HEMATOLOGICAL PROFILES AND ASSOCIATED FACTORS AMONG ADULT HIV POSITIVE INDIVIDUALS BEFORE AND ON HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT: A CROSS SECTIONAL STUDY IN MADDA WALABU UNIVERSITY GOBA REFERRAL HOSPITAL, SOUTHEAST ETHIOPIA

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A THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCIENCE, FACULITY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER SCIENCE IN CLINICAL LABORATORY SCIENCES, SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

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ABSTRACT

Background: Hematological abnormalities are common in human immunodeficiency virus (HIV) positive individuals and increase the risk of morbidity and mortality. It is stated in different literatures that the prevalence of hematological abnormalities due to HIV before highly active antiretroviral therapy (HAART) initiation is more prevalent than after HAART initiation. However, there is limited data on the profiles of hematological abnormalities in HIV positive adult individuals in Southeast Ethiopia.

Objective: This study was aimed to determine the hematological profiles and associated factors among adult HIV positive individuals before and on highly active antiretroviral treatment at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia.

Methods: A facility based cross-sectional study was conducted involving adult HIV positive individuals from April 1 to June 30, 2018 at Madda Walabu University Goba Referral Hospital.

A total of 308 HIV positive adult individuals were included in this study. Data on socio-demographic characteristics and clinical data of the study subjects were collected using structured questionnaire. Hematological and immunological parameters were measured. Stool examination for intestinal parasites and blood film for hemoparasites were done. Data were entered into EpiData 3.1 and analyzed using SPSS V-20.0 statistical software. P- Values<0.05 were considered as statistically significant.

Results: The prevalence of anemia, leukopenia and thrombocytopenia was 31.8%, 18.2% and 11.4% before HAART while 14.6%, 24% and 4.5% in HIV positive individuals on HAART, respectively. There were significant differences between on mean values of RBC, Hgb, Hct, MCV, MCH, RDW, TLC, PLT and CD4+counts between pre-HAART and on HAART. CD4 count<200cells/µl (AOR=4.2, 95% CI :(1.4-4.8) was the only independent risk factor of anemia and leukopenia in HIV positive individuals before HAART. WHO clinical stage IV (AOR=2.4, 95% CI :(1.4-4.8), female (AOR=2.6, 95% CI: 1.3-4.8), HAART regimen (ZDV, 3TC, NVP (AOR=3.2, 95% CI :(2.2-7.5) and having intestinal parasites infection (AOR=2.5, 95% CI :(1.1-7.4) were found to be significant predictors of anemia on HAART HIV positive individuals.

Conclusion: There was a decline in the prevalence of anemia and thrombocytopenia among HIV positive individuals after ART initiation. There was differences prevalence of hematological abnormalities between HIV positive individuals before and on HAART. Hematological abnormalities in HIV positive individuals before and on HAART had different risk factors.

Therefore, regular follow-up are necessary to prevent hematological abnormalities.

Keywords: Hematological profile, associated factors, HIV, adult, HAART, Southeast Ethiopia

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AIDS	Acquired Immune Deficiency Syndrome
ANC	Absolute Neutrophil Count
AOR	Adjusted odd Ratio
BMI	Body Mass Index
CD	Cluster Differentiation
cART	Combined Antiretroviral Treatment
COR	Crude Odd Ratio
EDTA	Ethylenediamine Tetraacetic Acid
EFV	Efavirenz
FACS	Fluorescence-activated cell sorting
HAART	Highly Active Anti Retroviral Therapy
Hct	Hematocrit
Hgb	Hemoglobin
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NVP	Neverapine
RBC	Red Blood Cell
RDW	Red cell Distribution Width
TDF	Tenofovir
TLC	Total Lymphocyte Count
3TC	Lamivudine
WBC	White Blood Cell
ZDV	Zidovudine
CV	Coefficient of Variation

1. INTRODUCTION

1.1. Background

Human immunodeficiency virus is a retrovirus that infects cells of the immune system, destroying or impairing their functions, which leads to the occurrence of opportunistic infections, immunological and hematological complications(1). It enters into the host immune system is mainly through interactions between CD4 cells and the presence of chemokine co receptors (1, 2). The virus is transmitted through viral shedding at mucosal surface lesions in contact with infected body fluids such as blood, semen or vaginal secretions and activates immune system which is characterized by the release of proinflammatory cytokines and chemokine, polyclonal B-cell activation and progressive CD4 depletion(1).

Infection with HIV has been associated with a broad range of clinical outcomes involving the hematopoietic system. The infection influences all hematopoietic cell lines leading to a spectrum of hematological abnormalities, such as peripheral blood cytopenias, bone marrow and as well as the coagulation pathways(3).

Globally, the HIV remains a serious challenge and continues to take its toll particularly on vulnerable populations like HIV infected individuals and malnourished childrens. It has a profound impact on disease progression and mortality (4). Hematological complications have been documented to be one of the most common causes of morbidity and mortality in HIV infected patients. The impact of HIV infection that can be found in the peripheral blood and bone marrow includes anemia, leukopenia & thrombocytopenia(5). Both antiretroviral treated and untreated individuals have different hematological abnormalities (6).

Anemia is the most common hematological abnormality in HIV infected individual. The prevalence of anemia in HIV infected individual’s ranges from 1.3% to 95% depending on the stages of HIV disease, sex, age, pregnancy status and presence of opportunistic infections. Several factors play a role in the development of anemia in patients with HIV including, chronic disease, opportunistic infections, parasitic infection, changes in cytokine production with subsequent effects on hematopoiesis; decreased erythropoietin concentrations and administration of chemotherapeutic agents. Other mechanisms for HIV-associated anemia, although uncommon, include vitamin B_{12} deficiency, autoimmune destruction of red blood cells and certain nutritional deficiencies (7-9).

The consequences of untreated anemia may lead to multisystem disabling symptoms, fatigue,

increased risk of HIV dementia, poor quality of life and exacerbates poverty in communities with a high HIV prevalence. On the other hand, survival time in HIV positive individuals may be enhanced after recovery from anemia (7, 8). Different studies showed that, normocytic-normochromic anemia are the dominant type of anemia followed by microcytic anemia (6, 7, 10-12).

Thrombocytopenia is the second frequent complication of HIV infection which is found in 3-40% of individuals with HIV infected and can be occur at any stage of HIV infection(13). The possible mechanisms that have been reported are immune-mediated destruction of platelets by antibodies, cross-reacting antibodies that are directed toward HIV proteins, particularly gp120 and p-24(4). Others causes of thrombocytopenia in HIV infection including, infectious or neoplastic conditions that involve the bone marrow suppression and medications that cause generalized myelosuppression can produce thrombocytopenia(11, 13). It is also associated with ineffective hematopoiesis, from direct suppression of bone marrow progenitor cells by HIV infection or indirectly through excessive secretion of inflammatory cytokines induced by HIV and blunt hematopoiesis(14).

Leukopenia is another hematological abnormality that occurs in HIV infected individuals of which, neutropenia is the most common and accounts 10-30% of HIV patients, typically with advanced disease(6). HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony-stimulating factor, resulting in leukopenia and neutropenia(6, 11).

Antiretroviral treatment is known to profoundly suppress viral replication, increases CD4 cell count, delays disease progression and death. However, patients on highly active antiretroviral therapy commonly suffer from side effects of the drugs(12). Several common serious adverse effects associated with antiretroviral therapy, including AZT-associated hematological abnormality is observed. In Protease inhibitors, several case reports suggested an association between these drugs and increased frequency and severity of bleeding in patients with hemophilia(15). Although many drugs used for the treatment of HIV-related disorders are myelosuppressive, severe cytopenia is most often related to the use of zidovudine(13).

1.2. Statement of the Problem

Globally, an estimated 36.7 million people were living with HIV of which, 34.5 million were adults. 1.8 million People became newly infected &20.9 million people were accessing antiretroviral therapy. one million people died from AIDS-related illnesses (16).

In Sub-Sahara Africa, 23.5 million people were living with HIV and 300,000 people were newly infected. An estimated 10.3 million people were accessing antiretroviral therapy(16). In Ethiopia, Demographic and Health Survey (DHS) estimated the national adult HIV prevalence is 1.5%. The estimated numbers of people living with HIV were 798,960 (479,940 female and 310,020 male)(17).

Hematological abnormalities associated with HIV infection were among the most frequently reported clinical problems in the early years of the acquired immune deficiency syndrome (AIDS). The incidence of cytopenias correlated directly with the degree of HIV-induced immunosuppression and they were important reasons for the increased morbidity and mortality that were the hallmark of the early years of the AIDS (18, 19).

The infection influences all hematopoietic cell lines leading to a spectrum of hematological abnormalities, such as peripheral blood cytopenias, bone marrow and as well as the coagulation pathways(14). In individuals infected with HIV, hematological abnormalities are associated with increased risk of disease progression and death(20).

Anaemia, thrombocytopenia and leucopenia are among the most common hematological abnormalities that are identified in patients with HIV infection. The causes of these cytopenias are multifactorial, reflecting the connected and often additive effects of inflammation, infection, malnutrition, malignancy and drugs(21).

Globally, according to World Health Organization (WHO), the prevalence of anemia is 24.8%. It affects an estimated 2 billion people worldwide (WHO/UNAIDS report on anemia global 2011). It is the most common hematological abnormality associated with HIV infection. Although the burden of anemia in HIV/AIDS patients is not very well understood, it is estimated that about 1.3% to 95% of HIV patients depending on the stages of HIV disease, sex, age, pregnancy status and presence of opportunistic infections(7). In these patients, 22% of anemia is thought to be caused by several treatments given to the patients including the antiretroviral medications(22).

HIV-infected women have a higher incidence of anaemia than men, and HIV-infected African Americans are more likely to have anemia than other racial groups(23, 24). The reasons for this are probably multifactorial but may include a higher incidence of iron deficiency anaemia and other nutritional deficiencies and poorly controlled HIV replication(25).

Thrombocytopenia is the second frequent complication of HIV infection which is found in 3-40% of individuals and can be occur at any stage of HIV infection(13). Leukopenia occurs

also in HIV infected individuals of which, neutropenia is the most common and accounts 10-30% of the infection typically with advanced disease(6). HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony-stimulating factor, resulting in leukopenia and neutropenia(6, 11).

Hematological abnormalities have negative consequences on the health and quality of life of HIV infected individuals; increase the risk of morbidity and mortality. The early uses of HAART improve clinical, hematological and immunological characteristics of the patients and delay the progression of diseases and improve survival in HIV positive individuals(26).

Most studies described the prevalence of hematological abnormalities (especially anemia) in HIV positive individuals before and after ART initiation in a separate population. However, in Ethiopia the magnitude of hematological abnormalities and associated factors among adult HIV positive individuals before and on HAART are not well documented and no study done in the study area. Therefore, the aim of this study was to determine hematological profiles and associated factors among adult HIV positive individuals before and on HAART at Madda Walabu University Goba Referral Hospital.

1.3. Significance of the study

Hematological abnormalities are common in HIV positive individuals. Thus, the aim of this study was to determine hematological profiles and associated factors among adult HIV positive individuals before and on HAART at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia.

Results obtained from this recommendation are fundamental for health planers, stakeholders and care givers for evidence-based intervention and provides information about the change of hematological profiles which may contribute in improving the management of patients before and on HAART, and moreover, also provides information for the clinicians about the need of hematological tests in HIV positive individuals.

2. LITERATURE REVIEW

2.1. Prevalence of anemia and associated risk factors

Study conducted in India on HIV positive individual showed the prevalence of anemia was 46% and increased in lower CD4 counts and common in untreated patient(27). Study conducted in India showed anemia at baseline was 65.5%(28). Study conducted in Nigeria showed anemia at baseline was 24.2%. The degree of cytopenia was directly related to the degree of immunosuppression(29).

Study conducted in Tanzania on HAART-naive adult patients showed the prevalence of anemia was 58.4% and 49% of study participants had CD4 counts<200 cells/µL(30). Another study performed in Iran indicated anemia at base line was 10% and most important associated risk factors were, female sex and stage of HIV infection(31). A cross-sectional study conducted in Cameroon at the Yaoundé University Teaching Hospital on ART naive HIV patients were done showed anemia was (62.9%). Participants with low CD4 counts and advanced clinical stages had greater occurrence of blood cytopenias(32).

A retrospective study conducted in Uganda on HIV naive patients, anemia at baseline was 47.8%. During follow-up, there were significant increase in Hgb, MCV, MCH, WBC and PLT counts. These changes in hematological parameters were associated with CD4 cell count, sex, BMI and age. Cytopenia was more prevalent in females, CD4 less than 200 cells/µL, those with lower and overweight BMI and presence of OI(oral Candidiasis) (14).

