showed better HDL profile among efavirenz than dolutegravir based ART users. The significant increase in the level of HDL concentration in our study might be related to increased HDL lipoprotein biosynthesis by dolutegravir than efavirenz based ART (Cagiarri et al., 2010). The increased levels of plasma TC, TG and LDL concentrations among efavirenz based ART users may lead to lipodystrophy, insulin resistance and central adiposity, thus increased risk of cardiovascular disease, atherosclerosis, coronary artery disease and ischemic stroke (Carr et al., 2013; Wohl et al., 2016).

The possible mechanism for better effects of dolutegravir than efavirenz might be due to the relative levels of serum HDL and TG with associated abnormalities (Abdel et al., 2018). Hypertriglyceridemia is an independent risk factor for cardiovascular diseases and insulin resistance independent of serum levels of HDL or LDL (Assmann et al., 2011), which is associated with elevated concentrations of atherogenic TG-rich remnant lipoproteins (Gurenfeld et al., 1992) and inflammatory biomarkers such as cytokines (Ridker et al., 2002). Therefore, clients on efavirenz based ART seem to have higher risk of cardiovascular diseases compared with those on dolutegravir based ART (Abdel et al, 2018).

On the other hand, increased level of HDL is associated with decreased risk of coronary artery disease (Adeole et al., 2010). HDL plays an important role in cellular immune response against HIV through the production of IL-2 (Cagiarri et al., 2010), or by its anti-inflammatory and anti-oxidant properties that alter the course of HIV infection (Buchacz et al., 2018). The anti-inflammatory property is achieved by inhibiting monocyte transmigration in response to oxidized LDL (Navab et al., 2014), and prevents the expression of cell surface adhesion molecules by

activated endothelial cells (Cockerill et al., 2011; Xia & Ryens 2011). HDL has several potential for antiatherogenic properties, for instance, cholesterol is transported from peripheral tissues such as the cells in the arterial walls to the liver by HDL components, where it is used for a composition of lipoproteins and in synthesis of bile acids, steroid hormones, or fat-soluble vitamins (Sellmeyer et al., 2013; Vanwijk JP et al., 2015).

Several studies have shown that HDL inhibits the expression of cell surface adhesion molecules by activated endothelial cells such as VCAM-1, ICAM-1, and E-selectin (Cockeril, 2011). The inhibition of adhesion molecule expression is associated with a reduction in the mRNA levels of these proteins (Cockeril, 2011). It also inhibits endothelial cell sphingosine kinase, which catalyzes a key step in the pathway by which TNF-α stimulates the expression of endothelial cell adhesion molecules (Viswambharan et al., 2014). However, TG increases inflammatory activities through several pathways (Navab et al., 2014).

Besides the above possible conditions, inflammation might be the possible explanation for the variation in lipid profile among patients on dolutegravir and efavirenz based ART. Dolutegravir based ART possess greater anti-inflammatory activity which might be associated with relatively higher level of HDL compared with efavirenz based HAART (Cockeril, 2011).

Chronic inflammation among ART naïve clients is characterized by prolonged inflammatory activities followed by simultaneous destruction and repair of the tissue due to inflammatory process (Kaur et al., 2016). The chronic inflammation may be due to the viral infection, opportunistic infections and autoimmune destruction (Mujawar et al., 2016). During the inflammatory process, memory B cells and natural killer cells possess abnormal function, T cells, platelets and neutrophil got activated, monocyte and macrophages become pro-inflammatory and their activation is associated with systemic inflammation (Sellmeyer & Grunfeld, 2013).

The inflammatory process initiated by viral infection can reduce HDL concentration (Torriano et al., 2016), and HIV-1 decreases plasma HDL by preventing the cholesterol-dependent efflux transporter ATP binding cassette protein A1 (ABCA1) in macrophages (Sherer, 2013). The inflammatory process may also be characterized by an elevation of interferon-γ levels originating from lymphocytes and macrophages (Wei et al., 2015). IFNγ levels are elevated at early stages of infection and are also correlated with the presence of hypertriglyceridemia (Grunfeld et al.,

2009). TNF-α is another potent proinflammatory cytokine whose concentrations increased in HIV-1 infected ART-naïve patients (Brenchley et al., 2016). TNFα promotes lipid peroxidation and disturbances in the metabolism of free fatty acids and inhibits lipolysis which is mediated by hormones (Fris et al., 2013). Chronic inflammation also leads to high plasma levels of total cholesterol and oxidized low density lipoprotein which is correlated with markers of inflammation and immune activation through time (Cagiarri et al., 2010).

Successful HAART suppresses viral replication at different stages of HIV life cycle, improves immunological status of HIV infected individuals, delays drug resistance and improves inflammatory conditions (Brenchley et al., 2016). For example, INSIs induced a significant gradual increase in CD4 T cell count, IL-10, IL-4 and TGF-β, INF-γ (anti-inflammatory cytokines), decrease in viral load, TNF-α, IL-6 (Pro-inflammatory cytokines) approaching to those in HIV uninfected subjects (Leite et al., 2019). This activity of INSIs shows their efficacy to decrease inflammation and immune activation more than NNRTIs (Roff et al., 2014). The inflammatory markers seen among HIV positive clients who were on INSIs were lower than those on NNRTIs based ART (Brown et al., 2009). This might be associated with the better lipid profile among clients on dolutegravir than efavirenz based ART (Pettit et al., 2012).

6.2. Complete blood count

Highly active antiretroviral drugs are associated with clinically significant hematologic disorders such as derangement of hematopoiesis, immune-mediated cytopenias and altered blood clotting mechanisms (Nacoluma et al., 2017). These abnormalities may be due to the virus itself, sequel of opportunistic infections, malignancies and secondary to HAART (Green et al., 2017).

Generally, the overall WBC count is important to monitor various medical conditions such as autoimmune diseases, immune deficiencies and blood disorders which may be due to infection, lack of response to treatment or any abnormality (Al Alaska et al., 2011). Antiretroviral drugs have been reported to cause various degree of haematologic and biochemical consequences and peripheral cytopenias (Ibeh, 2013).

This study found statistically significant ($p = 0.002$) decline in total WBC (9.57 ± 3.73 to 7.48 ± 2.52 in TLE and 8.88 ± 2.52 to 6.93 ± 2.41 in TLD), neutrophil (68.75 ± 10.14 to 60.73 ± 9.88 in TLE and 66.77 ± 11.78 to 56.55 ± 9.40 in TLD with $p = 0.009$) basophil and eosinophil counts

as compared to their the baseline value. These findings are consistent with Kibaru et al. (2015), which noted the occurrence of altered number of WBC post HAART administration. However, these findings are contrary to the findings of Cockeril and his colleagues who did not observe any change pre and post HAART administration (Cockeril et al., 2011).

The possible mechanism responsible for leucopenia might be largely due to the significant reduction in neutrophil count, which may be due to suppression of the bone marrow and ineffective granulopoiesis (Kimura et al., 2017), which is caused by inflammation in the tissues as a result of exposure to the drugs (Fimhammer et al., 2010). Dolutegravir and efavirenz based ART might exhibit direct leucopenic suppressive effect on matured granulocytes in the peripheral system or on the myeloid progenitor cells in the bone marrow (Al Alaska et al., 2011). The neutropenia might also be caused by the presence of antigranulocyte antibodies, secondary to accumulation of ART metabolites which probably attack and destroy granulocytes (Wei et al., 2015).

Lymphocyte count after administration of either of the treatment regimens increased (22.43 ± 6.58 to 27.62 ± 6.58 in TLE and 21.45 ± 4.94 to 29.45 ± 6.98 in TLD groups). This may be due to compensatory activities in favour of endogenous cell provisions over virus mediated cell killing (Baker et al., 2017), which might show improvement of the immune system (Green et al., 2017). Studies such as Mohri et al. (2012); Grossman et al. (2015) and Baker et al. (2017) support the positive effects of antiretroviral drugs in lymphoid tissue activity in response to the immune system activation following HAART administration. In contrary to our findings, some other studies like Al Alaska et al. (2011) and Green et al. (2017) found decrement in lymphocyte count post HAART administration due to suppressive activity of the antiretroviral drug.

This study also recorded significant improvements in erythroid cell line and indices. RBC count (3.79 ± 0.78 to 3.97 ± 0.70 in TLE and 3.93 ± 0.77 to 4.12 ± 0.83 in TLD), Hgb (12.76 ±2.65 to 13.34 ±2.09 in TLE and 12.84 ± 2.75 to 13.90 ± 2.56 in TLD), HCT (34.42 ± 6.99 to 35.76 ± 5.42 in TLE and 34.37 ± 6.92 to 37.13 ± 6.42 in TLD), MCV and RDW-SD were significantly higher after HAART administration compared from the baseline. These findings are consistent with previous studies by Ogunbusuyi et al. (2015); Sullivan et al. (2018). In contrary to this, other study findings such as Akinbami et al. (2010); Chmaier et al., (2011) showed a decline in the erythroid lineage due to the toxic effect of medication metabolites on the bone marrow.

