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Assessment of Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Months in Yabelo Hospital, south East Ethiopia.

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This is to certify that the thesis prepared by Girma Ashenafi, entitled:

Assessment of Immunohematological Outcome Among Adult HIV Patients Taking Highly Active Antiretroviral Therapy.for at least Six Months in Yabelo Hospital, south Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
BMI	Body Mass Index
CBC	Complete blood count
CD4	Cluster of differentiation 4
DNA	Deoxyribonucleic acid
ETB	Ethiopia Birr
HAART	Highly active antiretroviral therapy
Hgb	Hemoglobin
HCT	Hematocrit
HIV	Human immunodeficiency virus
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
OI	Opportunistic infection
PLT	Platelets
RBC	Red blood cell
RNA	Ribonucleic acid
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
WBC	White blood cell:
WHO	World Health Organization
YGH	Yabelo General Hospital

Abstract

Background*:* Immunohematological parameters are key tools for evaluating antiretroviral treatment and prognosis during follow up in Human immunodeficiency virus infected patients. Clinical response to highly active antiretroviral therapy in resource-limited settings is monitored with CD4 + T cell counts and some hematologic indices. Comparing baseline immunohematological parameters of infected patients with their change after they take treatment for at least six months is very useful in evaluating treatment success.

Objective: To assess changes in immunological and haematological parameters in HIV infected patients on HAART for at least Six months at the antiretroviral therapy clinic of Yabelo Hospital, Borena, Ethiopia.

Methods: A cross sectional study was conducted from February to July 2021 using convenient sampling method to recruit **333** HIV infected adults who were on follow-up for at least six months at the ART clinic of Yabelo hospital. Socio-demographic and clinical characteristics data were collected using pre-tested structured questionnaire. Venous blood samples were collected and processed following standard procedures for determining CD4+ T cell count and complete blood count using FACSPresto and Sysmex XE 2100 automated haematology analyser, respectively. Data analysis was performed using SPSS version 25. Bivariate and multivariable analyses were conducted to identify factors significantly associated with the outcome variable. P –value < 0.05 was considered as significant.

Result:- The prevalence of anemia(47.4%),leucopenia(73.3%),neutropenia(58.3%), lymphopenia (76.9%) and thrombocytopenia(3.3%) before starting of highly active antiretroviral therapy was declined to 23.1%, 36.4%, 23.4%, 35.7% and 2.4% after initiation of highly active antiretroviral therapy, respectively; there was also significant decrease in the rate of Immunosuppression (62.2% to 20.7%). Except MCHC, there was a significant improvement in the common hematological parameters after use of highly active antiretroviral therapy for at least six month.Treatment interruption, presence of extra pulmonary TB ,sex and BMI were factors associated with anemia after ininitation of highly active antiretroviral therapy.

Conclusion: Immunohematological profile of the patients has improved after initiation of highly active antiretroviral therapy. Treatment interruption, presence of extra pulmonary TB, female sex and Body mass index are factors associated with anemia. Early initiation of highly active antiretroviral therapy is helpful in decreasing hematological abnormalities in HIV infected patients.

Key words: HIV, Highly Active Antiretroviral Therapy, CBC, CD4.

1. Introduction

1.1 Background

Human immunodeficiency virus (HIV) infection is a state of intense depletion in immune system, covering a stages of disease ranging from acute syndrome seen with primary infection, extended asymptomatic state, to an advanced disease or acquired immunodeficiency disease syndrome (1).

Acquired Immunodeficiency Syndrome (AIDS) for first time was reported in the United States of America in 1981 and its causative agent, HIV was isolated in 1983 from a patient manifesting lymphadenopathy. HIV infection become globally pandemic infection causing over sixty million individuals to be infected so far with twenty million deaths and greater than twenty million orphaned children and it is believed that most of the persons newly infected each day, live in developing countries. The greatest impact is observed in low- and middle-income countries where an estimated 90% of the global HIV-infected population live; sub-Saharan Africa is the most affected area of world while South-East Asia is the second most affected (2, 3).