A cross section study was conducted in Arba Minch Town showed the overall prevalence of anemia at base line of ART initiation was 52.3%; with 28.1%, 22.9% and 1.3% mild, moderate and severe anemia, respectively. Male (male 62.4% vs. female 46.7%), low CD4 count and history of TB were associated with higher risk of baseline anemia(33). A cross sectional study conducted in Hawassa University Referral Hospital indicated the prevalence of anemia before and after ART initiation was 23.4% and 12%, respectively. The prevalence of anemia was higher in males than females(before77.8vs.22.2%) and after HAART65.2vs. 34.8%) (34).

A cross sectional study conducted in Black Lion Specialized Hospital, Addis Ababa, Ethiopia. Prevalence of anemia before and after ART initiation was 41.9% and 11.4%, respectively. There are significance differences in CD4 + T cell count, RBC count, Hgb values and RBC indices in HIV patients before and after ART initiation. WHO clinical stages and CD4+ T cell counts were found to be associated with the prevalence of anemia before ART initiation

Normocytic normochromic anemia was present in 71% of the cases before ART and 58.6% of the cases after HAART. The prevalence of macrocytic normochromic anemia before and after ART initiation was 4.7% and 27.6%, respectively(35).

A cross sectional study conducted at Minillik II Hospital ART clinic on HIV infected patients who were talking ART and had follow up. The prevalence of anemia before ART was 52.6% and after the initiation of ART was 37.3% of this prevalence of anemia was higher in female than in males in both cases before 70.25% vs. 29.75% and after treatment 69.23% vs. 30.77%. All grades of anemia were consistently more common with AZT- based regimes relative to d4T- based therapy(36).

Study conducted in Zewditu Memorial Hospital on HIV patients showed the overall prevalence of anemia was 22.2%; with 52.5%, 42.5%, and 5.0% patients had mild, moderate, and severe anemia. The baseline anemia was 35.5%. There was significant increase in severity and prevalence of anemia in those with CD4counts<350cells/μl, HAART naive, intestinal parasites/helminthes infection(37). A cross sectional study conducted in Yekatit 12 Hospital on HIV naïve patients showed anemia at base line was 18.9%(38).

A retrospective cohort study conducted in Zewditu Memorial Hospital showed the prevalence of anemia at baseline was 42.9% with 79%, 15.6% and 5.3% mild, moderate and severe anemia, respectively. The prevalence was significantly decreased to 20.9% at 6 months and to 14.3% at 12 months after ART initiation. Male sex, WHO clinical stages III/IV and TB co-infection were risk factors of anemia at base line. Male, TDF based, ages and CD4<200cells/µl were risk factors of anemia after 6 months and 12 months of treatment (39).

Study conducted in Gondar on HIV patients on pre and post treatment. The prevalence of anemia was 42.8% before and 18.9% after ART initiation. Age <5 years, advanced WHO clinical stages and CD4 %< 25 were risk factors for anemia before HAART. Opportunistic infection was risk factor of anemia after ART initiation (9). A retrospective study conducted in University of Gondar Hospital showed prevalence of anemia before and after ART initiation was 21.2% and 11.5%, respectively. There was significance difference in CD4 cell count, Hgb and Hct values on patients before and after ART initiation. Opportunistic infection and CD4 cell count were associated with prevalence of anemia before ART initiation(40).

A retrospective study conducted involving ART naive HIV infected individual at Gondar University Hospital. The overall prevalence of anemia was 35%. Female had significantly

higher prevalence of anemia than males (62% Vs 38%). One-third of HAART naïve were anemic and the increase with decreased CD4 cell (41).

A retrospective cohort study conducted in Dessie Referral Hospital on ART patients. The prevalence of anemia before ART initiation was 34.4%; with 20.5%, 12.3 % and 1.6% mild, moderate and severe anemia, respectively(42). A cross sectional study conducted in Gondar University Hospital on adult HIV infected individual HAART naïve and on HAART. Prevalence of anemia was 11.7% on HAART and 29.7% HAART naive patients. There was significance difference in total WBC, RBC, Hgb, MCV, MCH, MCHC, MPV and CD4 counts between patients on HAART and HAART naïve(43).

Study conducted at Felege-Hiwot Referral Hospital indicated prevalence of anemia was 65%(44). Study conducted in Hiwot Fana Specialized University Hospital on ART follow up patients showed the prevalence of anemia 54.4% and was a declined to 39.2% after ART initiation(45).

A cross sectional study conducted in Jimma University Specialized Hospital on HAART naive and HAART experienced showed the overall prevalence of anemia was 23.1%. The prevalence of anemia in HAART naive and HAART experienced persons was 29.9% and 16.2%, respectively. Presence of opportunistic infections, CD4 counts <200cells/ µL and rural residence were found to be predictors of anemia for HAART naive participants. HAART regimen (ZDV/3TC/NVP) and the duration of HAART were found to be predictors of anemia for HAART experienced groups(46).

2.2. Prevalence of thrombocytopenia and associated risk factors

Study conducted in India on HIV infected persons showed the prevalence thrombocytopenia was 24% and increased in lower CD4 count and common in untreated patient (27). Study conducted in Tanzania on ART-naive adult patients showed the prevalence of thrombocytopenia was 14.4%. About 49% of study participants has CD4counts<200 cells/µL(30). Study conducted in India showed that thrombocytopenia at baseline was 7%(28). A cross sectional study conducted in Nigeria thrombocytopenia at enrollment was16.1% (29). A cross sectional study conducted in Uganda thrombocytopenia at base line was 8.3%. Cytopenia was more prevalent in females, lower CD4 count and lower BMI(14).

A cross-sectional study in Cameroon at the Yaoundé University Teaching Hospital on ART naive HIV patients were done showed thrombocytopenia at base line was 27.1%. Advanced WHO clinical stage and CD4 <200cells/µl were risk factors (32).

Study conducted in Gondar on adult HIV infected HAART naive and on HAART showed the prevalence of thrombocytopenia was 4.1% on HAART and 9% HAART naïve patients (43). Another study conducted in Yekatit 12 Hospital on HIV naïve patients of showed the prevalence of thrombocytopenia at base line was 8.5% (38).

2.3. Prevalence of leukopenia and associated risk factors

A cross sectional study conducted in Nigeria showed leucopenia at base line was 26.8%(29). Study conducted in India on HIV infected patients showed the prevalence of leukopenia was 25% and increased in lower CD4 count and common in untreated patient(27). Study conducted in Tanzania on ART-naive adult patients showed the prevalence of leukopenia was 23.6% and 49% of study participants has CD4 counts <200 cells/µL(30).

Study conducted in Uganda showed leukopenia at baseline was 24.3%,cytopenia was more prevalent in females, lower CD4 count and lower BMI(14). A cross-sectional study done in Cameroon at the Yaoundé University Teaching Hospital on HIV patients HAART naive showed leukopenia at base line was 34.6% and was associated with advanced WHO clinical stage and CD4<200cells/µl(32).

A cross sectional study conducted at Minillik II Hospital ART clinic, on HIV infected patients who were talking ART and had follow up showed neutropenia events were consistently more common with AZT- based regimes relative to d4T- based therapy(36).

Study conducted in Gondar on adult HIV infected HAART naive and on HAART showed the prevalence of leukopenia, neutropenia and lymphopenia was 35.9%, 28.3% & 2.1% on HAART patients, respectively &16.6%, 14.5%&2.1% HAART naïve patients, respectively (43). Another study conducted in Yekatit 12 Hospital on HIV naïve patients showed neutropenia at base line was 2.8% (38).

There are many studies conducted on hematological abnormalities and risk factors among HIV positive individuals before and after ART initiation separately. However, in Ethiopian studies showed the magnitude and severity of hematological abnormalities occur due to complications in both before and after ART were not well addressed and no study conducted in the study area. Therefore, the aim of this study was to determine the hematological profiles

and associated factors among adult HIV positive individuals before and on HAART at Madda Walabu University Goba Referral Hospital.

2.4. Conceptual frame work

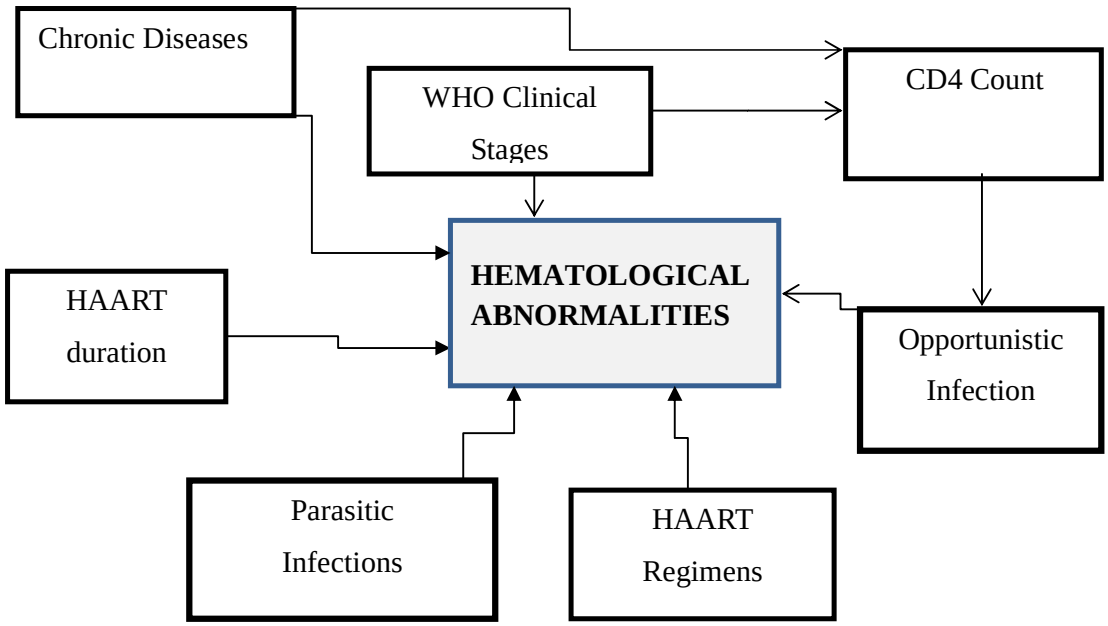

Figure 1: Conceptual frame work of hematological profiles and associated factors among adult HIV positive individuals at Goba Referral Hospital, Southeast Ethiopia April to June, 2018

3. OBJECTIVES

3.1. General objective

- The aim of this study was to determine hematological profiles and associated factors among adult HIV positive individuals before and on HAART at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia.

3.2. Specific objectives

- To determine hematological profiles of adult HIV positive individuals before HAART
- To determine hematological profiles of adult HIV positive individuals on HAART
- To compare hematological profiles of adult HIV positive individuals before and on HAART
- To assess the associated factors of hematological abnormalities of adult HIV positive individuals before and on HAART

4. MATERIALS AND METHODS

4.1. Study Setting

This study was carried out in Madda Walabu University Goba Referral Hospital, Goba City Southeast Ethiopia. It is 446km far away from Addis Ababa in Oromia Region, Bale zone, Goba Woreda. This city has an altitude of 2,743 meters above sea level. The 2007 national census reported a total population for Goba of 32,025, of whom 15,182 were men and 16,843 were women; 4,797 or 6.13% of its population were urban dwellers(47).

The hospital services as a teaching and referral hospital for 974,625 populations in Southeast part of Ethiopia since 2007 E.C. It provides HIV/AIDS interventions including free diagnosis, treatment and monitoring. There were 2,224 HIV positive individuals on follow up of which, 1928 were adults and 296 were children. (Report document 2017/2018 of Goba Referral Hospital ART clinic).

4.2. Study design and Study periods

A facility based cross sectional study design was conducted from April 1 to June 30, 2018.

4.3. Population

4.3.1. Source population

All adult HIV positive individuals who were attending at Madda Walabu University Goba Referral Hospital

4.3.2. Study population

All adult HIV positive individuals who were on follow-up at the ART clinic of Goba Referral Hospital during the study period and fulfilled inclusion criteria.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

HIV positive adult participants who were on follow up during the study period, voluntarily participated in the study and had complete record of baseline information including hematological and immunological parameters.

4.4.2. Exclusion criteria

HIV positive adult pregnant women, patients who had traumatic injury or surgical interventions resulting in blood loss during the study period or within 3 months prior to the study period, those who are on vitamin and iron supplements for known hematological abnormalities at the time of sampling, HAART experienced for less than 3 months and HIV positive individuals blood transfused within the last three months.