Thrombocytopenia was considered as an early haematological abnormality that may show disease progression and the occurrence of abnormal bleeding (Mc Millan, 2007). The result in this study showed a significant decrease in platelet count in TLE and TLD compared with the baseline, which is consistent with the findings of Brown et al. (2009) and Leite et al. (2019) who recorded increased prevalence of thrombocytopenia after HAART administration. The possible explanation might be due to increased platelet destruction secondary to anti-platelet antibody deposition of immune complexes on platelets (Kasturi et al., 2016), and decreased platelet production (Kasturi et al., 2016). However, the results of this study was not in line with the findings of a study by Enawgaw et al. (2014), which revealed a decrease in the incidence of thrombocytopenia after six months of antiretroviral therapy.

Comparison of neutrophil count in dolutegravir based ART revealed statistically significantly (p-value = 0.009) lower levels when compared with the efavirenz group, which in turn leads to higher prevalence of leukopenia in the former group (24%) than the later(16%). These findings are in agreement with previous studies like Asgeir et al. (2011); Afari (2015) and Simon et al. (2017). This reduction in granulocyte count might be due to the higher rate of abnormal granulopoiesis and anti-granulocyte antibody as a result of ART metabolite deposition (Friis et al., 2013). However, studies by Castro & Goldani (2016), showed higher prevalence of neutropenia among efavirenz than dolutegravir based ART users.

This study also revealed that dolutegravir has a more positive influence on erythroid cell lines compared with the efavirenz based ART group, with better RBC count, Hgb, HCT and other RBC indices. The prevalence of anemia was statistically significantly (p=0.049) higher in the efavirenz group (22.6% vs. 10.7%). This finding is supported by studies by Afari et al. (2015) and Cohn et al. (2018), while it was opposed by Taremwa et al., (2015), which noted a better RBC indices among clients taking efavirenz than dolutegravir based ART. This study also showed dolutegravir groups recorded significantly lower platelet count (p= 0.033) and indices (p=0.022) than efavirenz groups, with higher prevalence of thrombocytopenia. This corroborates with the findings of Scadden et al., (2015). This might be due to an accumulation of ART metabolites on Platelete progenitor cells (Taremwa et al., 2015).

6.3. Viral load levels

The main goal of ART is to achieve sustainable viral load suppression among HIV seropositive clients (USAID, 2019). To achieve the third 95 protocol of WHO (2016), an appropriate ART regimen has to be selected mainly based on their abilities to virological suppression rate and the time taken for the suppression. That's why this study is intended to make a comparison between the two drug regimens.

In our study, we found that the average viral load values was statistically significantly (p= 0.001) decreased (23484.71 ± 3994 to 3778.49 ± 163.56 copies/ml in efavirenz and 24467 ± 3926 to 1816.56 ±116.71 in dolutegravir groups) compared with the baseline values. Initially, 13.3% in TLE and 10.6% in TLD arm were virally suppressed (viral load < 1000 copies/ml). However, after 6 to 12 months of treatment, 86.7% of the former and 94.6% of the later became virally suppressed, the difference from baseline is being statistically significant ($\chi 2$ =53.77, p= 0.0001). This viral suppression is much higher than the viral suppression rates reported by Martinz et al. (2016), but lower than studies by Jielin et al. (2019); Cohn et al. (2018).

In comparing the two drug regimens, this study revealed clients on dolutegravir based ART had lower (1816.56 ±116.71) mean viral load values than efavirenz (3778.49 ± 163.56 copies/ml) arm, with no significance variation (p= 0.399) between them. It was also showed that 86.7% (20% undetected) of clients on efavirenz and 94.6% (29.3% undetected) of those on dolutegravir based ART achieved virological suppression ($\chi 2$ (df = 1, n = 75) = 2.836, ρ = 0.092). This comparable viral load suppression between the two groups is supported by studies like Connell et al. (2010); Rockstroh et al., (2013) and Caitlin et al. (2019). However, some other studies found more viral load suppression rate among dolutegravir than efavirenz based ART users (Van Lunzen et al., 2012; Jacobson JS et al., 2016). It was also reported by other studies that efavirenz based ART had greater tendency to viral suppression than dolutegravir (Rosen et al., 2016); Walmsley et al., 2016). The variation may be due to the difference in genetics, geography and nutrition (Podany et al., 2017). Again, as per the study, 13.3% of patients on efavirenz and 5.3% on dolutegravir based ART users remained virally unsuppressed after six to twelve months of treatment.

The inflammatory activity could be related to virological suppression rates (Funderburg et al., 2016). For instance, the proportions of inflammatory markers such as sTNF-RI, sTNF-RII, TNF-α, IL-6, and adhesion molecules (sVCAM-1 and sICAM-1) decreased by half for every 1000 copies/ ml viral load suppression (Brown et al., 2009). In another observational study, IL-6, interferon γ-inducible protein-10, and monokine induced by interferon γ decreased significantly in patients with suppressed viral load (Hattab et al., 2014). It was also seen, among young adults who were on ART for 1 year who achieved viral RNA <1000 copies/ml, the levels of pro inflammatory cytokines were decreased significantly (Rudy et al., 2015). In HIV seropositive persons initiating potent ART with suppressed viral load, it was shown in a decreased in IL-6, TNF-α, sVCAM-1, sICAM-1, E- selectin, and P-selectin compared with those having higher viral load levels (Calza et al., 2016). It was also noted in a study that, the inflammatory markers such as sTNF-RI, sCD163, and D-dimer and IL-6 were higher among clients taking efavirenz than dolutegravir based ART (Funderburg et al., 2016).

7. CONCLUSION

This study seeks to evaluate serum lipid profile, CBC, and viral load levels to compare between HIV seropositive patients taking dolutegravir and efavirenz based ART.

Compared with efavirenz, dolutegravir based ART showed a better lipid effect with low prevalence of dyslipidemias, and therefore can be used as a preferred first line therapeutic regimen among aging population with other risk factors for metabolic syndrome and cardiovascular disease. Besides, dolutegravir showed significant improvements in erythroid and lymphoid lines with reduced proportions of anaemia. Both treatment regiments showed comparable efficacy in viral load suppression.

8. RECOMMENDATION

In this study we found that the prevalence of dyslipidemia in both treatment subjects was higher. Therefore, we recommend the health care providers to perform baseline and routine serum lipid test and to assess changes in cardiovascular event, provide health promotion activities on the use of life style modifications and appropriate therapeutic switches when needed. The prevalence of various types of cytopenia varies with the type of HAART. Thus, we recommend evaluation of hematologic parameters at regular interval and initiate appropriate treatment regimen based on laboratory findings. It is also important to address the issue of enhanced adherence counseling and periodic viral load determination to identify virological failure. We also recommend researchers to perform intensive research on different biochemical & hematologic parameters in both treatment regimens

9. STRENGTHS AND LIMITATIONS

9.1. Strengths

This study has the following strengths. It is the first report in Ethiopia in comparing serum lipid profile, CBC and viral load levels between patients taking efavirenz and dolutegravir based ART. Besides, samples run in duplicates.

9.2. Limitations

The present study has some limitations. As initial lipid values were not recorded, we cannot made comparisons from baseline. Dyslipidemia and cytopenia can be caused by opportunistic infections, malignancies and other silent health problems, which this study was unable to rule out these asymptomatic conditions. Again, we had no bone marrow aspirates, thus we lack bone marrow hemopoetic picture. In spite of these limitations, this study contributes to understand the possible effects of Efavirenz and Dolutegravir based ART on the investigated parameters.

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11. ANNEXES

Annex I: Information sheet

ADDIS ABABA UNIVERISITY

COLLEGE OF HEALTH & MEDICINE

DEPARTEMENT OF MEDICAL BIOCHEMISTRY

Good morning/ afternoon?

My name is **Bedlu Sahlu Hailemariam** Currently I am MSC student at Addis Ababa University, Department of Medical Biochemistry. I am conducting a research to assess serum lipid profile, Complete blood count, and viral load between HIV patients taking dolutegravir and efavirenz based ART at Wolkite health Center, Southern Ethiopia.

The main purpose of the study is to perform laboratory investigations which help to put a base line clinical data for the country regarding CBC and lipid profile and establish a functioning patient monitoring system to enable effective chronic HIV care, therapy and prevention; To attain this purpose your honest and genuine participation is very important and highly appreciable. I, therefore, kindly request you to participate in the research.