Highly active antiretroviral therapy (HAART) is a combined therapy used in HIV positive patients to radically lengthen the time to development of AIDS and the time to death in HIV infected persons. Major HAART mechanism of action is inhibition of plasma HIV levels which helps an increase in CD4 T-lymphocyte count and function (3-6). The aim of HAART is to inhibit viral replication while minimizing toxicities and side effects associated with the available drugs. By using HAART, it is promising to promote growth in children and prolong the survival of all HIV infected patients, reduce their morbidity and improve their quality of life (17).

Immunohematological profiles are key tools for evaluating treatment and prognosis over a time of flow up in HIV infected patients. Clinical response to ART in resource-limited settings is monitored with CD4 + T cell counts and RBC indices (8-10). Immunological values such as CD4 counts are used as the best forecaster of the risk of developing AIDS-related complications, additionally also used to monitor disease progression; assess prognosis; handpick HIV patients for trials and monitor therapy.

But only counting CD4 did not serve as complete immunological parameter to measure prognosis and antiretroviral therapy. Viral load and haematological parameters are useful to monitor therapy or measure prognosis (11, 12).

Variety of HIV strains are found at different regions in the world with different response to immunohematological profiles (13-15). The length of time HIV patients taking HAART has been shown to have influence on understanding the kinetics of viremia and CD4 counts (16). Using immunohematological parameters data from other regions and countries as a control in monitoring of HIV patients in other local setting might be inaccurate. Many challenges could compromise the effectiveness of HAART, including failed/incomplete treatment responses (17), drug interaction and toxicity, drug resistance (18, 19), lost to follow up and early mortality (20). Hence monitoring HAART outcome at each locality will be useful for generating information for improving the management of ART patients in the respective sites.

Immunohematological abnormalities are due to diverse reasons which include immune-mediated destruction of cells, direct cytopathic effects of the virus, secondary to various infections and neoplasms, and drug toxicity (4). The most prevalent haematological disorder observed in adults with HIV infection is anemia. Incidences of anemia are particularly high in patients with late stages of the disease and a reduced CD4 cell count.

In HIV-infected person T cells affected by viruses as it sites for replication intern it reduce the growth of bone marrow progenitors, thus suppressing hemopoiesis (9).

Anemia and neutropenia are generally caused by inadequate blood cell production because of bone marrow suppression by HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment (14-15).

Among hematological abnormalities Neutropenia is commonly observed after development of AIDS, and has been associated with definite types of HAART medications used to treat HIV infection (17). Colony growth of the progenitor cells decreased in patients taking HAART, this leads to decreased production granulocyte and monocytes produced by the infected cells known to suppress neutrophil production (18-19).

On another hand thrombocytopenia could seen from increased platelets destruction and decreased platelet production by the HIV-infected megakaryocytic cells in patient infected by HIV (17- 19).

Like other immunohematological abnormalities decreased CD4 count also manifested due to HIV, as this virus use CD4 receptor as site of attachment and replication. The CD4 cell count is important measures of the efficacy and effectiveness of HAART among HIV participants enrolled in HIV care and treatment programs.

HIV/AIDS affects economic growth by reducing the availability of human capitalWithout proper prevention, nutrition, health care and medicine that is available in developing countries, large numbers of people are falling victim to AIDS. People living with HIV/AIDS will not only be unable to work, but will also require significant medical care. The forecast is that this will probably cause a collapse of babies and societies in countries with a significant AIDS population (17).

Ethiopia peoples are very vulnerable to HIV, with an estimated 15 percent of the population are at risk of getting it. HIV/AIDS is one of the key challenges for the overall development of Ethiopia, as it has led to a seven-year decrease in life expectancy and a greatly reduced workforce (19).