4.5. Sample Size Determination and Sampling Technique

4.5.1. Sample size determination

Sample size was determined using single population proportion formula based on the following assumption;

P =29.7% proportion of anemia at baseline from previous study(43)

$Z_{(1-\alpha/2)}$ = 1.96 = value of the standard normal distribution corresponding to a significance level of α (1.96 for 2-sided test at the 0.05 level)

d =margin of error 5% n=desired sample size (when population > 10,000)

NF= desired sample size (with population < 10,000)

$n= \underline{Z^2\ p\ (1-p)}/\ d^2$

$n= (1.96)^2 x 0.297\ (1-.297)/\ (0.05)^2 = 320.7 \approx 321$ HIV positive individuals

Since the source population is <10,000. So, correction formula was applied.

N=the estimate of the population size

Total HIV positive individuals on follow up were 2,224

NF = n/1+ (n/N)

NF = 321/1+ (321/2,224) =308

4.5.2. Sampling technique

All study participants available during study period were included consecutively.

4.6. Variables

4.6.1. Dependent Variable

Hematological abnormalities

4.6.2. Independent Variables

Age, sex, educational status, marital status, residence, family size, occupation, average monthly family income, type of HAART regime, CD4 count, opportunistic infection, body mass index, history of chronic diseases, duration on HAART, intestinal parasitosis, hemoparasites, WHO clinical stages.

4.7. Data Collection Techniques

4.7.1. Socio-demographic and clinical data collection

Data on the socio-demographic and clinical characteristics of the HIV positive individuals were collected using structured questionnaire by interviewing and reviewed of medical records by three BSc-nurses. Past medical history and base line data were collected by reviewing patient's records. Data collectors were trained with the objective of standardizing the data collection instrument and providing them with basic skill of extracting the data both from the ART log book as well as patients follow up cards.

4.7.2. Anthropometric Measurements

Weight of each study participant was measured by using portable digital scale to the nearest 0.1 kg. A fixed base calibrated height scale was used for the height measurement to the nearest 0.1 cm. Body Mass Index (BMI) was calculated as weight in kilogram divided by the square of height in meter. All anthropometric measurements were taken twice and the average value was used for analysis (values were used to compute BMI).

4.8. Specimen Collection, Processing and Analysis

4.8.1. Blood sample collection and examination

Four (4ml) of venous blood specimen was collected from vein puncture using vacutainer method into ethylenediamine tetraacetic acid (EDTA) containing test tubes by an experienced laboratory technologist from each participant and sent to the hematology laboratory for analysis. The blood sample was for hemoparasites, hematological and immunological

parameters (CD4+cells); Hematological parameters (Total and differential WBC, red cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) and platelet (PLT) were performed by CELL-DYN 1800 automated hematology analyzer (*Abott Laboratories Diagnostics Division, USA*). CD4+ count was measured by BD FACSPresto^{TM} machine **(***BD Biosciences, NJ, USA*). Specimens were run within an hour of collection in order to provide accurate results for hematological parameters and for CD4+T count. Two drops of whole blood from EDTA anticoagulated venous blood was placed onto a clean and labeled microscopic slide for preparation of thick and thin blood film. Dried thin slide was fixed by absolute methanol, stained with 10% Geimsa for 10 minutes and later were examined for hemoparasites.

4.8.2. Stool sample collection and examination

Sufficient amount of stool sample (approximately one gram) of fresh well-formed and semi formed stool or one table spoonfuls of watery stool was collected from each participant using clean, dry, leak proof and wide-mouthed caps and sent to the medical parasitology laboratory and examined for intestinal parasitic infections using direct wet mount technique microscopically.

4.9. Quality Assurance

To assure the quality of data, questionnaires were translated to local language; Afaan Oromoo and Amharic and back translated to English. Three days training was given for the data collectors on the data collection tools, methodology and maintaining quality to minimize technical and observer biases by principal investigator. All data were checked for completeness, clarity and consistency by the principal investigator immediately after data was collected. The principal investigator was responsible for overall supervision.

All the laboratory activities were done strictly following manufacturer's instruction. Standard Operational Procedures were implemented for all laboratory procedures. All reagents used were checked for their expiry date and prepared according to the manufacturer's instructions. A control reagent was used for the hematology analyzer (high, medium, and low) and BD FACS Presto machine (integrated reagent quality control) to check the accuracy and precision of the results. Background was also checked for hematology analyzer.

4.10. Data Processing and Statistical Analysis

Data were entered into EpiData version 3.1(EpiData Association, Odense Denmark) and exported to SPSS-version 20.0 (Armonk, NY: IBM Corp) statistical software for analysis. Descriptive statistics (Minimum, maximum, mean, range and standard deviation) were appropriately applied in the course of analysis. Proportions and percentages were calculated for categorical variables. Bivariate analysis was done to see association of each independent variable with an outcome variable. Variables with P-value<0.25 by the bivariate analysis were candidate for the multiple logistic regression model to identify the effect of each independent variable with dependent variable and to avoid confounding variables. Paired T -test was used to assess mean differences for continuous variables between HAART naive and on HAART HIV positive individuals using SPSS V-20 statistical software. All P-value<0.05 was considered as statistically significant.

4.11. Operational Definitions

Anemia: Hemoglobin value <13g/dl for adult males and <12gm/dl for non pregnant women; the value is less than 12 g/dl. Severity was graded as mild: 11-11.9 g/dl for women and 11-12.9 g/dl for men; moderate: 8-10.9 g/dl for both sexes; and < 8 g/dl as severe anemia for both sexes(48).

Adult: a person whose age is ≥18 year

Hematological abnormality: consists of anemia, leukopenia, neutropenia, lymphopenia and thrombocytopenia.

Normocytic: MCV(80-100fl), microcytic MCV<80fl, macrocytic MCV>100fl and hypochromic MCHC value <31 g/dl(46).

Leukopenia: Total leukocyte count<$4x10^3$/µl, neutropenia: absolute neutrophil count <$2.5x10^3$/µl, lymphopenia: total lymphocyte count<$1.5x10^3$/µl and thrombocytopenia: platelet count <150 $x10^3$/µl(49).

Before highly active antiretroviral therapy: HIV positive individuals who are not yet started taking highly active anti retroviral drugs.

On highly active antiretroviral therapy: HIV positive individuals who are taking highly active antiretroviral drugs at least for three months.

4.12. Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board of Jimma University Institute of Health. Permission to conduct the study was obtained from the Madda Walabu University Goba Referral Hospital clinical director. After describing the study aim, risks, benefits of study participants and right to withdraw from the study, written informed consent was taken from each study participant. All abnormal and positive results of study participants' were timely reported to the physician for proper management.

4.13. Dissemination and Utilization of Findings

The finding of the study was submitted to School of Medical Laboratory Science, Faculty of Health Sciences, postgraduate and research coordinating office and the copy of the result was submitted to Madda Walabu University Goba Referral Hospital. Efforts will be made to publish the research on local or international reputable peer reviewed journals. The finding will be presented on different scientific conferences.

5. RESULTS

5.1. Socio-Demographic characteristics of the Study Participants

A total of 308 adult HIV positive individuals of which, 160 (51.9%) males and 148 (48.1%) females were involved in this study. The overall mean age was 38.8±11.9 years, within the range of 18-80 years of age. About 113(376.7%) of the individuals were within 30-39 years age (Table -1).

Table 1: Socio-demographic characteristics of HIV positive individuals at Madda Walabu University Goba Referral Hospital Southeast Ethiopia April to June, 2018

Variables	On HAART		Before HAART	
Sex of respondent's	N	%	N	%
Male	160	51.9	160	51.9
Female	148	48.1	148	48.1
Age in years				
18-29	36	11.7	37	12.0
30-39	116	37.7	111	36.0
40-49	111	36.0	112	36.4
≥50	45	14.6	48	15.6
Residence				
Urban	55	17.9		
Rural	253	82.1		
Educational level				
Unable to read and write	42	13.6		
Able to read and write	58	18.8		
Elementary	138	44.8		
Secondary	29	9.5		
Certificate &above	41	13.3		
Occupation				
Farmer	56	18.2		
Housewife	43	14.0		
Private	54	17.5		
Daily laborer	65	21.1		
Government employee	77	25.0		
NGO employee	13	4.2		
Marital Status				
Single	70	22.7		
Married	147	47.7		
Divorced	44	14.3		
Widowed	47	15.3		

Average monthly-income(ETB					
	<1000	148	48.0		
	1000-1999	88	28.6		
	≥2000	72	23.4		
Size of household					
	<2	52	16.9		
	2-5	102	33.1		
	6-10	127	41.2		
	>10	27	8.8		

N=number %=Percentage ETB=Ethiopian birr

5.2. Clinical characteristics of the study participants

From the total of 308 HIV positive individuals on HAART, 14(4.5%) had one or more opportunistic infections. The dominant opportunistic infection were, 7(2.3%) Candidiasis, 3(1%) Herpes Zoster, 2(0.6%) pneumonia and 2(0.6%) tuberculosis. They were screened for history of chronic diseases of which, 20(6.5%) diabetic mellitus, 18(5.8%) hypertension and 3(1%) tuberculosis. From the total of 308 HIV positive individuals before HAART, had one or more opportunistic infections of which, Candidiasis 8(2.6%), Herpes Zoster 6(1.9%), pneumonia 6(1.9%), tuberculosis 3(1%) and skin infection 3(1%) and 179(58.1%) opportunistic infections were not recorded (Table- 2).

The stool samples of 308 HIV positive individuals were done for detection of intestinal parasites. One or more intestinal parasites were detected in 101(32.8%) of HIV positive individuals on HAART. The dominant intestinal parasites were, ova of *Ascaris lumbricoids* 42(13.6%), Hookworm 24(7.8%), *Trichuris trichiura* 15(4.9%), Teanea species 11(3.6%) and *Strongyloides stercolaries* 9(2.9%). Blood films were done for the detection of hemoparasites for all study participants and 5(1.6%) was positive for hemoparasites of which, 4(1.3%) *Plasmodium vivax and* 1(0.3%) *Plasmodium falciparum* (Table-2)

Table 2: Clinical characteristics of HIV positive individuals at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Variables	**On HAART**		**Before HAART**	
Opportunistic infection	N	%	N	%
No	294	95.5	103	33.4
Yes	14	4.5	26	8.4
Not recorded			179	58.2

History chronic disease					
	No	267	86.7		
	Yes	41	13.3		
CD4 in cells/ul					
	<200	43	14.0	106	34.4
	200-500	107	34.7	125	40.6
	>500	158	51.3	77	25.0
Body Mass Index (kg/m^2)					
	<18.5	50	16.2	152	49.4
	18.5-24.9	244	79.2	115	37.3
	≥25	14	4.6	25	8.1
	BMI not recorded			16	5.2
WHO clinical stages					
	I	245	79.5	204	66.2
	II	35	11.4	70	22.7
	III	21	6.8	22	7.2
	IV	7	2.3	12	3.9
Hemoparasites					
	No	303	98.4		
	Yes	5	1.6		
Intestinal parasite					
	No	207	67.2		
	Yes	101	32.8		

Note: N=number %=percentage BMI=Body Mass Index

The study participants were taking different classes of antiretroviral treatment of which 71.8% were ZDV based therapy (ZDV, 3TC, NVP 52% and ZDV, 3TC, EFV 19.8%) and the rest were TDF based therapy ZDV based therapy which accounted (figure-2).

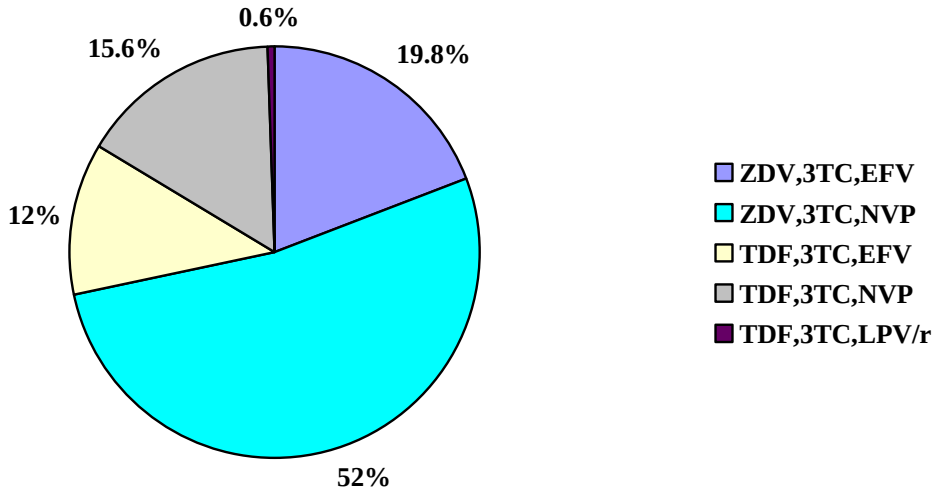

Figure 2: Percentage of HAART used in HIV positive individuals at Madda Walabu University Goba Referral Hospital Southeast April to June, 2018

About 38.6% of study participants were on HAART duration of 49-60 months followed by 19.5% in months of 25-36 months (figure-3).