Please be assured that all the information gathered and the results of laboratory investigations will be kept strictly confidential and your name shouldn't be written on any of the questionnaire page. Only the researcher has the access of the information and used it for the study purpose only. You have a full right and decision to not respond all the questions or partly.

Thank you, have a nice day

Data Collector: Name ……. Signature …….Date ……..

Supervisor: Name ……. Signature …….Date …….

Annex II: Consent form

Everything about the study has been explained to me by the data collector. I understood the objective of this study. I also understood the risk and benefit of participation in this study. I agree to participate in the study and I here approve my agreement with my signature.

Participants: Name …… Signature …….Date …….

Data Collector: Name ……. Signature …….Date ……..

Investigator: Bedlu Sahlu Hailemariam

Phone Number: +251937874455

Annex III: Questionnaire: English version

Part I – Socio demographic characteristics

A. Unique ART Number ----------

B. Age in years? ----------

C. Gender: 1. Male 2. Female

D. Residence 1. Urban 2. Rural

E. Marital status: 1. Single 2.Married 3. Separated 4. Divorced 5.Widowed

F. Educational status: 1. Illiterate 2. Primary 3. Secondary 4. Diploma & Above

G. Religion: 1. Orthodox 2. Muslim 3. Protestant 4. Catholic 5. Other……..

H. Do you have History of Cardiovascular diseases: 1. Yes 2. No

I. Do you have history of alcoholism: 1. Yes 2. No

J. Do you have history of smoking: 1. Yes 2. No

K. Do you have history intravenous (IV) drug 1. Yes 2. No

L. Do you have history of using of lipid-lowering drugs: 1. Yes 2. No

M. Is there any drug that you are using? 1. Yes 2. No

N. If yes for question number 13, specify the type of drug you are using……

Part II – HIV/ART related questions

A. Duration of HIV infection is confirmed ……

B. Is there any illness currently: 1. Yes 2. No

C. If yes for question number 2, specify the type of illness ……

D. Are you diagnosed with TB positive? 1. Yes 2. No

E. If yes for question number 4, when……..

F. Are you currently taking anti TB drugs? …….

G. The type of ART currently used: …….

Part III Previous clinical data (from patient card)

A. WHO stage ……..

B. CD4 count ……..

C. Viral load …….

D. Opportunistic infections ……

E. Change of ART …….

Part IV – Physical findings

A. Blood pressure ……..

B. Pulse Rate ……

C. Temperature ……..

D. Weight ……..

E. Height ……..

F. Body Mass Index (BMI) …….

G. Waist Circumference…….

Part V – Laboratory findings

A. Complete Blood Count

1. Total WBC Count…….
2. Neutrophil Count ……..

3. Eosinophil Count ……
4. Basophil Count ……..
5. Lymphocyte Count ……
6. Monocyte Count …….
7. Platelet count ……..
8. RBC Indices
    - ✓ HCT ……
    - ✓ MCV ……
    - ✓ MCH ……
    - ✓ MCHC …….
    - ✓ RDW ……

B. Lipid profile

1. Total Cholesterol ……
2. Triglyceride …….
3. HDL – Cholesterol …….
4. LDL – Cholesterol …….
5. HDL – Cholesterol: Total Cholesterol…….

C. Viral Load: …….

D. Fasting Blood Sugar: ……

Annex IV: Questionnaire: Amharic version

የታሳታፎ ፍቃድና መተማመኛ ቅፅ

የታሳታፎ ፍቃድ

ሰላም ጤና ይስጥልኝ በድሉ ሳህሉ እባላለሁ። በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ በ ባዮኬሚስትሪ ትምህርት ክፍል የሁስተኛ ድግሪ ተመራቂ ተማሪ ስሆን Comparative assessment of serum lipid profile, Complete blood count, and viral load between HIV patients taking Dolutegravir and Efavirenz based ART at Wolkite health Center, Southern Ethiopia በሚል ርዕስ ጥናት እያጠናሁ አገኝለሁ።

የአርስዎ በዚህ ጥናት ላይ የሚኖርዎት ትሳትፎ ሙሉ በሙሉ በግል ፈቃደኝነት ላይ የተመሰረተ ሲሆን ለመሳተፍ አልያም ላለመሳተፍ ቢወስኑ እንኳ በዚህ ጤና ተቋም ዉስጥ የሚያገኙት ማንኛዉም አገልግሎት አይቋረጥም።

በዚህ ጥናት ለመሳተፍ ቃፈደኛ ከሆኑ አንዳንድ ቃስ መጠይቆችን ለመመለስና በስተመጨረሻ መጠነኛ የደም ናሙና ለመስጠት መስማማት ይጠበቅብዎታል።

የአርስዎ ተሳትፎ በአርስዎና በወገንዎ ደም ዉስጥ የሚገኘውን የቅባት፣ የደም ሴልና የቫርሱን መጠን ከሚወሰድው መድሀኒት ጋር ያለዉን ተያያዝነት እና ስጋት በማወቅ ህሙማን በለማራጭና በተሻለ ሁኔታ እንዲረዱ ያግዛል።

በዚህ ጥናት መሳተፎ የሚያስክትለዉ ጉዳት ባይኖርም መጠነኛ የደም ናሙና ከእጆዎ በምወሰድበት ጊዜ አነስተኛ የህመም ስሜት ሊኖር ይችላል። ነገር ግን ናሙናዉ የሚወሰደዉ በከፋተኛ ጥንቃቄ ልምድ ባለዉ ባለሙያ ስለሆነ ህመሙ በጥቂት ደቂቃዎች ዉስጥ ይተዋል።

ከአርስዎ የሚወሰድው መረጃና ናሙና ጥቅም ላይ የሚዉለዉ ለጥናቱ ለላማ ብቻ ነዉ። ቀጥሎም የተሰበሰበዉ መረጃ ለስራዉ አግባቢነት ላላቸዉ ጥቂት ሰዎች ብቻ የሚደርስ ሲሆን የራስዎን ማንነት የሚገልጡ መረጃዎች ማለትም ስም ፤ አድራሻ ፤ የስልክ ቁጥር እና የመሳሰሉትን ግን አያካትትም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለመለየት የሚረዳ መለያ ቁጥር ብቻ ጥቅም ላይ ይዉላል።

መተማመኛ ቅፅ

ከሳይ የተዓፈዉን ሀሳብ ፤ የጥናቱን ዓላማ፤ በጥናቱ ዉስጥ በመሳተፌ የሚገኝዉን ጥቅምና ጉዳት በግልጽ ተረድቻለሁ።

ስለዚህ በዚህ ጥናት ዉስጥ ለመሳተፍ ፍቃደኛ ነኝ።

ስም፡ .....

አደደለሁም፡ .....

የተሳታፊው ፊርማ፡ .....

.

የአጥኚዉ ፊርማ፡ .....

.

ለለ ፍቃደኝነትዎ አመሰግናለሁ!!!

ክፍል አንድ ፡ የማህበረሰብና ስነህዝብ ባህርያት በተመለከተ

1. የፀረ-ኤች አይ ቪ መለያ ቁጥር .....

2. ዕድሜ .....

3. ፃታ፡ 1. ወንድ 2. ሴት

4. የመኖርያ አካባቢ፡ 1. ገጠር 2. ከተማ

5. የጋብቻ ሁኔታ፡ 1. ያላገባ 2. ያገባ 3. የተለያየ 4. ባለቤቱን በሞት ያጣ

6. የትምህርት ሁኔታ፡ 1. አልተማርኩም 2. ሁለተኛ ደረጃ 3. ዲፕሎማና ከዛ በላይ

7. ሀይማኖት 1. ኦርቶዶክስ 2. እስልምና 3. ፕሮቴስታንት 4. ካቶሊክ 5. ሌላ.....

8. የታወቀ የልብ ህመም አልብዎ፡ 1. አው 2. አይደለም

9. አልኮል ይጠቅማሉ፡ 1.አው 2. አይደለም

10. ሲጋራ ያጨሳሉ፡ 1. አው 2. አይደለም

11. በደም ስር የሚወሰዱ መድሀኒቶችን ይጠቀማሉ፡ 1. አው 2. አይደለም

12. ኮሌስትሮልን ለመቀነስ የሚረዱ መድሀኒቶችን ይጠቀማሉ፡ 1. አው 2. አይደለም

13. እየተጠቀሙ ያሉት መድሀኒት አለ፡ 1. አው 2. አይደለም

14. እየተጠቀሙ ያሉት መድሀኒት ካለ የምን .....