1.2 Statement of the Problem

Human immunodeficiency virus continues to be a global challenge. Globally an estimated 38(31.6- 44.5) million people were living with HIV in 2019, about 17 million male and 19.2 million female. Death rate decreased to 39% when compared with 2010 estimate which may be attributed due tom increase in people receiving HAART over time. Depending on WHO region, Africa region is the highly affected region with 25.7% million people living with HIV in 2019 (13, 17)**.**

In Ethiopia in 2018 around 690,000 people were living with HIV and in this year the number of new HIV infections has also decreased, from 29,000 to 23,000 in the same period. Out of people livings with HIV, 65% were on treatment. Among them, 66% of all adults above 15 years of age living with HIV were on treatment. When disaggregated by sex, 65% of women and 66% of adult men are on treatment (19, 21).

Highly active antiretroviral therapy is a treatment used in HIV infection and produce spectacular drop in mortality, morbidity, disability and health care utilization. Uninterrupted use of HAART has resulted in continued suppression of HIV virus RNA replication, which is resulting in ongoing increases in CD4 T-lymphocyte count. However, the long term use of HAART might be decreased by emerging drug resistance which may contribute to treatment failure. HIV drug resistance can be caused by different factors; among those factors the virus

factors, which is its high replication capability and error-prone nature of the viral enzyme reverse transcriptase and host factors like poor adherence, low absorption and other pharmacokinetics factors (13, 17, and 19).

Highly active antiretroviral therapy has great effect in improving the immuohematological profile of HIV infected Persons. Although several studies have quantified the gains in CD4 cell counts over the first two years HAART initiation (22, 23).Immunohematological parameters of HIV positive patients after six month of initiation of HAART have not been documented in study area. Which might be useful for physician and health sector policy makers to assess the success of treatment.Understanding the changes in the immuno-hematological profile trends during treatment can help the care givers and physician to reduce most of the adverse effects that occur on HIV positive patients in our community?

1.3 Significance of the study

The study focuses on outcome of hematological and immunological measurements before and after initiation of HAART in Adult HIV positive patients. The current study has major importance to convey information about immunohematological outcome in HIV infected patients in the area in general. As the study assesses change in hematological parameters before and after initiation of HAART, it provides information to clinicians for improving management of HIV infection. This is because specific interventions other than antiretroviral treatment may be indicated for its correction. This will also help hospital management and staff to plan appropriately regarding supplies and drugs needed for the diagnosis and treatment.

The result can also be utilized as an input by policy makers for improving HIV Positive adult treatment outcome of HIV/AIDs patients in the South Region of Ethiopia, specifically Borena zone and it is also aimed to fill gap in knowledge concerning immunohematological outcome in HIV patients taking HAART in my study area. Moreover, this study can serve as a reference material for further researches with regards to immuno-hematological outcome in adult HIV infected patients.

2. Literature Review

Acquired Immunodeficiency Syndrome (AIDS) for first time was reported in the United States of American in 1981 and its causative agent, human immunodeficiency virus (HIV) was isolated in 1983 from a patient manifesting lymphadenopathy. HIV infection become globally pandemic infection causing over sixty million individuals to be infected so far with twenty million deaths and greater than twenty million orphaned children and it is believed that most of the individuals newly infected each day, live in developing countries (2).

2.1 Immuno-Hematological changes in HIV positive patients

In patient living with HIV hematological changes are among the most common abnormalities reported in different literature at different part of world. The Study conducted in Indian shows that 91.4% of HIV infected patients had a decrease in hemoglobin level (which shows anemia), 26.8% depletion in WBC count (leucopenia) and 21.7% thrombocytopenia (24).

Similarly like Parintha SS and coworkers, another study conducted in India also suggest that hematological abnormalities are common in all stages of HIV infection from the total 150 patients 70-80% developed anemia (low RBC and hemoglobin) 10-15% patients manifested low neutrophil count(neutropenia), low CD4 count (<200 cell/ul) and thrombocytopenia also observed (25). Additionally a study conducted in India shows anemia in 40.1% of the total population, low platelet count in 3.74%, WBC count was decreased in 5.88% of patients while eosinophilia was seen in 22.99% (26).

A descriptive observational study conducted in Islamabad showed that a significant positive correlation between hemoglobin concentration and CD4 Lymphocyte counts in HIV infected patients (27).