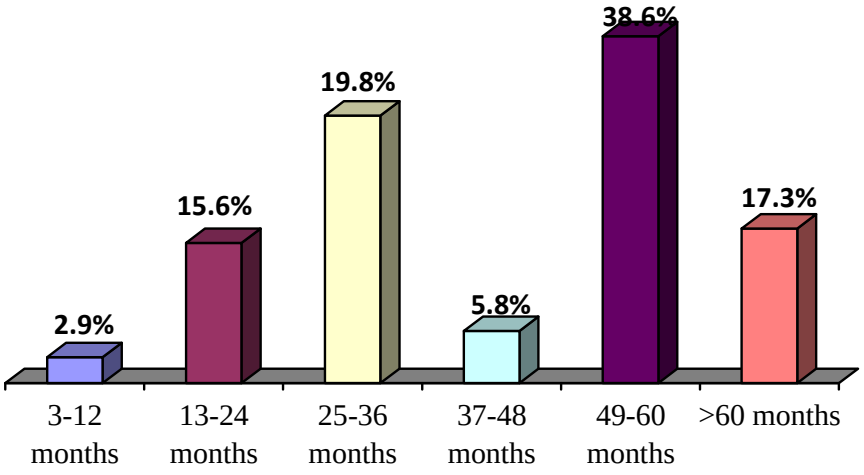

Figure 3: HAART duration of HIV positive individuals at Madda Walabu University Goba Referral Hospital, Southeast April to June, 2018

5.3. Hematological Parameters

There were significant differences between mean values of RBC, RDW, TLC, Hgb, Hct, MCV, MCH and PLT count before and on HAART HIV positive individual ($p<0.05$).

RBC and RDW count was higher before HAART while TLC, Hgb, Hct, MCV, MCH and PLT were higher in HIV positive individual on HAART ($p<0.05$) (Table -3).

Table 3: Hematological parameters of HIV positive individuals at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Parameters	Before HAART (Mean±SD)	On HAART (Mean±SD)	T-value(95%CI)	P-Value
WBC($X10^3$/µL	5.6±2.6	5.7±1.6	-1.21(0.13-(-0.56))	0.716
TLC($X10^3$/µL)	1.9±1.9	2.2±0.9	2.86(0.067-0.37)	**0.001***
ANC($X10^3$/µL	3.1±1.9	3.0±1.9	-0.71(-0.42-0.12)	0.481
RBC($X10^6$/µL	4.2±0.8	4.0±0.8	-1.86(-0.26-0.01)	**0.044***
Hgb (g/dl)	12.2±2.5	13.6±2.8	7.08(1.09-1.93)	**0.001***
Hct (%)	34.6±6.8	38.7±7.6	7.10(2.99-5.29)	**0.001***
MCV (fl)	90.9±11.9	94.2±9.9	3.79(1.57-4.96)	**0.001***
MCH (pg)	32.3±5.0	33.3±5.1	2.56(0.23-1.78)	**0.011***
MCHC (g/dl)	34.8±2.5	35.0±3	0.88(-0.23-0.60)	0.380
RDW (CV %)	15.2±1.4	14.3±2.1	-5.99(0.14-(-1.15))	**0.001***
PLT($X10^3$/µL)	242.4±109.7	273.6±117	3.47(13.48-50.00)	**0.001***

Note: ***=** indicates the level of significance ($p<0.05$), fl=femtoliter, pg=picograms

The prevalence of hematological abnormalities among HIV positive individuals

Hematological abnormalities were presented in HIV positive individuals before and on HAART. The prevalence of anemia, leukopenia, neutropenia, lymphopenia and thrombocytopenia in HIV positive individuals before HAART were, 31.8%, 18.2%, 15.6%, 2.9% and 11.4%, respectively and while, 14.6%, 24%, 17.9%, 3.6% and 4.5% in HIV

positive individuals on HAART, respectively.

From the total anemic patients before HAART, 52(53.1%) had normocytic normochromic anemia followed by 30(30.6%) microcytic hypochromic anemia. About 29(64.4%) macrocytic normochromic anemia and 12(26.7%) normocytic normochromic anemia presented in patients on HAART. Macrocytosis (MCV>100fl) was common on HAART as compared to before HAART which was 29(64.4%) and 16(16.3%), respectively.

Table 4: The prevalence of hematological abnormalities of HIV positive individual at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Abnormalities	**Before HAART**	**On HAART**
Anemia	98(31.8%)	45(14.6%)
Male	54(55.1%)	13(28.9%)
Female	44(44.9%)	32(71.1%)
Severe anemia	3(3.1%)	1(2.2%)
Moderate anemia	42(42.9%)	8(17.8%)
Mild anemia	53 (54.1%)	36(80%)
Leukopenia	56(18.2%)	74(24%)
Male	26(46.4%)	34(46.9%)
Female	30(53.6%)	40(54.1%)
Neutropenia	48(15.6%)	55(17.9%)
Male	22(45.8%)	27(49.1%)
Female	26(54.2%)	28(50.9%)
Lymphopenia	9(2.9%)	11(3.6%)
Male	4(44.4%)	6(54.5%)
Female	5(54.6%)	5(45.5%)
Thrombocytopenia	35(11.4%)	14(4.5%)
Male	22(62.9%)	6(42.9%)
Female	13(37.1%)	8(57.1%)

5.4. Immunological Parameters

The overall mean CD4 count was 442±270 cells/µl and the mean was 363.7±275.6 and 520.2±264.6, respectively in HIV positive individual before and on HAART.

CD4 count was statistically higher in HIV positive individual on HAART as compared to before HAART HIV positive individuals (P=0.001) (Table 5).

Table 5: Immunological parameters of HIV positive individuals at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Variables	Before HAART (Mean±SD)	On HAART (Mean±SD)	P-Value
CD4 (cells/µL)	363.7±275.6	520.2±264.6	**0.001***

Note: "*"= indicates the level of significance (p<0.05)

CD4 Count and Hematological Abnormalities

The minimum, maximum and overall median value of CD4 count was 14cells/µl, 2000 cells/µl and 403cells/µl, respectively. The prevalence of anemia, leukopenia and neutropenia increased with decreased in CD4 counts (p<0.05). The prevalence of anemia before HAART with CD4 count<200 cells/µl was 47.2% higher than CD4 count between 200-500 and CD4 count>500 were 28% and 16.9 %, respectively.

The prevalence of leukopenia before HAART with CD4 count<200 cells/µl was 23.6% higher than CD4 count between 200-500 and CD4 count>500 were 22.4% and 3.9%, respectively. The prevalence of neutropenia before HAART with CD4 count<200 cells/µl was 19.8% higher than CD4 count between 200-500 and CD4 count>500 were 19.2% and 3.9%, respectively.

There was statistically significant association between different categories of CD4 count and percentage of anemia, leukopenia and neutropenia in HIV positive individuals before HAART. However, there was no significant association between hematological abnormalities and different categories of CD4 count in HIV positive individuals on HAART (p<0.05) (Table -6).

Table 6: Association of CD4 counts with cytopenias in HIV positive individuals at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

HIV positive individuals before HAART						
Variable	Anemia N (%)	Leukopenia N (%)	Neutropenia N (%)	Lymphopenia N (%)	Thrombocytopenia N (%)	Total
CD4 count cell/µl						
<200	50(47.2%	28(23.6%)	24(19.8%)	2(1.9%)	15(14.2%)	106
200-500	35(28%	25(22.4%)	21(19.2%)	6(4.8%)	14(11.2%)	125
>500	13(16.9%	3(3.9%)	3(3.9%)	1(1.3%)	6(7.8%)	77
P-value	**0.001***	**0.001***	**0.005***	0.263	0.772	
HIV positive individuals on HAART(X^2-Test)						
CD4 count cell/µl						
<200	4(9.3%)	9(20.9%)	6(14%)	1(2.3%)	3(7%)	43
200-500	19(17.6%	19(17.6%)	18(16.7%)	3(2.8%)	4(3.7%)	107
>500	22(13.9)	46(29.3%)	31(19.7%)	7(4.5%)	7(4.5%)	158
P-value	0.783	0.176	0.274	0.785	0.367	

Note: *= indicates the level of significance (p<0.05)

HAART=Highly Active Antiretroviral Treatment HIV=Human immune Deficiency Virus

5.5. Factors associated with hematological abnormalities

Multivariable logistic regression analysis was done for all explanatory variables with p< 0.25 in binary logistic regression analysis. WHO clinical stages, sex, types of HAART regimens and intestinal parasitosis on HAART HIV positive individuals and CD4 cell count and age before HAART HIV positive individuals were analyzed multi logistic regression.

HIV positive individuals before HAART with CD4 count <200cells/µl had 4.2 times more likely developing anemia as compared to those who had CD4 count>500cells/µl (AOR=4.2, 95% CI:(1.4,4.8) (p=0.04). HIV positive individuals before HAART with CD4 count

<200cells/µl had 3.2 times more likely developing leukopenia as compared to those who had CD4 count>500cells/µl (AOR=3.2, 95% CI: (2.8-7.9) (p=0.005) (Table -7).

Table 7: Factors associated with predictors of anemia and leukopenia of HIV positive individuals before HAART at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Variables	Anemia N (%)	COR(95% CI)	P-value	AOR(95% CI)	P-value
CD4 count(cells/µl					
<200	56(47.2%)	4.4(1.2,4.8)	**0.001***	4.2(1.4,4.8)	**0.04***
200-500	90(28%)	1.91(1.14,4.6)	0.09	2.1(2.3,6.3)	0.098
>500	64(16.9%)	1.00		1.00	
Age (year)					
18-29	10(10.2%)	1.0(1.0,2.7)	0.21	0.4(0.24,5.6)	0.28
30-39	27(27.6%)	4.1(3.1,5.7)	0.17	0.9(0.71,2.7)	0.07
40-49	48(49%)	1.2(1.1,1.46)	0.05	2.7(3.2,5.4)	0.82
≥50	13(13.3%)	1.00		1.00	
Leukopenia N (%)					
CD4 count(cells/µl					
<200	81(23.6%)	7.6(7.24-8.67)	**0.001***	3.2(2.8-7.9)	**0.005***
200-500	57(22.4%)	2.9(1.26-3.67)	0.058	0.12(0.16-1.67)	0.09
>500	74(3.9%)	1.00		1.00	
Age(year)					
18-29	10(10.2%)	1.1(1.0,1.4)	0.03	0.1(0.21,2.50)	0.064
30-39	15(15.3%)	2.5(1.65,3.95)	0.08	2.9(0.21,2.40)	0.21
40-49	12(12.2%)	3.0(2.9,6.7)	0.24	1.8(0.22,0.48)	0.53
≥50	19(19.5%)	1.00		1.00	

Note: *= indicates the level of significance (p<0.05),

COR=Crude odd ratio, AOR=Adjusted odd ratio, 1.00=reference group

Females were 2.6 times more likely of developing anemia compared to males (p=0.048, AOR=2.6, 95% CI=1.3-4.8). HIV positive individual with intestinal parasite infection was 2.5 times more likely of developing anemia compared to those without infections (p=0.048, AOR=2.5, 95% CI=1.1-7.4). HIV positive individual with WHO clinical stage IV was 2.4

times more likely of developing anemia compared to others clinical stages(I,II&III) (p=0.038, AOR=2.4, 95% CI=1.4-4.8) and patients on ZDV, 3TC, NVP therapy was 3.2 times more likely of developing anemia compared to others HAART regimens (p=0.001, AOR=3.2, 95% CI=2.2-7.5).

These factors were computed for others hematological abnormalities (leucopenia, neutropenia, lymphopenia and thrombocytopenia); however, they were not found to be statistically significant (p>0.05) (Table -8).