ክፍል ሁለት፡ ከ ፀረ-ኤች አይ ቪ እና ተያያዥ ጉዳዮች ጋር በተያያዘ

1. ቫይረሱ ከተገኘብዎት ምን ያህል ጊዜ ይሆናል? .....
2. በአሁን ሰአት የሚያሞት ነገር አለ? .....
3. የሚያሞት ነገር ካለ? ይገልፁ .....
4. የቲቢ በሽታ ትገኝቶቦታል? 1. አው 2. አይደለም

5. የቲቢ በሽታ ከ ተገኝቦት ምቼ?.....

6. በአሁን ሰአት የቲቢ በሽታ ህክምና እየወሰዱ ነው? .....

7. የትኛውን ፀረ-ኤች አይ ቪ መድሀኒት ነው የሚጠቀሙት? .....

ክፍል ሶስት፡ የቀድሞ ክሊኒካል መረጃ

1. በአለም ጤና ድርጅት መስፈርት የበሽታው ደረጃ .....

2. ቫይረሱ የሚያጠቃው የነጭ ደም ሴል ቁጥር.....

3. የቫይረሱ ብዛት/ ጫና.....

4. ተጨማሪ የጤና ችግር.....

5. የፀረ-ኤች አይ ቪ ለውጥ .....

ክፍል አራት፡ በምርመራ ወይም በልኬት የሚገኙ/ የሚታወቁ

1. የደም ግፊት መጠን .....

2. የልብ ትርታ መጠን .....

3. የሙቀት መጠን .....

4. የክብደት መጠን .....

5. ቁመት .....

6. የክብደትና ቁመት ንፅፅር መጠን .....

7. የወገብ ልኬት .....

ክፍል አምስት፡ የላቦራቶሪ / የደም ናሙና ዉጤት በተመለከተ

1. Total WBC Count.....

2. Neutrophil Count .....

3. Eosinophil Count …….

4. Basophil Count ……..

5. Lymphocyte Count …….

6. Monocyte Count ……..

7. Platelet count ……..

8. RBC Indices

    a. HCT …….

    b. MCV …….

    c. MCH …….

    d. MCHC …….

    e. RDW …….

ክፍል ስድስት፡ የደም የ ሲፒድ መጠን

1. Total Cholesterol …….

2. Triglyceride ……..

3. HDL — Cholesterol …….

4. LDL — Cholesterol ……..

5. HDL — Cholesterol: Total Cholesterol…….

የቫይረሱ ብዛት/ ጫና……..

የደም የስኳር ምጠን …….

Annex V: Standard operating Procedures

Standard operative Procedure for serum lipid Profile by Roche Hitachi 704 Chemistry Analyzer

Analyte: Total Cholesterol, HDL-Cholesterol, Triglycerides, and LDL-Cholesterol

Matrix: Serum

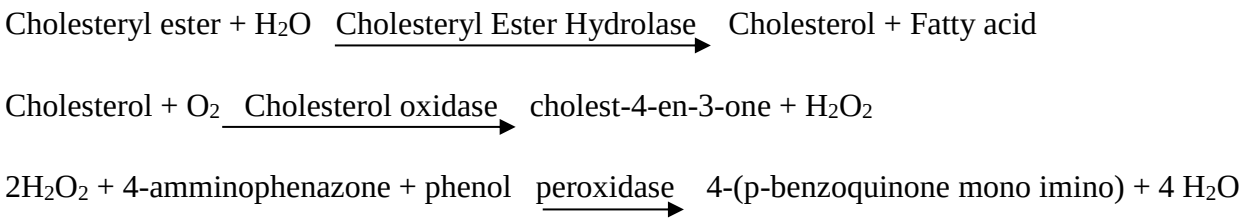
Description

The Roche Hitachi 704 Chemistry Analyzer is a fully automated, discrete, and computerized chemistry analyzer. It uses serum; urine, plasma, and CSF samples to determine quantitative and qualitative tests on a wide range of analytes. In addition it is capable of performing potentiometric and photometric assays.

Test principles

Total cholesterol determination

Cholesterol was measured enzymatically in serum in a series of coupled reactions that hydrolyzed cholesterol esters and oxidized the 3-OH group of cholesterol. Esterified cholesterol was hydrolyzed to free cholesterols by cholesterol esterase. The free cholesterol then oxidized to form hydrogen peroxide, which later reacts with phenol and 4 -amino antipyrine in the presence of peroxidase catalyst to produce a red coloured quinoneimine dye complex. The absorbance was measured at 500nm wave length and proportional to cholesterol concentration present in the sample. The color intensity is proportional to cholesterol concentration. Desirable cholesterol levels were considered to be below 200mg/dL. The reaction sequence is as follows:



Serum triglycerides level determination

Triglycerides are measured enzymatically in serum using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol using lipoprotein lipases. The glycerol is then phosphorylated by adenosine triphosphate (ATP) by glycerol kinases (GK) to form glycerol-3 -phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is then oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). A red colored product will be formed by peroxidase (POD) catalyzed reaction of 4-amnioantipyrine and phenol with hydrogen peroxide (H_2O_2), and the optical density will be read at 540 nm wave length which will be proportional to the concentration of triglyceride present in the sample. Desirable level will be considered when TG level is less than 150 mg/dL (DHHSP, 2000): The reaction sequence is as follows:

Triglycerides $\xrightarrow{\text{Lipases}}$ glycerol + fatty acids

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

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{glycerophosphate oxidase}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + 4-aminoantipyrine $\xrightarrow{\text{peroxidase}}$ quinoneimine +HCl +H_2O + 4-chlorophenol

Serum High density lipoprotein cholesterol level determination

HDL is measured directly in serum with the following basic principle: The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-cholesterol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg 2+, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The absorbance is then measured at 600 nm. The reaction sequences are summarized as follows:

ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO4 $\longrightarrow$ soluble non-reactive complexes with apoB-containing lipoproteins

HDL-cholesteryl esters PEG-cholesteryl esterase $\longrightarrow$ HDL-unesterified cholesterol + fatty acid

Unesterified cholesterol + O_2 PEG-cholesterol oxidase $\longrightarrow$ cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H_2O + H+ peroxidase $\longrightarrow$ qunoneimine dye + H_2O

Serum Low density lipoprotein cholesterol level determination

The Friedewald formula was used to calculate LDL concentration, which uses measured values of total cholesterol, triglycerides and HDL-cholesterol. The formula is given as: LDL-cholesterol = total cholesterol - HDL-cholesterol – (TG/5); Where TG/5 can be taken as an estimate of VLDL-cholesterol and all values were expressed in mg/dL (Friedewald et al., 1972). Desirable levels of LDL-chol are those below 130 mg/dL in adults (DHHSP, 2000).

Safety precautions

All personnel working in the laboratory must wear gloves and laboratory coats. All used gloves, vials, pipettes and other items that come in contact with specimens are disposed of in a Biohazard bag. Do not pipet samples by mouth. Avoid any contact with serum. Cover any scratches or cuts on fingers with adhesive plasters and hands and wear gloves before handling serum. Store all samples in sealed containers. In order to minimize the formation aerosols, do not leave samples open to the atmosphere longer.

Specimen collection, storage and handling

Recent food intake exerts little effect on plasma total cholesterol concentration. Plasma triglycerides, however, increase in postprandial plasma to an extent that is related to the fasting triglyceride levels and the amount of fat intake. This is due to the appearance of chylomicrons in the circulation after a fat-containing meal. Chylomicrons are normally cleared within 9-12 hr. and no chylomicrons should be present after a 12 hr. period of fasting. Transient decreases in HDL-chol and LDL-chol also occur, the magnitude of which depends on the fat content of the meal. Triglyceride is measured only in specimens drawn from participants who have fasted at least 9 hours before venipuncture.

Serum vs. plasma: In general, anticoagulants exert osmotic effects in which water leaves the cells and enters the plasma, thus diluting the plasma and lowering the concentrations of non-diffusible

components. The magnitude of this effect depends on the anticoagulant used and its concentration. Serum cholesterol and triglyceride concentrations are about 3-5% higher in serum than in EDTA plasma, although no significant serum-plasma difference was observed for HDL. Thus, the serum concentrations of lipids and lipoproteins probably reflect more accurately the subjects' physiological state at the time of venipuncture. Serum is used for measuring lipids and lipoproteins in NHANES 2001-2002, as it has been for previous HANES surveys.

Sample volumes: The sample volumes required are as follows: total cholesterol and/or triglyceride, 0.5 ml; HDL measured with the direct method, along with total cholesterol, 0.2 ml. Any sample remaining after analyses are complete are returned to -80 oC, and subsequently sent to the NHANES 2001-2002 serum bank as directed by NCHS.