Study conducted in Cameron concludes that anemia, leucopenia and thrombocytopenia are all strongly and directly related to CD4 cell counts and WHO disease stage. The lowest prevalence of blood cytopenia was noted among WHO clinical stage 1. The frequency and severity of hematological manifestation increased with the decline in CD4 Counts (28).

In Nigeria prospective studies conducted suggested that low RBC and hemoglobin (anemia) with 43.7%. Low WBC counts 30.3%, thrombocytopenia 11.55%, neutropenia 26%, immunologically reduced CD4 count were reported (29).

A study from Gondar showed that the prevalence of immunohematological abnormalities of HIV positive individuals during the first visit revealed 19% and 12.2% of the study participants had leucopoenia and thrombocytopenia, respectively (30).

2.3 Immuno-hematological changes in HIV positive patients after highly active antiretroviral therapy

Highly active antiretroviral therapy (HAART) is a combined therapy used in HIV positive patients to radically extend the time to development of acquired immunodeficiency syndrome (AIDS) and the time to death in human immunodeficiency virus infected persons. Its major mechanism of action is suppression of plasma HIV levels which helps an increase in CD4 T-lymphocyte count and function (7).

Significant improvement of CD4 count and haematological parameters after taking HAART has been reported from various studies in Ethiopia. These include study from University of Gondar Hospital (5) and Zewditu Memorial Hospital in Addis Ababa (6, 22). Significant improvement in haematological derangements caused by HIV has been reported as it is shown by a study in Minelik II Hospital and Black Lion Hospital where there was significantly reduced burden of anaemia (23, 31).

Understanding the changes in the immune-haematological profile during treatment can help the care givers and physician to prevent most of the adverse effects that occur on HIV positive patients in our community. This study aimed to assess the immunohematological parameters of HIV positive adults on HAART for at least six months of duration with the expectation to improve the ARV treatment outcome of HIV patients in the study area. There is no published study conducted in this particular study setting concerning the current topic.

3. Objectives

3.1 General objective

To determine immunological and hematological outcomes among Adult HIV infected patients on HAART for at least six months at the ART clinic of Yabelo Hospital, South Ethiopia, from February to July 2020.

3.2 Specific objectives:-

- To determine haematological parameters changes among Adult HIV patients before and after taking Highly Active Antiretroviral Therapy for at least 6 months.
- To assess change in immunological (CD4) parameter among Adult HIV patients taking Highly Active Antiretroviral Therapy for at least 6 months.
- To compare immunological and hematological change before and after taking HAART for at least 6 months on the common immuno-hematological abnormalities.
- To identify factors associated with anemia among patients taking HAART for at least six months.

4. Materials and Methods

4.1 Study area

The study was conducted at the ART clinic of Yabelo General Hospital (YGH) which is situated at Yabelo town, Borena, Ethiopia. It is located at 567 km South of Addis Ababa. Yabelo town is the capital of Borena zone; has a latitude and longitude of $5^0 35^{'}$N $38^0$15'E / 5.583^0N 38.250^0E, with an elevation of 1,716 meters above sea level. It has average annual rainfall of 648mm. According to the city population centre it has a total population of 27,820 in the year 2007 E.C. Yabelo town has one governmental general hospital and one health centre. The facilities provide services for about 1,296,475 catchment population. The facilities provide ART service for HIV positive individuals in one ART unit (32). The ART clinic serves a total of 2507 HIV patients who are on follow up.

4.2 Study design and Period

A hospital based cross sectional study using both primary and secondary data was conducted from February- July 2020.

4.3 Population

4.3.1 Source population

The source populations were all HIV infected patients attending the HAART follow-up clinic of Yabelo General Hospital during the study period.

4.3.2 Study population

All volunteering HIV infected adult patients attending the ART clinic of Yabelo hospital during the study period and who were on HAART for at least six months were study population.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- ✓ 18 years and above
- ✓ Those who were on ART for at least six months and had follow up.
- ✓ Patients having complete CBC and CD4 count at the base line.

4.4.2 Exclusion criteria

- ✓ Pregnant women
- ✓ Those taking vitamins and iron supplementation at the time of sampling,

- ✓ Individuals who had received a blood transfusion for the last 4 months
- ✓ Patients with underlying hematological malignance and other chronic disease.