Table 8: Factors associated with predictors of anemia of HIV positive individuals on HAART at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia April to June, 2018

Variables	Anemia (%)	COR(95% CI)	P-value	AOR(95% CI)	P-value
Intestinal parasites					
Yes	35(77.8%)	10.4(9.3,15.4)	**0.022***	2.5(1.1,7.4)	**0.048***
No	10(22.2%)	1.00		1.00	
Sex					
Female	32(71.1%)	3.1(1.8,3.9)	**0.04***	2.6(1.3,4.8)	**0.048***
Male	13(28.9%)	1.00		1.00	
WHO clinical stages					
I	2(4.4%)	1.00		1.00	
II	7 (15.6%)	3.0(2.05,4.7)	0.02	0.2(0.4,3.3)	0.1
III	5(11.1%)	3.8(2.2,3.9)	0.007	0.4(0.02,1.2)	0.5
IV	31(68.9%)	5.6(4.1,7.8)	**0.029***	2.4(1.4,4.8)	**0.038***
HAART regimens					
ZDV,3TC,EFV	3(6.7%)	19.3(15.3,23.7)	0.5		
ZDV,3TC,NVP	23(51.1%)	5.6(3.6,8.2)	**0.045***	3.2(2.2,7.5)	**0.001***
TDF,3TC,EFV	8(17.8%)	3.6(0.02,1.3)	0.9		
TDF,3TC,EFV	11(24.4%)	3.4(0.1,2.2)	0.85		
TDF,3TC,LPV/r		1.00		1.00	

Note: "*"= indicates the level of significance (p<0.05)

COR=crude odd ratio AOR=adjusted odd ratio CI=confidence interval

6. DISCUSSION

The prevalence of anemia and thrombocytopenia were higher before HAART HIV positive individuals and while leukopenia was higher in adult HIV positive individuals on HAART. CD4 count<200cells/µl was identified as significant risk factors of anemia and leukopenia before HAART while HAART regimen (ZDV, 3TC, NVP), WHO clinical stage IV, female and intestinal parasites were identified as significant predictors of anemia in HIV positive individuals on HAART.

In the current study, there were statistically significant differences on mean values of RBC ($4.2\pm0.8x10^6$ vs. $4\pm0.8x10^6$/µl), RDW (15.2±1.4 vs. 14.3±2.1%), hemoglobin (12.2±2.5 vs. 13.6±2.8g/dl), MCV (90.9±11.8 vs. 94.2±9.9fl), MCH (32.3±5 vs.33.3±5.1pg) & CD4cells (363.7±275.6cells vs. 520.2±264.6cells/µl) count before and on HAART, respectively. This is concurrent with studies conducted in Black Lion Specialized Hospital(35), Gondar University Hospital(43) and Jimma University Specialized Hospital (46). There were also statistically significant differences on mean values of hematocrit (34.6±6.8vs.38.7±7.6%), TLC ($1.9\pm1.1x10^3$ vs. $2.2\pm0.9x10^3$/µl) and platelet ($242.2\pm109.7x10^3$vs. $273.6\pm117x10^3$/µl) counts before and on HAART, respectively. But, these parameters were not found to be statistically significant in others studies conducted in Gondar University Hospital(43) and Jimma University Specialized Hospital(46).The difference might be different in HAART regimens.

In the current study, prevalence of anemia was 31.8% and 14.6% before HAART and on HAART, respectively. This indicates that prevalence of anemia was higher in untreated patients ($p<0.05$). This is consistent with studies conducted in Jimma University Specialized Hospital 29.9% and 16.2% (46), Gondar University Hospital 29.7% and 11.7%(43), Hawassa University Referral Hospital 23.4% and 12%(34), Gondar University Hospital 21.2% and 11.5%(40), Minillik II Memorial Hospital 52.6% and 37.3%(36), Gondar Northwest 42.8% and 18.9%(9), Black Lion Specialized Hospital 41.9% and 11.4%(35) and Zewuditu Memorial Hospital 42.9% and 14.3%(39),respectively. The possible explanation could be that, the decrease in the prevalence of anemia after ART initiation is attributed to the positive effect of HAART on the differentiation and survival of erythrocytes, decreases the viral load and reduces the frequency of opportunistic infection(50, 51).

The prevalence of anemia before HAART was 31.8% which is concurrent with findings reported in Gondar University Hospital 35% (41), Zewditu Memorial Hospital 35.5%(37), Jimma University Specialized Hospital 29.9% (46), Dessie Referral Hospital 34.4% (42) and

Gondar University Hospital 29.7%(43). But, higher than studies conducted in Iran10%(31), Nigeria 24.2%(29), Yekatit 12 Hospital18.9%(38), Hawassa University Referral Hospital 23.4%(34) and Gondar University Hospital 21.2%(40) and lower than studies conducted in India65.5%(28),Cameroon 62.9%(32),Hiwot-Fana Specialized University Hospital 54.4%(45) and Arba Minch Town 52.3%(33). The reasons for the observed differences might be due to difference of socio-demography, genetic variation, presence of parasitic infections, history of chronic diseases and nutritional difference.

In the current study, the prevalence of anemia on HAART was 14.6% which is in agreement with studies conducted in Jimma University Specialized Hospital 16.2%(46),Gondar University Hospital 11.7%(43), Black Lion Specialized Hospital 11.4%(35),Gondar University Hospital 11.5%(40), Hawassa Referral Hospital 12%(34) and Zewuditu Memorial Hospital 14.3%(39). But, lower than the findings reported in India 46%(27), Minillik II Memorial Hospital 37.3%(36) and Hiwot-Fana Specialized University Hospital 54.4%(45). The reasons for the observed differences might be due genetically difference, awareness of the patients toward causes of anemia, nutrition difference and parasitic infections.

In this study, the most common type of anemia was normocytic-normochromic anemia. This is comparable with studies conducted in Northwest Gondar(52) and Gondar University Hospital(43). Macrocytic-normochromic anemia was found to be the second common type of anemia. This concurrent with findings of India(27), Black Lion Specialized Hospital(50) and Northwest Gondar(52). This could be due to the effect of AZT on the MCV, as the majority of anemic patients were receiving Zidovudine based regimens(53).

Macrocytosis was common in HIV positive individuals (64.4%) on HAART than before HAART (16.3%). This is probably due to the effect of Zidovudine based therapy which is responsible for the development of macrocytosis and associated with marrow toxicity (53).

In the current study, prevalence of leukopenia was 18.2% and 24% before HAART and on HAART, respectively. This could be that Zidovudine causes leukopenia by suppression of bone marrow and cytotoxicity of T cells(54). The prevalence of leukopenia before HAART was 18.2% which is concurrent with the studies reported in Uganda 24.3%(14), Nigeria 26.8%(29), Tanzania 23.6% (30) and Gondar University Hospital 16.6%(43). But, lower than study conducted in Cameroon 34.6%(32). The prevalence of neutropenia and lymphopenia before HAART was 15.6% and 2.9%, respectively and neutropenia is similar with study conducted in Gondar University Hospital 14.5%(43).

In the current study, the prevalence of leukopenia on HAART was 24% in agreement with study conducted in India 25% (27) and lower than Gondar University Hospital 35.5%(43). The prevalence of neutropenia and lymphopenia on HAART was 17.9% and 3.6% respectively. Neutropenia is lower than study conducted in Gondar University Hospital 28.3% (43) and lymphopenia is in agreement with study conducted in Gondar University Hospital 2.1%(43). The reasons for the observed differences might be due to the difference in clinical condition of patient, study population, sample size and study design variation.

In the current study, the prevalence of thrombocytopenia before HAART was higher than on HAART (11.4% vs.4.5%). The possible explanation could be that AZT can rapidly increase platelet count in patients with HIV(19, 55). This is concurrent with studies conducted in Uganda 8.3% (14), Nigeria 16.1% (29), Tanzania 14.4% (30), Gondar University Hospital 9% (43) and Yekatit 12 Hospital 8.5% (38) and lower than studies conducted in India 24%(27) and Cameroon 27.1% (32). But, the prevalence of thrombocytopenia on HAART was 4.5% which is concurrent with study conducted in Gondar University Hospital 4.1%(43). The reasons for the observed differences might be due to platelet immune destruction by spleen, clinical condition of patient, ineffective platelet production and study population.

In this study, the prevalence of anemia was associated with WHO clinical stage IV in HIV positive on HAART. This is concurrent with studies conducted in Cameroon(32), Uganda(14), Felege-Hiwot(44), Black Lion Specialized Hospital(35) and systematic review and meta-analysis(56). This could be explained as, the advanced WHO clinical stage the higher the prevalence of anemia(57).

The prevalence of anemia was associated with HAART regimen (ZDV, 3TC, NVP) in HIV positive individual on HAART. This is in agreement with studies done in Cameroon(32), Jimma University Specialized Hospital(46) and Minillik II Memorial Hospital(36). This might be due to hematological toxicity of antiretroviral treatment.

The prevalence of anemia was associated with female in HIV positive individual on HAART. This consistent with studies conducted in Iran(31), Uganda(14), Minillik II Memorial Hospital(36) and Gondar Northwest (9). The higher prevalence of anemia in female could be explained by due to large attribution of menstrual blood loss and drains on iron stores that occur with pregnancy and delivery(58). The prevalence of anemia also associated with intestinal parasitic infection. This agrees with study in Zewuditu Memorial Hospital (39).

CD4 count<200cells/µL was found to be associated with anemia, leukopenia and neutropenia before HAART HIV positive individual. This is concurrent with studies conducted in India (59), Cameroon (32), University of Gondar Hospital (40), Jimma University Specialized Hospital (46), Black Lion Specialized Hospital (35), Arba Minch Town (33),Northwest Gondar(52), systematic review and meta-analysis(56) and Jimma University Specialized Hospital(46). The risk of having CD4 count<200 cells/µl was increased before ART initiation, because, before ART initiation patients have less immunity towards the HIV and the virus attacks CD4 cells easily. This may indicate the increases of immunological deterioration and also, may be caused by direct and indirect effect of HIV infection, toxicity of the drugs and opportunistic infections (26, 33) and in the advanced stage of the disease, the blood cell counts were lower than the early stage of the diseases(60, 61).

7. LIMITATIONS OF THE STUDY

Data on viral load was not included in this study. Because the test is not done at the study site. The causes of hematological abnormalities in HIV positive individuals are multi-factorial. So, this study inability to identify all causes hematological abnormalities.

8. CONCLUSIONS

There was a decline in the prevalence of anemia and thrombocytopenia among HIV positive individuals after ART initiation. This study has shown that the most common hematological abnormalities in HIV positive individuals were anemia, leukopenia and thrombocytopenia. The prevalence of anemia and thrombocytopenia were higher before HAART while leukopenia, neutropenia and lymphopenia were higher in HIV positive individual on HAART. The normocytic normochromic anemia was common before HAART HIV positive individuals while macrocytic normochromic anemia was common in HIV positive individuals on HAART and mild anemia was the commonest type of anemia. There were statistically significant differences on mean values of RBC, Hgb, Hct, MCV, MCH, RDW, TLC, PLT and CD4 cell counts between before and on HAART HIV positive individuals.

HIV positive individuals before HAART had hematological abnormalities with risk factors different from on HAART HIV positive individuals. The CD4 count<200cells/µl was only the risk factor of anemia, leukopenia and neutropenia in HIV positive individuals before HAART. Anemia, leukopenia and neutropenia decreased as CD4 count increased and no statistically significant association was found between CD4 counts and hematological abnormalities in HIV positive individual on HAART. Female, WHO clinical stage IV, HAART regimen (ZDV, 3TC, NVP) and intestinal parasites infection had statistically significant association with anemia in HIV positive individuals on HAART. Therefore, routine monitoring of risk factors for hematological abnormalities and early initiation of ART is beneficial to reduce the magnitude of hematological abnormalities.

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9. RECOMMENDATIONS

Based on these findings, it is recommended that all HIV positive individuals should be screened and treated for hematological abnormalities to reduce the morbidity and mortality. Physicians giving care for HIV positive individuals should regularly investigate and treat hematological abnormalities in HIV positive individuals before and on HAART. HIV positive individuals with history of intestinal parasite infection, who are taking AZD containing ART, have low CD4 count and advanced WHO clinical stages should be carefully screened and treated for hematological abnormalities.

Therefore, further longitudinal studies with long term follow-up are needed to explore more on the causes of hematological abnormalities.

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11. ANNEXS

11.1. Annex One: Structured Questionnaires’

1.1. Data collection Questionnaire’s (English Version)

Title of the study: Hematological profile and associated factors among HIV positive adult individuals before and on antiretroviral treatment at Madda Walabu University Goba Referral Hospital.

Introduction to the study: This study is aimed to determine the hematological abnormalities and associated factors among HIV positive adult individuals before and on highly active antiretroviral treatment which is the major cause of morbidity and morbidly, and provide recommendation possible prevention and controlling level on the problem and help them for effective intervention plan in the future.