Serum shipment and storage container: In NHANES 2001-2002, plastic, screw top cryovials are used to ship and store serum. Different size vials are used for 3-5 year old children and those >5 years old. Serum can be stored at -20°C in a non-self- defrosting freezer for up to 4 weeks. For longer storage (> 4 weeks) they should be maintained at -80°C or lower. Total cholesterol, triglyceride and HDL-cholesterol are stable for at least one year at -80 ^{o}C or lower.

Specimen handling

Collect blood into a glass tube such as a red top Vacutainer® blood collection tube. Allow the blood to stand for 45 min at room temperature to allow complete clotting and clot retraction. A shorter period may result in incomplete clotting and secondary clots may form later. During the clotting period leave the collection tube sealed. Then, Centrifuge the samples at 1,500 x g for 30 min at 4°C. It is preferable to use a refrigerated centrifuge for this purpose, but an unrefrigerated centrifuge can be used if necessary. Finally, samples should be kept frozen at -20°C.

Reagents required for operating instrument

Working solution (NaOH and Hitergent): Add 20 mL Hitergent to deionized water and brings to 1,000 mL. The components of Cholesterol High Performance System Pack Reagents (Roche Diagnostics, Indianapolis, IN) include (taken from package insert): Cholesterol Reagent (16 x 50 mL) 75 mmol/L PIPES buffer, pH 6.8 10 mmol/L Mg2+ 0.2 mmol/L Sodium cholate 0.15 mmol/L 4-Aminophenazone > 4.2 mmol/L Phenol > 0.5 U/mL Cholesterol esterase Cholesterol

oxidase, Peroxidase 1% Fatty alcohol-polyglycol ether Buffer, unspecified stabilizers, unspecified preservative The reagent is supplied as a solution and is ready to use. After being opened, the reagent is stable for 28 days at 2-12 °C, or 7 days at room temperature. Protect reagent from light.

Triglyceride Reagents: The components of the Triglycerides (GPO) System Pack include (from package insert): 50 mmol/L PIPES buffer, pH 6.8 40 mmol/L Mg++ 0.20 mmol/L Sodium cholate > 1.4 mmol/L ATP > 0.13 mmol/L 4-Aminophenazone 4.7 mmol/L 4-Chlorophenol 1 μmol/L Potassium hexacyanoferrate (II) 0.65% Fatty alcohol polyglycol ether > 5.0 U/mL lipoprotein lipase, glycerol kinase, glycerophosphate oxidase, Peroxidase unspecified preservative The reagent is supplied as a solution and is ready for use. When opened, the solution is stable for 14 days at 2-12° C, or 7 days at room temperature (15-25° C).

Direct HDL-cholesterol method (a) The Direct HDL-cholesterol reagents, R1 and R2 contain the following components (from package insert): R1 Cyclodextrin/Buffer, supplied as a solution, ready to use. 0.5 mmol/l α-cyclodextrin 0.5 g/l dextran sulfate 7.0 mg/ml magnesium sulfate (MgSO4) 0.3 g/l EMSE 10 mmol/l MOPS (3-morpholino-propane sulfonic acid) buffer, pH 7.0) unspecified preservative

Storage and Stability:

Store cholesterol and triglyceride reagents on the analyzer at two to twelve degree centigrade The solution is stable for 4 weeks at 2-12° C or 7 days at 20-25° C when protected from light and contamination by microorganisms. The reagent is stable for 14 days at 2-12° C or 7 days at room temperature. Store unopened heparin at 20-25° C. Stable until the expiration date printed on the bottle label. Direct HDL-cholesterol. R1 Reagent is stable unopened up to the stated expiration date.

Acceptable values

Total Cholesterol: 200 -239mg/dL

Triglycerides: 150 -199mg/dL

LDL-c < 130 mg/dL

HDL-c > 60 mg/dL

TC/ HDL < 5

Limitations of the method

The following information applies to both cholesterol and triglyceride. Hemolysis: No interference up to 200 mg/dL hemoglobin. Lipemia: No interference unless lipemia is marked, generally over 1,000 mg/dL. Turbid specimens are automatically flagged by the analyzer, and are reanalyzed after dilution with 0.15 M NaCl. Note that in NHANES 2001-2002, specimens with TG > 600 mg/dL are diluted with 0.15 M NaCl and reanalyzed. Bilirubin: No interference up to 12 mg/dL. The color reactions used to measure cholesterol and triglyceride are unaffected by common interfering substances such as uric acid, creatinine, and glutathione. Hemolysis: No significant interference from hemoglobin up to a hemoglobin index of 1000. Lipemia: No significant interference from triglycerides up to 1,200 mg/dl. Ascorbic Acid: No significant interference from ascorbic acid up to 15 mg/dl.

Hematology Standard Operating Procedures for fully Automated Hematology Analyzer – HEMAX 330

Analyte: It measures the following 18 hematologic parameters: WBC (Lymphocyte, Monocyte, and Granulocyte) Platelet (PLT count, PDW, PCT), RBC, HGB, HCT, MCV, MCH, MCHC, RDW, and MPV).

Matrix: Whole blood collected into EDTA tubes

Method: Hemax 330 hematology analyzer, which is a fully automated cell counter for in vitro diagnostic use

Storage and transport temperature: 2 - 8ºC up to 6 hours

Description:

Hemax 330 hematology analyzer is a fully automated cell counter for in vitro diagnostic use. It can process one sample within a minute with a specified accuracy and reproducibility; it can as well withstand a maximum of about 2000 sample capacity including histograms.

It requires no manual operations for aspirating blood, dilutions, measuring, calculations, print-outs and computer transfer of data. It measures the following 18 hematologic parameters: WBC (Lymphocyte, Monocyte, and Granulocyte) Platelet (PLT count, PDW, PCT), RBC, HGB, HCT, MCV, MCH, MCHC, RDW, and MPV).

Test principle

Hemax 330 hematology analyzer is a fully automated cell counter for in vitro diagnostic use, which was first, calibrated using three controls Low, Normal and High levels from Humatrol then patients samples were run to obtain the full haemogram report. The non-coagulated blood collected in ethylene diethyl trichloracetic acid (EDTA) tubes were placed on a roller which facilitated mixing and each sample was then fed into the open system machine and sucked by a probe. Complete blood cell counts report was obtained and printed out**.** The hemoglobin concentration is measured photometric and hematocrit value, red cell indices mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) calculated automatically.

It used electrical impedance method to determine WBC, RBC, PLT and differential counts. As the cell pass through small aperture, a change in electrical resistance occurs generating an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of WBC, RBC, and PLT counted and the intensity of each pulse is essentially proportional to the cell volume

Procedure

Switch on the analyzer: Turn ON the analyzer with the "rear power button".

SAMPLE TEST

- ✓ Ensure proper sample mixing in a sample tube by shaking the tube up and down for 3-5 minutes.
- ✓ Don't shake the tube acutely and the sample waiting for test should be stored at a room temperature and only valid for testing within 4 hrs.
- ✓ Before counting please ensure you select the right *"MODE*" of testing and have the correct sample ready to avoid aperture blockage.
- ✓ Follow the recommended test procedure.
    - ➢ Put sample tube under the aspiration pipette, press the "ASPERATE KEY".
    - ➢ A status on the top of the screen displays "testing……." When the analyzer starts to analyzer the sample.
    - ➢ Incase testing on "pediluted - peripheral blood mode " prepare one tube and put it under the needle ,click "diluent" press "aspirate key" then 700ul diluent will be dispensed. Accurately add 20ul peripheral blood and mix completely. Finally aspirate the liquid /sample for testing.
- ✓ On the test window click "profile", edit information of patient before or after the test.
- ✓ At sample test window click "print" button to print the test report if necessary.
- ✓ Click "shut down "interface on the window when shutting down the instrument, when the display turns "black screen" switch off the analyzer.

Responsibilities:

- ✓ Hematology department personal are required to be knowledgeable of this procedure.

- ✓ New employees are trained and assessed for competence before they can handle patient sample.
- ✓ The head of the department must resolve any problem with the process and difficulties in using this SOP.

Specimen requirements:

- ✓ About 2ml of venous blood collected into EDTA tubes.
- ✓ Specimens should be transported at room temperature 18 - 26ºC and can be store in the refrigerator of 2 - 8ºC up to 6 hours. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis.

Specimen reception:

Reception of request and sample should be recorded, and record time of reception. Pay attention to sample identification and labeling of tubes.

Criteria for rejection haematologic specimens

- ✓ Inadequate identification
- ✓ Insufficient quantity
- ✓ Inappropriate container
- ✓ Inappropriate transport/storage.
- ✓ Unknown duration of delay
- ✓ Clotted sample

Quality control procedures:

1. At the beginning of each work shift all parameters are tested with blood control.

2. The 3 levels include: Abnormal Low, Normal, Abnormal and High

3. Controls are stored at 2-8ºC and brought to room temperature on a roller mixer before use.

4. Controls are gently inverted many times according to the manufacturer's instruction before use.

5. From the RUN screen, press [SPECIMEN TYPE].

6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key.