4.5 Study Variables

4.5.1 Dependent variables

- ➢ Hematological parameters
- ➢ Immunological parameter (CD4 count)

4.5.2 Independent variables

- ➢ Age, sex, residence, educational status, marital status, BMI, opportunistic infection, occupational status, history of treatment interruption and WHO clinical staging.

4.6 Measurement and Data collection

4.6.1 Sample size determination

The sampling includes all HIV infected patients that were attending HIV unit of Yabelo hospital during study period, except children and pregnant women. The sample size (n) was determined using the following statistical formula for single proportion:

$n=Z^2P(1-P)/d^2$ Where as:-

n = sample size

d = margin of error between the sample and the population (0.05)

Z = 95% confident interval (1.96) P = 0.5, was use to get maximum and representative sample size.

$n = (1.96)^2 \times 0.5 (1- 0.5)/ (0.05)^2 =$**384**

Since study population (N = **2507** obtained from the hospital medical record) is < 10,000, it needs adjustment. By using correction formula for finite population, the final sample size was calculated as:

Final sample size (nf) **=** n /1+ (n/N)

nf = N/ (1+ (384/2507))= **333**

Thus, a total of 333 HIV infected individuals who were enrolled at Yabelo General Hospital ART unit and fulfilling the eligibility criteria were included in the study using convenient sampling method.

4.6.2 Sampling method

Convenient sampling method was used among the eligible participants until the required sample size was reached after explaining the study aim to get their informed consent also to use their baseline CBC and CD4 count data.

4.6.3 Data collection procedure

The base line clinical data was collected from electronic data base (AMR smart care) by the help of data collectors who works in the ART unit and current immunohematological profile was collected by taking blood sample from each study participant. Data on socio demographic factors were collected using structured questionnaire by interview.

4.6.3.1 Sample collection

Approximately 4 mL whole blood was collected by trained staff from the cubital vein with a vacutainer system in K_3EDTA test tube. Samples were transported from ART clinic to Yabelo general Hospital laboratory and analyzed within 8 hours of collection according to manufacturer's instructions; laboratory principles & procedures used are attached (Annex **4**).

4.6.3.2 Laboratory analysis

Complete blood count analysis

Hematological analysis was done using Sysmex XE-2100 (Sysmex, Kobe, Japan) automated hematology analyzer. The Sysmex hematology analyzer XE-2100 can analyze and output the results for 32 hematological parameters from whole blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the DIFF scatter gram. Hemoglobin is measured using photometry principle.

CD4+T cell count

The CD4+T cell count was done by FACSPresto (Becton Dickson, San Jose, CA) POC analyzer. The multicolor (3-color) fluorescent photo-microscopy and light absorbance detection were used to measure CD4 and Hemoglobin respectively. The added whole blood

sample to an inlet of the cartridge which contains CD3 APC, CD4 PECy™5, CD14 PE, and CD45RA APC is stained at the incubation of 18 minutes. Then the sample is illuminated from two LEDs the excitation sources. Then the resulting images are captured through a microscope lens system by a digital camera assembly. Finally, the captured images are analyzed by software and results are reported to the operator.

4.7 Data quality assurance

The quality of data was assured by properly designing the instrument for its simplicity and clearness for collecting data using structured questionnaire that was prepared in English and translated to the local language and it was pre-tested (5%) in areas other than the actual data collection site (Bule Hora Hospital). During data collection each and every activity were monitored and evaluated for its correctness and completeness. Standard Operating procedure (SOP) which was prepared according to the Ethiopian Public Health Institute (EPHI) guide lines and the hospital SOP were strictly followed during the course of reagent selection and specimen processing.

Three level whole blood controls (High, Normal, Low) were used to ensure the qulity of hematological parameters. Food CD4 count, 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. Quality control was done by inbuilt quality control system in the machine during switch on the analyzer. Post analytic quality was ensured by proper interpretation and documentation of both hematological parameters and CD4 count data. All wastes were disposed as per SOPs.