The involvement in the study is based on your voluntary and you have the right to refuse to participate in the study, and the confidentiality of the information gathered will be kept and only used for this study. The result of the laboratory find will be communicated to your physician or care giver.

Direction:-Please encircle the number of your answer or correctly fill in the black space provided for open ended questioners

Part One: Socio-Demography Data

Sno	Questions	Answers
1	Identification	Code number.....
2	Sex of patients	1.Male 2.Female
3	Age of patients in years	
4	Where do you live?	1. Urban 2.Rural
5	What is your educational status?	1.unable to read and write 2. able to read and write 3. Elementary School 4. Secondary school 5. Certificate and above
6	What is your occupation?	1. Farmer 2. Housewife 3. Private 4. Daily laborer 5. Governmental Employee 6. NGO employee 7. Other, specify _____
7	What is your current marital status?	1. Single 2. Married 3. Divorced 4. Widowed
8	What is your monthly family income? (in ETB)	
9	What is the size of your household?	

PART II: CLINICAL DATA

Sno	**Questions**	**Answers**
10	WHO clinical stage(from ***patient card***)	1. I 2.II 3. III 4.IV
11	Opportunistic infection If **yes,** go to 12	1. Yes 2. No
12	Which one?	1. Herpes Zoster 2. Candidiasis 3. Pneumonia 4. Tuberculosis 5. Other.....
13	Have you chronic illness? If **yes,** go to 14	1. Yes 2. No
14	Which one?	1. TB 2. DM 3. Hypertension 4. other ...
15	Type of HAART regime(from card)	1. ZDV, 3TC, EFV 2. ZDV, 3TC, NVP 3. TDF, 3TC, EFV 4.TDF, 3TC, NVP 5.TDF, 3TC, LPV/r 6. Others ...
16	Duration of HAART(in months)	
17	Body Mass Index (BMI in kg/m^2)	Height (m)..... Weight (kg).....

PART THREE: LABORATORY DATA

Laboratory results will be attached with these questionnaires

Complete blood count, CD4 count, hemoparasites and stool examination

የጥያቄዎች ዝርዝር መዋቅር (የአማረኛጥያቄ)

ክፍል አንድ: የመልስሰጪዋየማህበራዊ፡ኢኮኖሚያዊናስነ-ህዝባዊባህሪን የተመለከተመረጃ

ተ.ቁ	ጥያቄ	ምላስ
1	መለየ	1) ኮድ ቁጡር..... 2) ያታ
2	እድሜ	**ምእመት**
3	**የበሽተኛዉ ፃተ**	1) ወንድ 2)ሴት
4	የት ትኖራለህ/ሽ?	1)**ከተማ** **2)ገጠር**
5	የትምህርት ደረጃዎ ምንድነው?	1)**ያልተማሬ** **2)አንደኛ ደረጃ ት/ቤት** **3)ሁለተኛደረጃት/ቤት** **4)ሰርትፍኬትና ከዛበላይ**
6	ሥራዎ ምንድነው?	1)**ገበሬ** **2)**የቤትእመቤት 3) የግልሰራተኛ 4)የመንግስት ሰራተኛ 5) ቀንሰራተኛ 6)መንግስታዊያልሆነ 7) ሌላ(ይገለጽ) ------
7	አሁን ያለዎት የጋብቻ ሁኔታ ምንድነው?	1) ያላገባች 2) ያገባች4) ባሏየሞተባት 3) ተለያይተውየሚኖሩ 5)የተፋታች
8	ወርሀዊ የቤተሰብዎ ገቢ ስንት ነዉ	**በወር ፈ**(ETB) **.....**
9	የቤታችሁ መጠን ምን ያህል ነው?	

ክፍል *ሁለት*: Clinical Data

ተ.ቁ	ጥያቄ	ምላሽ
10	የዓለም የጤና ድርጅት ደረጃ	1)I 2)II 3)II 4)IV
11	**የአፖርቹንስትክ አንፊክችን የዞክ(ሽ) የቃል?** መልሱ አዎን ከሆነወደጥያቄ12 ይሂዱ	0) የለም 1) አዎን
12	**ምን ኤይነት ነዉ?**	1) **ሳንባ ነቀርሳ** **2) አልማዝ በላጭራ** **3) ከንዲድያሲስ** **4)ንሞንያ** **5) ሌላ** (ይገለጽ)
13	ሥር የሰደደ በሽታ አለብዎት? መልሱ አዎን ከሆነወደጥያቄ14ይሂዱ	0) **አይደለም** **1)** አዎን
14	**ምን አይነት ነዉ?**	1) **ደምግፊት** **2) ሱከርበሽታ** 3) **ሰንባ-ነቀርሳ** **4) ልላ**(ይገለጽ)
15	**የወረ-ዔድስ በሽተ መዳንት አይነት**	1. ZDV, 3TC, EFV 2. ZDV, 3TC, NVP 3. TDF, 3TC, EFV 4.TDF, 3TC, NVP 5.TDF, 3TC, LPV/r 6. Others ...
16	በ HAART ምን ያህል ጊዜ ይቆያሉ?	
17	የሰውነት ስብስብ ኢንዱስትሪ (ኪሎ ግራም / ኪ.ሜ)	ቁመት(ሜ) ክብደት(ኪ.ግ)

ክፍል ሶስት: የላቦራቶሪ ውጤቶች **ከዘሂ ጥያቄ ጋር ይተሳራል**

Gaaffiilee (Hiikkaa Afaan Oromoo)

Kutaa 1ffaa: Gaaffiilee Haala Jireenyaa Hawaasaa Fi Diinagdee

Lakk	Gaaffiilee	Deebii
1	Eenyummaa dhukkubsataa	Lakk.addaa.....
2	Saala	1) dhalaa 2) dhiira
3	Umrii dhibamaa waggaadhan	
4	Body mass index (kg/m2)	Dheerina(m) ulfaatina(kg)
5	Iddoon jireenyaa keessaniin eessaa?	1) Magaala 2) Baadiyyaa
6	Sadarkaan barnoota keessanii meeqaadhaa?	1) hin baranne 2) sadarkaa 1ffaa 3) sadarkaa 2ffaa 4) sartifikeetii fi isaa oli
7	Hojii maalii irratti bobbaatanii jireenya maatii gaggeessituu?	1) qotee bulaa 2) haadha manaa 3) hojii dhuunfaa 4) qacaramaa mootummaa 5) hojjataa guyyaa 6) mit-mootummaa 7) Kan biro.....
8	Yeroo ammaa haallii gaa'ela keessaa akkami?	1) fuudhe 2) hin fuune 3) hiike 4) kan manatti hafe(tee) 5) Kan irraa du'e(te) 6) Kan biro (ibsi).....
9	Ji'aan qarshii meeqa argattuu?	
10	Baay'innii miseensa maatii meeqaa?	

Kutaa 2ffaa: Odeeffannoo Daatawwan kilinikaalaa

Lak	Gaaffiilee	Deebii
11	Sadarkkan WHO meeqarra jirtaa?	1) I 2)II 3)III 4)IV
12	Dhibee oporchuunistikii isin qabee beekaa?	1) lakkii 2) eeyyeen
13	Gaaffii lakk.**12**ffaa **eeyyeen** yoo tahe,isa kamii?	1)dhibee daranyoo sombaa 2) dhibee waan lafaa 3)dhibee fangasii 4) dhibee afuuffee qilleensaa 5) Kan biro (ibsi).....
14	Dhibee namarraa turu si qabee beekaa?	0) lakkii 1) eeyyeen
15	Gaaffii lakk.**14**ffaa **eeyyen** yoo tahe,isa kamiidhaa?	1) dhibee dhiibbaa dhiiga 2) dhibee sukkaaraa 3) Dhibee daranyoo sombaa 4) Kan biro (ibsi).....
16	Akaakuu qorsa dhibee Eedsii(kardi irraa)	
17	Qorsa dhibee eedisii erga fudhachuu eegaltee ji,a meeqaadhaa?	

Kutaa 3ffaa: Qorannoo laaboraatorii -waraqaa gaaffiilee kana waliin walitti hidhama.

11.2. Annex Two: Data Extraction Form for Base Line

Sno	Variable	Categories
Part One: Socio-Demographic and Baseline Characteristics		
1	ART Unique Number(code no)	
2	Age in years	
3	Sex	1.Male 2.Female
4	CD4	cells/µl
5	RBC	cells/µl
6	Hgb	g/dl
7	Hct	%
8	MCV	fl
9	MCH	gm/dl
10	MCHC	pg
11	RDW	%
12	WBC	cells/µl
13	Total lymphocyte count	cells/µl
14	Absolute neutrophil count	cells/µl
15	Platelet	cells/µl
16	WHO stage	1) I 2)II 3)III 4)IV
17	Body Mass Index(BMI in kg/m^2)	
18	Opportunistic infection if **yes**, go to 19 question	1) Yes 2) No 3) Not recorded
19	Which one?	1. Herpes Zoster 2. Candidiasis 3. Pneumonia 4. Tuberculosis 5. Other.....

11.3. Annex-Three: Laboratory Procedures

Complete Blood Counting by Cell-Dyn 1800 Automation Hematology Analyzer

Material required:

- Celldyn 1800 Diluents of 20 lit
- Celldyn 1800 Detergent of 20 lit
- Celldyn 1800 Lyse of 3.8 or 5 lit
- Celldyn 1800 Enzymatic cleaner
- Celldyn 1800 Calibrator
- Celldyn 1800 tri- level control reagents

Supplies

- EDTA anticoagulant tube(Vacutainer tube)
- Dry gauze
- Cotton Swab
- Vacutainer needle with holder
- 70% Ethanol alcohol or similar antiseptic
- Tourniquet
- Glove

Specimen type: EDTA ant coagulated whole blood. A minimum of 0 .5 ml must be collected for micro collection specimens. This ensures an adequate amount of blood for the 30 µl aspiration at open mode.

Specimen stability: A well-mixed whole blood specimen, collected in EDTA anticoagulant and run within eight hours after collection, provides the most accurate results for hematological parameters.

Daily start up procedure

- Confirm that the power plugs for the instrument and printer are inserted in to a grounded power out let
- Check waste containers if it is more than ¾ full discard it and replace it
- Set the printer switch to on. Confirm that the printer is installed and feeding correctly.
- Check to see that all reagent tubing is properly connected to the correct inlet and that there is sufficient volume of reagent in the reagent containers.
- Set the instrument power switch to on.

- When INITIALIZED is displayed in the Main Menu screen status box, press [PRIME/RUN] to bring the instrument to ready.
- Prior to running patient specimens and control reagents, run Background counts until result results are within appropriate specification.

➢ Running a background counts

➢ Perform a specimen run without placing a specimen under the sample aspiration Probe.

➢ Press the [RUN] key in the MAIN MENU screen. Then press the [SPECIMEN TYPE] key followed by [NORMAL BACKGROUND]

➢ Press the Touch plate. (Only reagents are cycled through the system.)

Note: All previous results displayed on the current RUN screen will be deleted completed

Repeat the procedure if the background Counts do not fall within the following acceptable ranges:

➢ WBC ≤ 0.5K/µl

➢ RBC ≤ 0.05M/µl

➢ HGB ≤ 0.1 g/dl

➢ PLT ≤10 K/ µl

Daily Quality control: On a daily basis before running patient specimens, perform Quality Control (QC) procedures.

Procedure for daily Quality control runs:

- From the MAIN MENU screen, press [RUN]
- From the RUN menu, press [SPECIMEN TYPE] followed by [QC TYPE]
- Select the desired level of control (Low, Normal, High, or Replicates)

Note: Prepare a permanent record (printed copy) of any files to be selected or purged, as required.

- Press [RETURN].
- Remove the cap from a well - mixed control specimen tube and place the open tube under the sample Aspiration probe. Raise the tube so that the end of the probe is deeply immersed in the specimen.
- Press the Touch Plate to activate the run.
- When the well-mixed control has been aspirated from the tube and the probe moves up through the Wash Block, remove the specimen tube and replace the cap.

Note: If a flow error, clog, or other fault message appears on the display screen during RUN cycle, press [CLEAR ORIFICE]

- Verify that control results are within your laboratory's acceptable limits.
- If the control results fall within acceptable limits, review the data for shifts or trends, record the results, and begin to process patient specimens.
- Press [PRINT REPORT] if a report of printed results is desired.
- Press [MAIN] to return to the MAIN MENU screen.