7. Control values must be within a three standard deviation otherwise the measurement has to be repeated, if the control still out of range:

a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.

b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable. If this does not solve the problem:

- ✓ Prepare new control(s) and try again.
- ✓ If the controls are still out, inform your supervisor to check the operator's manual, or recalibrate instrument and If controls are still out,. Contact Medical Maintenance where applicable, or servicing engineer.

8. All control data are managed using software that provides graphical reports (LeveyJennings graphs, and monthly cumulative histograms).

Limitations/ Interfering substance:

Verification of any "Abnormal" test result (including flagged results or results outside their normal range) is due to the following listed:

WBC White Blood Cells (Leukocytes): NRBC, Non-lysed Red Cells, Multiple myeloma, Hemolysis, Leukemia, Increased turbidity, Chemotherapy, Cry globulins

RBC Red Blood Cells (Erythrocytes): High WBCs, Agglutinated red blood cells, Cold agglutinins.

HGB (Hemoglobin): Turbidity of the blood sample which may be due to Elevated WBC or Elevated Lipids, Fetal bloods mixed with maternal bloods may produce a falsely elevated hemoglobin value.

PLT (Platelets): Very small erythrocytes, Agglutinated red blood cells, Giant platelets in excessive numbers, Chemotherapy, Hemolysis, RBC inclusions, Platelet agglutination.

Expected values of Complete blood count in adults:

Total WBC: 4000 - 10000 cells/ dL

Percentage of Neutrophil: 50-70%

Percentage of lymphocyte: 20 -40%

Percentage of monocytes: 3-12%

Percentage of eosinophils: 0.5-5%

Percentage of basophils: 0-1%

RBC count: 3.5-5 $X10^6$/ dL

HCT value: 37-47 %

Hemoglobin <13 - 16 g/dL **(men) and <12-15** g/dL **(women)**

RBC indices

MCV: 80-100 fL

MCH: 27-34pg

MCHC: 320-360g/L

RDW-CV: 11-16%

RDW- SD: 35-56fl

Platelete: 150, 000 – 450,000 / dL

Interpretation of the results:

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

Standard Operating Procedures for Viral load Determination for COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0

Description

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0 is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human EDTA plasma or from a cobas® Plasma Separation Card (PSC) dried plasma spot using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection. The test can quantitate HIV-1 RNA over the range of 20 - 10,000,000 copies/mL (33 to 1.67 x 107 International Units /mL in EDTA plasma and 738 – 10,000,000 copies/mL.

One copy of HIV-1 RNA is equivalent to 1.67 IU based on the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 group M and HIV-1 group O infected patients. The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

Principles of the test

The COBAS® TaqMan® instrument for specimen processing amplification, detection, and Quantification of HIV-1 RNA over 20copies/ml was used to determine HIV viral load by using the manufacturer's instructions with the use of the AmpliPrep version 2.0.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human EDTA plasma or from a PSC dried plasma spot. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target. The COBAS® AmpliPrep/COBAS®

TaqMan® HIV-1 test permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation. The Master Mix reagent contains primers and probes specific for both HIV-1 RNA and HIV-1 QS RNA. The detection of amplified DNA is performed using target-specific and QS-specific dual-labeled oligonucleotide probes that permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon.

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculates the HIV-1 RNA concentration in the test specimens by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

Target Selection

Selection of the target RNA sequence for HIV-1 depends on identification of regions within the HIV-1genome that show maximum sequence conservation among the various HIV-1 groups M subtypes and HIV-1 group O specimens. In order to address the high genetic variability of the virus, two regions of HIV genome are simultaneously targeted for amplification and detection by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. Two target-specific and one QS-specific dual-labeled oligonucleotide probes permit independent identification of the HIV-1 amplicon and of the HIV-1 QS amplicon. Accordingly, the appropriate selection of the primers and the dual-labeled oligonucleotide probes is critical to the ability of the test to amplify and detect the HIV-1 group M subtypes and HIV-1 group O.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The procedure processes 850 µL of plasma or SPEX-based extract from the PSC. The HIV-1 virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HIV-1 RNA from RNases in plasma. Protease and a known number of HIV-1 QS Armored RNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the HIV-1 RNA and HIV-1 QS RNA are bound to the surface of the magnetic glass particles.

Reverse Transcription and PCR Amplification

The reverse transcription and PCR amplification reaction is performed with the thermostable recombinant enzyme Thermus specie Z05 DNA Polymerase (Z05). In the presence of manganese ($Mn^{2+)}$ and under the appropriate buffer conditions, Z05 has both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon. Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow the downstream primers to anneal specifically to the HIV-1 target RNA and to the HIV-1 QS RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, Z05 polymerase extends the annealed primers forming DNA strands complementary to the RNA target.

Target Amplification Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. Following reverse transcription of the HIV-1 target RNA and the HIV-1 QS RNA, the Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the RNA:cDNA hybrids and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05 in the presence of Mn2+ and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, extends the annealed primers along the target template to produce double-stranded DNA molecules termed amplicons.

TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the two regions of the HIV-1 genome between the primers; the entire HIV-1 genome is not amplified Selective Amplification Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan®

HIV-1 Test, v2.0 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA

strands containing deoxyuridine34, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine.

Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55ºC, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 utilizes real-timePCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HIV-1 and HIV-1 QS-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 the HIV-1 and HIV-1 QS probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects.

During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' $\rightarrow$ 3' nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HIV-1 RNA and HIV-1 QS RNA are measured independently at different wavelengths. This process is repeated for a designated number of

cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HIV-1 RNA and HIV-1 QS RNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

Fundamentals of COBAS® TaqMan® Test Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is inherently quantitative over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HIV-1 titer of a specimen, the earlier the fluorescence of the reporter dye of the HIV-1 probes rises above the baseline fluorescence level. Since the amount of HIV-1 QS RNA is constant between all specimens, the fluorescence of the reporter dye of the HIV-1 QS probe should appear at a similar cycle for all specimens. In specimens where the QS fluorescence is affected, the concentration is adjusted accordingly. The appearance of the specific fluorescent signals is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a predetermined threshold (the Assigned Fluorescence Level), and starts the exponential growth phase of this signal.

HIV-1 RNA Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 quantitates HIV-1 viral RNA by utilizing a second target sequence (HIV-1 Quantitation Standard) that is added to each test specimen at a known concentration. The HIV-1 QS is a non-infectious Armored RNA construct, containing fragments of HIV-1 sequences with primer binding regions identical to those of the HIV-1 gag target sequence. The HIV-1 QS contains HIV-1 primer binding regions and generates an amplification product of the same length and base composition as the HIV-1 gag target RNA. The detection probe binding region of the HIV-1 QS has been modified to differentiate HIV-1 QS amplicon from HIV-1 gag target amplicon.

During the annealing phase of the PCR in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the

instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HIV-1 RNA and HIV-1 QS RNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HIV-1 RNA and the HIV-1 QS RNA. The lot-specific calibration constants provided with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 are used to calculate the titer value for the specimens and controls based upon the HIV-1 RNA and HIV-1 QS RNA Ct values.

WARNINGS AND PRECAUTIONS

- ✓ This test is for use with human plasma collected in the anticoagulant EDTA or from a PSC dried plasma spot.
- ✓ Do not pipette by mouth.
- ✓ Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- ✓ Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control vials.
- ✓ The use of sterile disposable pipettes and RNase-free pipette tips is recommended.
- ✓ Do not pool controls from different lots or from different bottles of the same lot.
- ✓ Do not mix reagent cassettes or controls from different kits.
- ✓ Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles
- ✓ Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.
- ✓ Do not use a kit after its expiration date.
- ✓ Safety Data Sheets (SDS) are available on request from your local Roche office.
- ✓ Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories

STORAGE AND HANDLING REQUIREMENTS

- ✓ Do not freeze reagents or controls.
- ✓ Store HIV-1 v2.0 CS1, HIV-1 v2.0 CS2, HIV-1 v2.0 CS3 and HIV-1 v2.0 CS4 at 2-8ºC. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 28 days at 2-8°C or until the expiration date, whichever comes first. HIV-1 v2.0 CS1, HIV-1 v2.0 CS2, HIV-1 v2.0 CS3 and HIV-1 v2.0 CS4 can be used for a maximum of instrument cycles, up to a maximum of 64 hours cumulative on board the COBAS® AmpliPrep Instrument. Reagents must be stored at 2-8°C between instrument cycles.
- ✓ Store HIV-1 H (+) C, v2.0, HIV-1 L (+) C, v2.0 and CTM (–) C at 2-8ºC. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.
- ✓ Store Barcode clips [HIV-1 H (+) C, v2.0 Clip, HIV-1 L (+) C, v2.0 Clip and HIV-1 (–) C Clip] at 2-30°C.
- ✓ Store PG WR at 2-30°C. PG WR is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.
- ✓ Store SPEX (used in PSC dried plasma spot procedure) at 2-8°C. SPEX is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.