4.8 Data analysis and interpretation

The data was coded, cleaned, entered and analyzed using SPSS version 25 statistical software. Descriptive analysis was done and results were presented as number and percentage or mean ± SD. Paired t-test was used for comparison of mean values of hematological parameters before and after HAART initiation. To identify factors associated with one of the common hematological abnormalities, anemia, bivariate analysis to calculate crude odds ratio with 95% CI was performed. For those variables with P=0.2 and below, multivariable logistic

regression analysis model was used to calculate adjusted odds ratio with 95% CI and identify factors which are independently associated with anemia. The results of the analysis were presented with tables and figures, where appropriate. P value of <0.05 at 95% confidence interval (CI) was used as level of statistical significances.

4.9 Ethical considerations

The study was conducted after being reviewed and approved by Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences, Addis Ababa University. After a letter of cooperation was sent to Borana Health Bureau from the Department of Medical Laboratory Sciences, the Institutional Review Board of the Bureau also approved the study. Then a letter informing Yabelo general Hospital administrators was written from the Institutional Review Board (IRB) and permission was obtained from the Hospital to conduct the study. All the information obtained from the ART center was coded and electronic files password protected to maintain confidentially throughout the study. Participants were on their routine ART follow up. The aim of the study and their right to decline was explained to them to use clinical records as well as provide sample for the current data.

4.10 Dissemination and utilization of results

The result of this study will be submitted to Department of Medical Laboratory Science; presented on thesis defense and to the hospital, health bureau and accordingly will be advocated for those who can implement it, example to the hospital‘s Management Board. The findings will be disseminated through presenting on conferences. In addition, an attempt will made to publish the paper in local or international journals.

4.11 Operational Definitions.

- **Anemia:** defined as hemoglobin concentration of <13g/dl for male and <12g/dl for female (26).
    - **Mild anemia**: hemoglobin value of 11 to 11.9 g/dl for female and 11 to 12.9 g/dl for male.
    - **Moderate anemia:** hemoglobin value of 8 to 10.9 g/dl for male and female.
    - **Severe anemia:** hemoglobin value of <8 g/dl for male and female.
- **Highly active antiretroviral Therapy (ART):** double or triple combination therapy designed to suppress the progression of HIV/AIDS (21).
- **Immuno-Hematological Parameters:** Include White blood cells, Red blood cells, Hemoglobin, Hematocrit, Red blood cell indices, Platelets and CD4+ T cells (26).
- **Leucopenia:** defined as total WBC count of less than 4000cells/µl(21).
- **Lymphopenia:** Total lymphocyte count of less than 1000cells/µl. (<20%)
- **CD4 Level (Immunosuppression):** in this study refers to CD4+T lymphocyte counts < 500 cell/µl (13).
- **Immunosuppression severity** was defined based on the CD4 count as per the WHO guideline (9).
    - CD4 count <200 sever immunosuppression
    - 200-349 moderate immunosuppression
    - 350-500cells/µl mild immunosuppression
- **Neutropenia:** Absolute neutrophil count less than 1500cells/µl (9).
- **Thrombocytopenia:** A platelet counts of less than 150,000cells/µ (7).

5. Result

5.1 Socio-demographic characteristics

A total of 333 individuals aged 18 years and above; of whom 170(51.1 %) males and 163(48.9 %) females were included in the study with 100% response rate. Majority of these participants 129(38.7 %) and 122(36.6 %) were in the age between 29-38 and 18-28 years of the age, respectively; with the mean age and standard deviation of 32.83 and 9.48 years. Concerning the marital status, education level and occupation of the participants,151(45.3 %) of them were married, 112(33.6 %) have no formal education and 85(25.5 %) were housewives. More than half of them were urban residents 195(58.6 %) (Table 1).

Table 1: Sociodemographic characteristics of HIV patients taking HAART for at least 6 months at Yabelo General Hospital, Borana Ethiopia, 2020.