Running patient specimen procedure: When the READY message is displayed on the RUN Screen, the instrument is ready to run specimens. To run patient specimens, proceed as follows:

- With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times.
- Remove the cap from the pre-mixed specimen tube.
- Place the tube under the aspiration probe and raise the tube so that the end of probe is deeply immersed in the specimen.
- Press the Touch Plate to activate the run.
- When the sample has been aspirated from the tube, the probe will move up through the Wash Block. Remove the specimen tube and Recap the tube
- After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the data
- If Automatic Graphics Printout has been specified in the SETUP menu, a report is printed according to the parameters selected during the setup procedure.

If Automatic Graphics printout has not been specified in the SETUP menu, press [PRINT REPORT] to obtain a copy of the results. The print report format is the only method to be used

Source of Error: If the sample is

- Hemolyzed 'Clotted
- Collected in improper tube
- Not mixed well and Small volume

(Source:-Cell-Dyn 1800 haematology analyzer user manual)

BD FACSPresto™ (absolute CD4)

Principle

The BD FACSPresto system includes ready-to-use, single-use disposable cartridges that contain dried-down reagents to simultaneously identify and enumerate CD4 T lymphocytes for absolute and percentage results from whole blood samples. In addition to performing absolute and percent CD4 tests, the BD FACSPresto cartridge also measures total hemoglobin concentration on the same sample and delivers all results concurrently. When blood is introduced into the BD FACSPresto™ Cartridge, the specific antibodies bind to the surface antigens on the T lymphocytes and monocytes during the incubation period. When the stained cartridge is inserted into the counter, the dedicated software identifies and counts the CD4+ T lymphocyte absolute and percentage cells, and calculates the hemoglobin concentration. The BD FACSPresto™ Cartridge also contains immobilized antibodies as a quality control measure which the instrument uses to ensure that the reagents are present and sufficient blood specimen volume has been added.

1. The fingertip is punctured with the lancet, and the first drop of blood is wiped. The second drop of blood is added into the inlet port of the cartridge. The finger is cleaned, the cartridge is capped, and a bandage is applied to the finger. For blood collected by venipuncture draw, a pipette is provided to collect blood from the tube and add it into the inlet port on the cartridge. No manual sample preparation is required for either collection method, saving.

2. Blood is added into the BD FACSPresto cartridge by finger stick or venipuncture draw. For finger-stick blood collection, the BD FACSPresto™ finger-stick collection kit comes with a lancet, bandage, alcohol pad, and nonwoven sponge.

3. Once the blood is added and the cartridge is capped, the blood picks up the dried reagent and flows along a channel inside the cartridge. At the end of the channel, a fill indicator ensures that the blood has properly flowed along the entire channel to its endpoint.
4. The operator places the BD FACSPresto cartridge on the work station outside the instrument for incubation.

5. The operator selects the timer on the screen that corresponds to the incubation slot on the work station. The on-board timer automatically counts down a preset incubation time of 18 minutes. Since incubation occurs outside the device, at room temperature, technicians gain greater flexibility.

6. Insert the cartilage into the door, results display within 2-3 minutes

Automated quality control and testing

Sensor inside the instrument detects the cartridge and draws it in, and the door closes. This automatically starts the reading process. The on-board reagent quality control feature verifies that reagent and a sufficient amount of blood are present in the cartridge.

(Source:-BD FACSPresto machine user manual)

Thin and Thick Blood Film by Geimsa Stain

Principle: Eosin and methylene blue in the solution contains various azure compounds such as thiamine and its methylene derivatives the staining reaction is oxidative. Therefore, the oxygen in water will initiate the reaction and ruin the stock stain.

Materials, Reagents &Equipment's

- Clear microscope slide
- Microscope
- Sterile lancet
- Staining jars (dish)
- Lens paper
- Methyl alcohol
- Immersion oil
- Buffer (ph7.1-7.2)
- Geimsa stock solution
- Timer
- Drying rack

Sample: One to two drop of whole blood from a capillary puncture or EDTA ant coagulated venous blood.

Evaluation of well-stained thin film

- The back ground should be clean and free from debris; the color of erythrocytes is a pale green pink.
- Neutrophil leukocytes have deep purple nuclei and well defined granules.
- The combination of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.
- Stippling should Shaw up as schuffner's dots in erythrocytes containing *P.vivax* or *P.ovale*, and Mauree's spots in erythrocytes containing the larger ring forms of *P.falciparum.*

Evaluation of well-stained thick film

- The back ground should be clean and free from debris, with a pale mottled-gray color derived from the laysed erythrocyte.
- Leukocytes nuclei are a deep, rich purple.
- Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In *P.vivax* and *P.ovale* infections the presence of schuffner's stippling in the ''ghost'' of the host erythrocyte can be seen especially at the edge of the film.

Evaluation of staining quality

- A malaria blood film is too pinkish suggests low pH or over stain.
- A malaria blood film is too bluish or purplish suggests that high pH or under-staining.

Procedure

A. Fixing the thin film

1. When the films are completely dry, fix ONLY the thin film by dipping if in absolute methanol for approximately 30 seconds. Care must be taken not to fix any point of the thick film.

2. Allow the film to dray.

B. Staining the thick and thin films

1. Gently pour 3% or 10% Geimsa working solution in the staining jar.

2. Put the slides in a rack inside the staining jar; the slides should be fully submerged /covered with the stain.

3. Stain for 30-45 minutes and 10-15minutes for 3% and 10% Geimsa working solutions, respectively.

4. Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain. Alternatively, gently immerse the whole jar in a vessel filled with clean water.

5. Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds. Pour water off.

6. Wipe the back of each slide with paper towels.

7. Dray the slides in a vertical position with the thin film down wards.

C. Focusing and scanning the blood film

- Place the blood film on the microscope stage, switch on the light and adjust the light source optimally by looking through the ocular and the 40x objective.
- Place the drop of immersion oil on the dray stained slide. To avoid cross contamination, ensure that the immersion applicator never touches the slide.

- Slowly change to the oil immersion objective, and a thin film of oil will form between the slide and the lenses.
- Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the 100x objective and use the fine adjustment knob to focus the lens should not be allowed to touch the slide.
- Examine the slide in a systematic fashion. Start at the left end of the thick film and begin reading at the periphery of the field and finish at the other end. When the field is read, move the slide right to examine adjacent fields.

D. Examining the thick blood film

1. Scan the thick film under oil immersion objective (100 xs) and ascertain whether a smear is positive or negative.

2. Use the WHO bench job aids in the diagnosis of plasmodium infections.

3. If positive, determine all species and stages present in the slide.

4. Read a minimum of 200 oil immersion fields before declaring a slide negative. If time permits, scan the whole thick film.

E. Examining the thin blood film

1. When species is doubtful on the thick film, or mixed infections are suspected, a careful examination of the parasite morphology should continue on the thin smear for verification.

2. If deferent species are observed, this should also be recorded.

Quality control

- Follow proper sample collection procedures.
- Glass slides must be clean and free from grease.
- Thin films must be prepared properly while draying protects blood films from dust, flies and insects.
- Do not dry exposed to direct sun light.
- Too thin a film may not have adequate quantity of blood for detection of parasites.
- Blood film spread unevenly on a grease slide makes examination difficult.
- Thin film too long, leaves less space for thick film.
- When fixing the thin film, take care the thick film do not touch by methanol.
- Wet slides are warped together and the slides stick to one another.
- Never add a pinch of EDTA powder directly to the sample tubes. High concentration of

EDTA leads to shrinking of RBC and destroys the structure of WBC and platelet

- Never add the blood before the EDTA solution is completely dried it will dilute the blood.
- Quality control of Geimsa stain is performed for every batch of the stain prepared and quality control results documented using the quality control form.
- Working solution of Geimsa stain should be changed depend on the climatic condition of that specific area at least every 8 hours.
- Test each stock prepared (one month after preparation) for optimum stain dilution and staining time using malaria smears prepared from actual specimens.
- Check pH of buffered water, and add appropriate correcting fluid.

(Source: District Laboratory Practice in Tropical Countries Part 2 Second Edition Monica Cheesbrough)

Parasitological Examination of Faeces

Direct Saline Preparation Techniques

Principle: Microscope slides made from patient specimens can be examined under low and high power for the presence of parasites.

Reagents and equipment

- Normal Saline
- Glass slides
- Cover slips
- Pipettes
- Gloves
- Microscopes

Sample: Ask the patient to pass the stool sample directly into a waxed cardboard or a plastic cup with a tight fitting lid. Collection of sample in a match box or on plant leaves is not a satisfactory method.

- About two grams of well-formed stool or one to two table spoonfuls of watery stool will suffice for a routine examination.

Microscopic examination: It is the simplest and easiest technique. A wet mount can be prepared directly from faecal material or from the concentrated specimens. The basic types of wet mounts that should be made from each sample include:

- Saline wet mount: It is used to detect worm eggs or larvae, protozoan trophozoites and cysts. In addition it can reveal the presence of RBCs and WBCs.

Procedure

- Apply the patient's sample to a small area on a clean microscope slide.
- Immediately before the specimen dries, add 1 or 2 drops of saline with a pipette. Mix with pipette tip.
- Cover the specimen with a cover slip.
- Examine the specimen with the low power objective (10 xs) and low light.
- Examine the entire cover slip for motile flagellates. Suspicious objects can be examined with the high power (40 xs) objective.
- Ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

Quality control: Check the saline. It should be clear with no visible signs of contamination.

Limitations: If the specimen is left at room temperature or held at refrigerator temperature for > 1 hour, the organisms will round up, lose their motility, and eventually die.

Source: District Laboratory Practice in Tropical Countries Part 2 Second Edition

Monica Cheesbrough)

11.4. Annex Four: Patients Information Sheets

Information sheet English version

This information sheet is prepared for individuals who are volunteer to participate in the study. The detailed explanation about what will be undertaken in the study is presented as follows and it is after reading the description that informed consent was obtained.

Title of the project: Hematological profile and associated factors among HIV positive adult individuals before and on taking antiretroviral treatment at Madda Walabu University Goba Referral Hospital, southeast Ethiopia

Name of Principal Investigator: Negesso Duguma Weyecha

Telephone number: +251921482538

E.mail: dugumanegesso@gmail.com

Description and Purpose of the study: Determining the hematological profile has paramount significance in reducing progression of morbidity and mortality. Such data are fundamental for health planers and care givers for evidence-based intervention, to guide future policy makers and would serve as baseline information for further studies at national level. Moreover, this study provides information for the clinicians for management hematological abnormality manifestation. Hence, this study will be conducted to determine hematological profile and associated risk factors among HIV positive adult individuals before and on taking antiretroviral treatment in Madda Walabu University Goba Referral Hospital.

Procedures:-Following your willingness, you are asked to sign a written consent and the following procedures will be undertaken

- You will provide us 15 minutes for interview
- Blood sample (4ml) and a gram of stool sample will be collected
- The blood sample will be analyzed for hemoparasite, hematological and immunological tests whereas; one gram of stool sample will be used for parasitological tests.

Risks and discomforts:-During sample collection we will follow standard operational procedures. The blood drawing may cause minor pain, at the place where blood is taken. However, this pain will no longer appear.

Benefits: - This study will benefit to the entire community since its success will aid in proper clinical decision making and treatment of patients. There is no direct financial benefit you get

by participating in this study. But, the test result will be delivered timely and appropriate intervention will be given.

Confidentiality:-Any information obtained during this study will be kept confidential. This is assured by avoiding use of any identifier and information will be recorded with code number. We release the result obtained from the study, it is in the way that avoids any identifier of you and if there is any identifier, there should be signed confirmation of you.

Voluntary participation: - Participation on this study is voluntary and you have the right to refuse participation at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put you at risk any present or future medical care or other benefits to which you otherwise entitled.

You may ask questions now and in the future if you do not understand something that is being done contact the investigator on above address.

For the success of the study, I will be asking you to give the correct answer for the respective questions. Thank you for your assistance!