Instrumentation and Software

- ✓ COBAS® AmpliPrep Instrument
- ✓ COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- ✓ Optional: cobas p 630 Instrument
- ✓ Optional: Docking Station
- ✓ AMPLILINK Software Version 3.3 or Version 3.4 Series
- ✓ Control Unit for the AMPLILINK Software, with printer
- ✓ Instrument and Software Manuals

Specimen Collection

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 is for use with plasma specimens. Blood should be collected in sterile tubes using EDTA as the anticoagulant and mixed adequately according to the tube manufacturer's instructions. Store whole blood at 2-25°C for no longer than 24 hours. Separate plasma from whole blood within 24 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer plasma to a sterile polypropylene tube.

Specimen Transport

Transportation of whole blood or plasma must comply with country, federal, state and local regulations for the transport of etiologic agents40. Whole blood must be transported at 2-25°C and centrifuged within 24 hours of collection. Plasma may be transported at 2-8°C or frozen at -20°C to -80°C.

Specimen Storage

Plasma specimens may be stored at room temperature (25-30°C) for up to 1 day or at 2-8°C for up to 6 days. Plasma specimens were shown to be stable for six weeks if frozen at -20°C to -80°C. It is recommended that specimens be stored in 1100-1200 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes. Plasma specimens may be frozen and thawed up to five times without a significant loss of HIV-1 RNA.

Results

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the HIV-1 RNA concentration for the specimens and controls. The HIV-1 RNA concentration is expressed in copies/mL or IU/mL, depending on the used TDF. The conversion factor between HIV-1 RNA copies/mL and HIV-1 IU/mL is 0.6 copies per IU.

Interpretation of Results

< 20 HIV RNA copies / ml = Undetected viral load

20 -1000 HIV RNA copies / ml = viral load suppression

1000 – 100000 HIV RNA copies / ml = high viral load

> 100000 HIV RNA copies / ml = very high viral load

PROCEDURAL LIMITATIONS

1. This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.

2. The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 has neither been evaluated with specimens containing HIV-1 group N, nor with specimens containing HIV-2.

3. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

4. The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 Master Mix reduces the risk of amplicon contamination. However, contamination from HIV-1 positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.

5. Use of this product should be limited to personnel trained in the techniques of PCR.

6. This product can only be used with the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

7. Though rare, mutations within the highly conserved regions of the viral genome covered by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 primers and/or probes may result in the under-quantitation of or failure to detect the virus.

8. Detection of HIV-1 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors, (i.e., age, presence of symptoms, and/or stage of the infection). While the clinical specificity of the test is 99.3% (95% CI = 98.2% to 99.8%), some low level false positive results in HIV-negative individuals have been noted.

9. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next; users perform method correlation studies in their laboratory to quantify technology differences.

Standard Operating Procedure for blood glucose determination using POCT – ASCENSIA® CONTOUR® BLOOD GLUCOSE METER

Description

The Ascensia® CONTOUR® Blood Glucose Meter is used to monitor blood glucose levels

PRINCIPLE OF THE PROCEDURE

When a blood sample is applied to the test strip, the glucose in the blood reacts with the chemicals on the test strip, producing a small electrical current. This current is measured and then a result is displayed by the monitor. The size of the current depends of the amount of glucose in the blood sample.

SPECIMEN REQUIREMENTS

Test sample: Whole blood (usually a capillary sample taken from the heel avoiding the posterior aspect).

Sample volume: 0.6μL. The meter will beep and the fifteen second countdown will commence when sufficient sample has been applied to the test strip.

HEALTH AND SAFETY

All patient samples are a potential infection risk. Follow appropriate procedures (e.g. use gloves). Cover cuts and abrasions on own hands and forearms with water-repellent 'island' type plasters. If hands do become contaminated with blood, wash immediately with soap and water.

Used lancets should be disposed of in a sharps bin. Any other used materials should be discarded into clinical waste in accordance with ward/site procedures.

EQUIPMENT AND INSTRUMENTATION

Blood lancing device: Ascensia® CONTOUR® Blood Glucose Meter

REAGENTS, STANDARDS, CALIBRANTS AND CONTROLS

Ascensia® MICROFILL® Test Strips

Ascensia® CONTOUR® Control Solutions (High and Low)

Method

Control Solution Testing

Remove a test strip from the bottle. Tightly close the bottle lid immediately after you have removed the test strip. NOTE: check the expiry and discard date. Make sure the test strip does not appear torn or damaged.

Hold the test strip with the grey end facing up. Insert the grey end into the test strip port on the meter.

The meter will turn on. A test strip with a flashing blood drop will appear letting you know the meter is ready to test.

Gently invert the control bottle to mix. Squeeze a small drop of control solution on a clean nonabsorbent surface. Do not apply control solution to the test strip directly from the bottle.

Immediately touch the tip of the test strip to the drop of control solution. Hold it in the drop until the meter beeps.

After the beep, you will see the meter countdown fifteen seconds until the result is complete and the control test result is displayed.

The meter will automatically recognize and mark √ the control result for you.

Compare your control test result with the Low and High control range values found on the bottom of the test strip box and make a record on the Quality Control Record Sheet for the meter.

Remove and discard the test strip.

Patient Testing

Wash your hands and dry them completely. Wear nitrile/latex gloves.

Select and prepare the sample site for blood collection.

Remove a test strip from the bottle. Tightly close the bottle lid immediately after you have removed the test strip.

Hold the test strip with the grey end facing up. Insert the grey end into the test strip port on the meter.

The meter will turn on. A test strip with a flashing blood drop will appear letting you know the meter is ready to test.

Immediately touch the tip of the test strip to the drop of blood.

Hold the tip of the test strip in the blood drop until the meter beeps.

After the beep, you will see the meter countdown the fifteen seconds until the test is complete and the result is displayed.

Record the glucose result in notes and include any action taken.

Remove and discard the test strip.

QUALITY CONTROL TESTING

Ascensia® MICROFILL® Control Solutions are used to check that the meter and test strips are working properly. Control Solution testing, using both High and Low, must be performed daily.

LIMITATIONS OF THE EXAMINATION

The Ascensia® CONTOUR® Blood Glucose Meter should not be used if the patient is severely dehydrated, hypotensive, in hyperglycemic-hyperosmolar state, in shock or in peripheral shutdown.

Fluoride/oxalate should not be used as a sample preservative if it is to be used on the Ascensia® CONTOUR®

Neonatal use: At glucose levels between 0.6mmol/L and 6.7mmol/L, Ascensia® MICROFILL® Test Strips are not significantly affected by Haematocrit levels in the range of 20% to 70%.

NB. The limitations are also provided in each box of test strips.

RECORDING AND CALCULATION OF RESULTS

All results are displayed in mmol/L. The analytical range is 0.6 – 33.3mmol/L (60 -126mg/dL). A result <0.6mmol/L (< 60mg/dL) will be displayed as LO and a result >33.3mmol/L (>126mg/dL) will be displayed as HI.