የመረጃ ወረቀት የአመርኛ ቅጂ

ይህ የመረጃ ወረቀት በጥናቱ ለመሳተፍ ፈቃደኛ ለሆኑ ግለሰቦች የተዘጋጀ ነው. በጥናቱ ውስጥ ስለሚካሄዱት ዝርዝር ማብራሪያዎች እንደሚከተለው ቀርቧል::

የፕሮጀክቱ ርዕስ-ሄሚቶሎጂካል ፕሮሞሽን እና ከኤች አይ ቪ / ከኤችአይቪ / ኤድስ ጋር ተያያዥነት ያላቸው ተፅእኖዎች በጋባ ሪፈራል ሆስፒታል ውስጥ በደም ምስራቅ ኢትዮጵያየ ዋ ና ተ መራ ማሪ ስ ም፡

የዋና ተቆጣጣሪ ስም: - Negesso Duguma Weyecha

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ኢሜል፡ dugumanegesso@gmail.com

የጥናቱ ዓላማ እና ዓላማ-- የሄሞቲሎጂያዊ ቅርፅን መለየት በሽታውን መጨመር ለመቀነስ ከፍተኛ ጠቀሜታ አለው:: እንደነዚህ ያሉት መረጃዎች ለጤና ፕላኖች እና ለንክብካቤ ሰጪዎች መረጃን መሰረት ያደረገ ጣልቃ ገብነት, ለወደፊቱ የፖሊሲ አውጭዎችን ለመምራት እና በብሔራዊ ደረጃ ለተጨማሪ ጥናቶች እንደ መሰረታዊ መረጃ አገልግሎት ሆነው ያገለግላሉ:: በተጨማሪም ይህ ጥናት ለኤች አይ ቪ ህመምተኞች በተደጋጋሚ የሂማቲክ ምርመራዎች ያስፈልጋቸው እንደሆነ ለክሊኒኮች መረጃ ይሰጣል. ስለዚህም ይህ ጥናት የሚካሄደው በቫይባ ሪፈራል ሆስፒታል ውስጥ የመድሃኒት ሕክምናን ከመውቀቁ

በፊት እና በኋላ በኤች አይ ቪ ህመምተኞችን እና ኤች.አይ.ቪ ውስጥ ያሉትን የተጋለጡ ምክንያቶችን ለማወቅ ነው::

ሂደቶች--አ ፈቃደኛነትዎን በመከተል የጽሁፍ ፈቃድ እንዲፈርሙ ይጠየቃሉ እና የሚከተሉት ሂደቶች ይካሄዳሉ::

• 15 ደቂቃ ቃለ መጠይቅ ያቀርቡልዎታል

• የደም ናሙና (4 ሚሊሊ) እና የቆዳ ናሙና ይወሰዳሉ

• የደም ናሙና ለሂሞራሰስ, ለሂሞቶሎጂ እና ለሞላው የሙከራ ምርመራዎች ይመረምራል. የሰገራ ናሙና ለገሰ-ቃላዊ ምርመራዎች ያገለግላል::

አደጋዎች እና አለመመቻቸቶች -በሙጣሙ ናሙናነት መሰረታዊ የአሰራር ሂደቶችን እንከተላለን. የደም ስዕሉ በደም ወሳኝ ቦታ ላይ ትንሽ ሕመም ሊያስከትል ይችላል. ነገር ግን ይህ ህመም ከአሁን በኋላ አይታይም::

ጥቅማ ጥቅሞች: - ስኬቱ ታካሚዎችን በሚወስኑ ተገቢ የሕክምና ውሳኔዎች እና ህክምና ላይ የሚያግዝ በመሆኑ ይህ ጥናት ለጠቅላላው ማህበረሰብ የሚጠቅም ይሆናል. በዚህ ጥናት ውስጥ በመሳተፍ የሚያገኙትን ቀጥተኛ ፋይናንሳዊ ጥቅማጥቅል የለም, ነገር ግን የፈተና ውጤቱ ወቅታዊ እና ተገቢ የሆነ ጣልቃገብነት ይጠቁማል::

ሚስጢራዊነት-- በዚህ ጥናት ወቅት የተገኘ መረጃ ሁሉ በሚስጢር ይያዛል. ይህ ማንኛውም መለያ ጥቅም ላይ በመዋል የተረጋገጠ ሲሆን መረጃ በቁጥር ቁጥር ይቀመጣል. ከጥናቱ የተገኙ ውጤቶችን እናስወግዳለን, ማንኛውም እርስዎን ለይቶ የሚያልፍበት መንገድ እና ምንም መለያ ከሌለ የእርስዎ የተፈረመበት ፊርማ ሊኖርዎት ይገባል::

በፈቃደኝነት የሚደረግ ተሳትፎ: - በዚህ ጥናት ላይ የሚሳተፈው በፈቃደኝነት ሲሆን በማንኛውም ጊዜ ተሳትፎ የማድረግ መብት አለዎት. ውሳኔዎ እርስዎ የሚገቡትን ጥቅማጥቅሞች ወይም ኪሳራ አያመጣሉም. ውሳኔዎ ለአሁን ወይም ለወደፊት ህክምናዎ ወይም ሌላ እርሶ ሊያገኙዋቸው የሚችሉ ጥቅሞችን ለአደጋ አያጋልጥዎትም::

እርስዎ አሁን ጥያቄዎችን ሊጠይቁ ይችላሉ, አሁን እየተከናወነ ያለው ነገር ካልገባዎ ከላይ ያለውን አድራሻ መርማሪውን ያነጋግሩ::

ለጥናቱ ስኬት ለጥያቄዎቹ ትክክለኛውን መልስ እንዲሰጡ እጠይቃለሁ. ለእገዛዎ እናመሰግናለን!

Ibsa Hirmaattota qo’annootif guca guutamu (Afaan Oromoo)

Guciin Kun Kan guutamu warren qo’annaa irratti fedhiin hirmaataniif Kan ooluu fi haallii qo’annaa sirritti erga ibsameefii booda Kan guutamufi Kan mallattaa’udha.

Mata-duree qo’annaa:-Qorannon bu’aa laaboraatorii *hematology* warreen vaayirasii HIVwaliin jiraatan kibba bahaa Itoopiyaatti hojjatama.

Maqaan qorataa:-Nageessoo Dhugumaa Wayyeechaa

Lak.bilbila:- +251921482538

E.mail:- dugumanegesso@gmail.com

Dhimmi-qu’anicha:-Bu’aan laboratorii warra vaayirasii HIV waliin jiraatan irraa argamu warra karooraa fayyaa baasanii fi gara fuulduraattii warra akka biyyaattii qorannoo adeemsisaniif akka bu’uuraattii gargaara.

Haala adeemsa qo ’annichaa: - qo’ aannaa irratti fedhiin hirmaachuu keessan mallattoo keessaniin nuuf ibsitaaniif ragaalee armaan gadii kanneen nuuf kennitan.

- Gaaffiilee afaaniitiif daqiiqaa 15 waliin turra
- Dhiiga 4ml fi boolii ni fudhanna
- Naamunaa fincaanii qorannoo ulfaa dalaguuf

Wantootni armaan olii Kun qorannoof kan barbaachisan tahu ni ibsina.

Sodaa fi miidhaa qabu:-Seeraa fi naamusa ogummaa fayyaa hordofuun waan dalagamuuf wanti na sodaachisu hin jiru. Haa tahu malee, dhukkubbiin xixiqqoon yeroo dhiiga fudhatamu namatti dhagahamuu Kan yoree xiqqoo turuufi miidhaa osoon hin geessisin kan badu tahu hubadhe.

Faayida qo’anniichaa fi kafaaltii hirmaataaf godhamu- qorannoo irratti hirmaachuuf kafaltii kan hin qabnee fi bu’aa qorannoo irraa argamuu fi tajaajila wal ’aansa argachuu ni danda ’u.

Iccitii hirmaataa eeguu-wantootni qorannoo irraa argaman hundinuu icciitiin kan eeggamaniifi ragaaleen argaman hundinuu maqaa keessaniin osoo hin tahiin lakkoofsa addaatiin/koodiin kan beekkamaniifi odeeffannoon hundinuu iccitiidhan warra ragaa funaanan biratti kan hafu tahuu isaa isiiniif ibsina.

Mirga fedhaan hirmaachuu- qorannoo irratti hirmaachuun fedhii kee qofa tahuu isaa beektee, yeroo barbaaddeettii qorannoo keessaa bahuu kan dandeessuu fi yeroo keessaa baatulee rakkoo tokkoo kan sirratti hin fidnee fi tajaajila argachuu qabduu hundumaa argachuu kan dandeessu tahuusaa. Waan himaattaniif galatoomaa!!

11.5. ANNEX-FIVE: CONSENT FORMS

Consent forms (English version)

Participant Code Number_____

Participant full name _____

I was informed fully in the language I understood about the aim of above mentioned research. I understood the purpose of the study entitled with “Hematologic profiles and associated factors among HIV positive adult individuals before and on taking antiretroviral treatment at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia”. I have been informed that, blood samples, urine and stool specimens were taken from me and there was minimal risk during sample collection. In addition, I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

Agree _____ Not agree_____

Therefore, I give my consent freely for my participation in this study.

Participant’s Name _____ signature _____ date_____

Investigator’s name _____ signature _____ date_____

Witness

1. Name _____signature _____ date _____
2. Name _____signature _____ date_____

የስምምነት ቅጾች (እንግሊዝኛ)

የተሣታፊ ኮድ ቁጥር _____

ተሳታፊ ሙሉ ስም _____

ከላይ ከተጠቀሱት ምርምሮችን ምርምር በተረዳሁበት ቋንቋ ሙሉ በሙሉ ተረድቼአለሁ. "በግብረ-ሰዶም ሆስፒታል ውስጥ በደቡብ ምስራቅ ኢትዮጲያ ውስጥ የፀረ-ኤሮራቫሮል ህክምናን ከመውጣታቸው በፊት እና ካደረጉ በኋላ በኤች አይ ቪ / ኤችአይቪ / ኤችአይቪ / ኤችአይቪ / ኤችአይቪ / ከተጋለጡ ግለሰቦች ጋር የተያያዙ ምክንያቶች" የደም ናሙናዎች እና የሱፍ ቁስሉ ከእኔ እንደሚወሰዱ ተነግሮኛል እናም በማጣቀሻ ቅደም ተከተል ወቅት አነስተኛ አደጋ ይኖረዋል. በተጨማሪም በጥናቱ ሂደት ውስጥ የተሰበሰበውን መረጃ በሙሉ በሚስጥር ይያዛል. ለመሳተፍ ወይም ለመጥቀስ ፈቃደኛ ካልሆንኩ የአሁኑ እና የወደፊት የሕክምና አገልግሎቶቼ ምንም አይሆኑም::

ተመስክሮ _____ አልስማማም _____

ስለሆነም, በዚህ ጥናት ውስጥ ለመሳተፍ የእኔን ነፃነት በነፃ ሰጥቼዋለሁ.

የተሳታፊው ስም _____ ፊርማ _____ ቀን _____

መርማሪ ስም _____ ፊርማ _____ ቀን _____

ምሥክር

1. ስም _____ ፊርማ _____ ቀን _____

2. ስም _____ ፊርማ _____ ቀን_____

UunkaaWalii galtee (Afaan Oromoo)

Lakk.addaa hirmaattota_____

Maqaan guutuu hirmaattota_____

Ani hirmaatan maqaan koo armaan olitti ibsame kun bu’aa fi miidhaan isaa erga sirritti natti himame fi miidhaan isaa baay’ee xiqqaa ta’uu isaa ergan hubadhee booda, naamunaa qorannoon laboratoriitiif kan oolu boolii, fincaanii fi dhiiga akkan kennuu fii dabalataaniis odeefannoo narraa argaman hunduu icciitiin akka qabaman nattii hiimameera.Akkasumas, gaaffileen gaafatamuuf deebii kennuu dhiisuu, hiirmachuu dhiisuu fi yeroon barbaadetti addaan kutuu akkan danda’uu bareen jira. Kana godhuu kiyyaaniis ammas ta’ee fuulduraaf fayyadamummaa tajaajila fayyaa kiyya irratti rakkoon tokkollee akka hin uumamanee hubadheen jira.

Walii galeera_____ **Walii hin gallee_____**

Kanaafuu qorannoo kana irratti fedhiin kootiin hirmaachuu koo nan ibsa.

Maqaa hirmaataa_____Mallattoo_____Guyyaa_____

Maqaa qoo’ataa_____Mallattoo_____Guyyaa_____

Ragaalee

1. Maqaa_____._Mallattoo_____Guyyaa_____

2. Maqaa_____ Mallattoo_____Guyyaa_____

DECLARATION

I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by Negesso Duguma Weyecha entitled "**hematological profiles and associated factors among adult HIV positive individuals before and on highly active antiretroviral treatment at Madda Walabu Goba Referral Hospital Southeast, Ethiopia**" I recommend that it can be submitted as fulfilling of the thesis requirement.

Name of the Principal Investigator	Signature	Date
Negesso Duguma Weyecha	_____	_____
Internal Accessory		
Dr Tilahun Yemane (MD, Associate Professor)	_____	_____
Approval of the first Advisor		
Lealem Gedefaw (MSc, Associate Professor)	_____	_____
Approval of the Second Advisor		
Girum Tesfaye (BSc, MSc)	_____	_____
Approval of the Third Advisor		
Wondimagegn Addisu (BSc, MSc)	_____	_____