Annex VI: Letters

Ethical Clearance

BIOCHEMISTRY DEPARTMENT
SCHOOL OF MEDICINE
ADDIS ABABA UNIVERSITY

ባዮኬሚስትሪ ት/ክፍል
ሕክምና ት/ቤት አዲስ አበባ
ዩኒቨርሲቲ

Ref. No. SOM/BCHM/๐๑๐/2012
Date: 12/3/2020

Departmental Research Ethics and Review Committee (DRERC) Decision

Meeting No. DRERC 02/20

Protocol Number: M.Sc. 05/20

Research Topic: **Comparative Assessment of Serum Lipid Profile, Complete Blood Count and Viral Load Suppression Between HIV Positive Patients Taking Dolutegravir and Evavirenz Based Anti-Retroviral Therapy at Wolkite Health Center, Southern Ethiopian, 2019-2020**	
Principal Investigator	**Bedlu Sahlu**
Institute:	Department of Biochemistry, School of Medicine. Addis Ababa University
Elements reviewed	Attached
Decision of the meeting	√ **Approved**
	Approved with Recommendation
	Disapproved

Obligation of the PI:-

- Should comply with national and international scientific and ethical guidelines
- All amendments and changes made in protocol and consent needs DRERC approval
- DRERC approval period from: 12/03/2020

Secretary

Sisay Addisu

Dept. of Biochemistry Medical Faculty A.A. ADDIS ABABA UNIVERSITY

Chairman

Dr. Solomon Genet

P.O Box: 9086
School of Medicine, AAU.
Addis Ababa, Ethiopia.
Email: biochemistry.som@aau.edu.et
FAX: 251-1-551-30-99
TEL: 251-011-550-52-48

Letter from department of Biochemistry

ADDIS ABABA UNIVERSITY
SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY

አዲስ አበባ ዩኒቨርሲቲ
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ቁጥር፡SOM/BCHM/112/2020

ቀን፡ መጋቢት 8/2012

ለ፡ ሚመለከተው ሁሉ

ጉዳዩ፡- ትብብር ስለመጠየቅ

ተማሪ በድሉ ግሀሉ በአዲስ አበባ ዩኒቨርስቲ በጤና ሳይንስ ኮሌጅ በባዮኬሚስትሪ ት/ክፍል የድህረ ምረቃ ተማሪ ሲሆን የምርምር ጽሁፉን "Comparative Assessment of Serum Lipid Profile, Complete Blood Count and Viral Load Suppression Between HIV Positive Patients taking Dolutegravir and Efavirenz Based Anti-Retroviral Therapy at Wolkite Health Center, Southern Ethiopia, 2019-2020" በሚል ርዕስ ስለሚሰራ በእናንተ በኩል የተለያዩ ለጥናቱ የሚያግዙ አስፈላጊ ጉዳዮች እገዛ እንዲደረግለት በትህትና እንጠይቃለን፡፡

ከሠላምታ ጋር

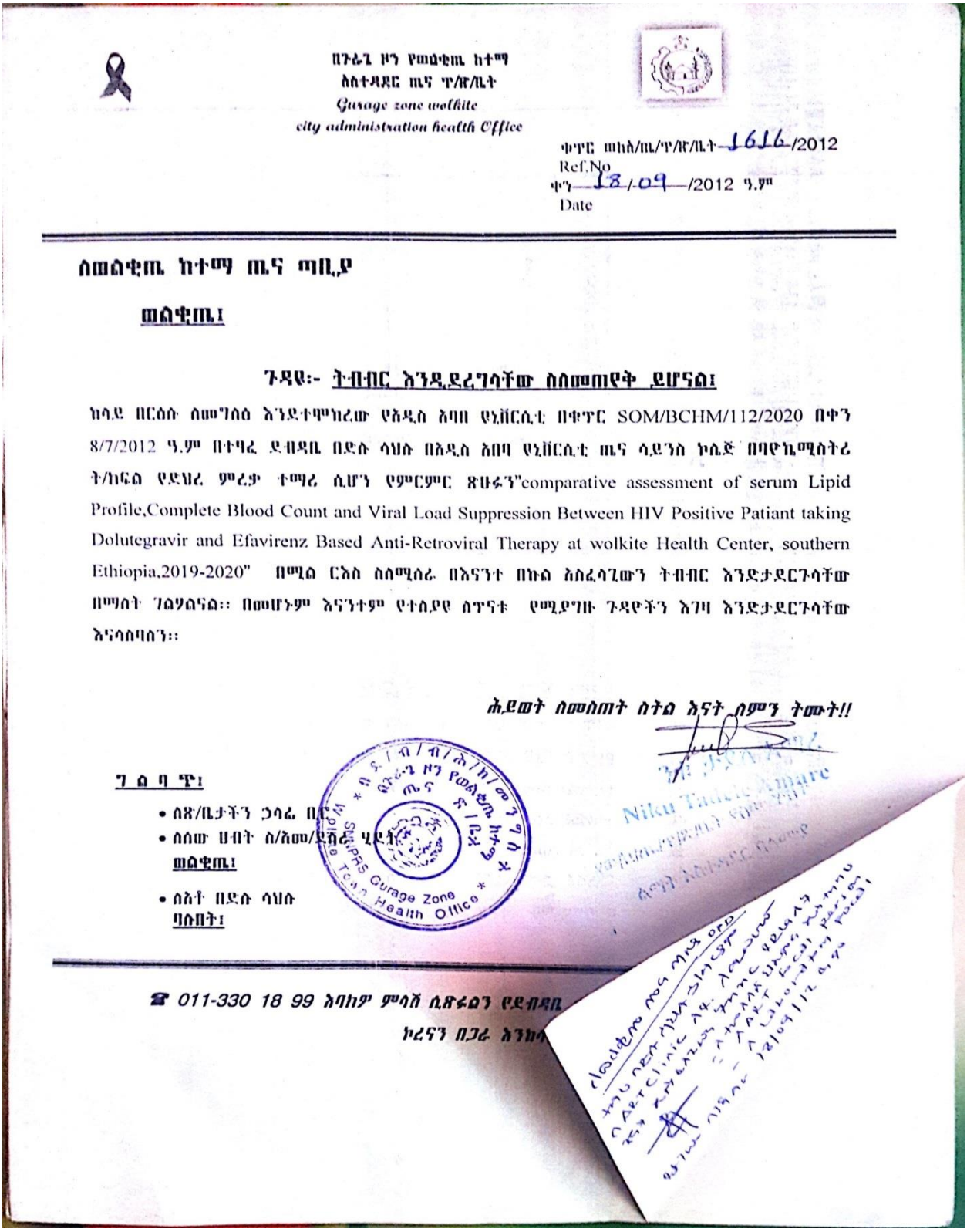
ማሪያ ደነቱ (ዶ/ር)
ባዮኬሚስትሪ ት/ክፍል ሀላፊ
ጤና ሳይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ
አዲስ አበባ

የአዲስ አበባ ዩኒቨርሲቲ Dept. of Biochemistry Medical Faculty A.A. ADDIS ABABA UNIVERSITY

P.O Box 9086
ADDIS ABABA ETHIOPIA
SCHOOL OF MEDICINE, AAU

Tel.251-011-550-52-48
FAX.251-1-551-30-99

Letter from Wolkite Health office



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አስተዳደር ጤና ጥ/ጽ/ቤት
Gurage zone wolkite
city administration health Office

ቁጥር ጠከአ/ጤ/ጥ/ጽ/ቤት 1616/2012
Ref.No
ቀን 18/09/2012 ዓ.ም
Date

ለወልቂጤ ከተማ ጤና ጣቢያ

ወልቂጤ፣

ጉዳዩ፡- ትብብር እንዲደረግላቸው ስለመጠየቅ ይሆናል፣

ከላይ በርዕሱ ለመግለፅ እንደተሞከረው የአዲስ አባበ ዩኒቨርሲቲ በቁጥር SOM/BCHM/112/2020 በቀን 8/7/2012 ዓ.ም በተላፈ ደብዳቤ በድሱ ሳህሉ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ በባዮኬሚስትሪ ት/ክፍል የድህረ ምረቃ ተማሪ ሲሆን የምርምር ጽሁፉን"comparative assessment of serum Lipid Profile,Complete Blood Count and Viral Load Suppression Between HIV Positive Patiant taking Dolutegravir and Efavirenz Based Anti-Retroviral Therapy at wolkite Health Center, southern Ethiopia,2019-2020" በሚል ርእስ ስለሚሰራ በእናንተ በኩል አስፈላጊውን ትብብር እንድታደርጉላቸው በማለት ገልፃልናል። በመሆኑም እናንተም የተሰያዩ ስጥናቱ የሚያግዙ ጉዳዮችን አገዛ እንድታደርጉላቸው እናሳስባለን።

ሕይወት ለመስጠት ስትል እናት ሰምን ትሙት!!

ግልባጭ፣

- ለጽ/ቤታችን ኃላፊ በ
- ለሰው ሀብት ስ/አመ/ደ/ሲ ሂሩት
  ወልቂጤ፣
- ለአቶ በድሱ ሳህሉ
  ባሉበት፣

Wolkite Town Gurage Zone Health Office

☎ 011-330 18 99 አባከዎ ምላሽ ሲጽፉልን የደብዳቤ
ኮረናን በጋራ እንከላ

Annex VII: Declaration

I, Bedilu Sahilu, declare that this research paper entitled: Comparative assessment of serum lipid profile, complete blood count and viral load levels between HIV positive patients taking dolutegravir and efavirenz based antiretroviral therapy at wolkite health center, southern Ethiopia, which I hereby submit for the degree of master of science in medical Biochemistry at Addis Ababa University, is my original work and has not yet been submitted for any degree at other University. All sources of materials used for this research have been fully acknowledged.

Bedilu Sahilu

Signature: _____

Date: _____