Variables	Frequency and percentile NO (%)
Age	
18-28	**122(36.6)**
29-38	**129(38.7)**
39-48	**53(15.9)**
49-58	**24(7.2)**
>=59	**5(1.5)**
Sex	
Male	**170(51.1)**
Female	**163(48.9)**
Religion	
Protestant	**97(36.6)**
Orthodox	**81(38.7)**
Muslim	**79(15.9)**
Catholic	**42(7.2)**
Wakefata	**34(1.5)**
Marital status	
Single	**70(21)**
Married	**151(45.3)**
Divorced	**54(16.2)**
Widowed	**58(17.4)**
Education level	
No formal education	**112(33.6)**
Can read and write	**49(14.7)**
Primary education	**87(26.1)**
Secondary education	**61(18.3)**
Higher Education	**24(7.2)**
Occupation	
House wife	**85(25.5)**
Employee	**54(16.2)**
Student	**54(16.2)**
Merchant	**76(22.8)**
Daily laborer	**36(10.8)**
Farmer	**16(4.8)**
Others	**12(3.6)**
Residence	
Urban	**195(58.6)**
Rural	**138(41.4)**

5.2 Clinical characteristics of HIV patients taking HAART for at least six months at Yabelo General Hospital, Borana, Ethiopia, 2020.

Before initiation of HAART about 151(45.3 %) of the study participants were in WHO clinical stage I while the rest of the patients were 95(28.5 %), 61(18.3 %), and 26(7.8%) were in WHO clinical stage II, III and IV, respectively.

But after taking HAART for at least six months almost all of the study participants were in WHO clinical stage I, 330(99.1%), the remaining 3 patients (0.9%) were in Stage II. The result revealed that there was increase in mean BMI of the study participants; 20.14 before starting of HAART and 22.19 after taking HAART for six months and above. It was found that 271(81.6%) of participants had no history of opportunistic infection. However, 18.6% of the patients (62(18.6%)) had opportunistic infections; of those patients, 31(50%) had pulmonary Tuberculosis, 18(29.04%) had severe bacterial pneumonia and 13(20.96%) had extra pulmonary tuberculosis. Functional status at base line indicated that 278(83.5 %) were working patients. Treatment interruptions were calculated to be 114 (34.2 %) at different points of follow up (Table 2).

Table 2 : Clinical characteristics of Adult HIV patients taking HAART for at least 6 months at Yabelo General Hospital, Borana, Ethiopia, 2020.

Variables	Frequency and percentile NO (%)
Initial WHO Clinical stage	
Stage I	**151(45.3**
Stage II	**95(28.5)**
Stage III	**61(18.3)**
Stage IV	**26(7.8)**
Current WHO clinical Stage	
Stage I	**330(99.1)**
Stage II	**3(0.9)**
Initial BMI	
18.5-24.99	**269 (80.8)**
<18.49	**54 (16.2)**
>25	**10 (3)**
Current BMI	
18.5-24.99	**225 (67.6)**
<18.49	**52 (15.6)**
>25	**56 (16.8)**
Treatment interruption	
Yes	**114(34.2)**
No	**219(65.8)**
Functional status at base line	
Working	**278(83.5)**
Ambulatory	**41 (12.3)**
Bedridden	**14 (4.2)**
History of opportunistic infection	
Yes	**62 (18.6)**
No	**271 (81.4)**
Type of opportunistic infection	
Severe bacterial pneumonia	**18(29.04)**
Pulmonary tuberculosis	**31 (50.0)**
Extra pulmonary tuberculosis	**13 (20.96)**

5.3 Hematological and immunological Values before and after initiation of HAART

The result has shown that there is change in hematological parameters among the study participants. WBC has changed from 4.106 ±0.970 to 4.995 ±1.34 (10^9/l) with p value of 0.0001; there was an increased in Hgb concentration from 12.38±1.77 to 13.22±1.81 (g/dl)and having p value of 0.0001.; MCV has changed from 87.57±11.63 to 89.99±8.87 (fl); and platelet change from 243.3±52.59 to 292.18±60.539 (10^9/l) with p value of 0.0001 and CD4 changed from 356.56±212.32 to 583.7±238.25 cells/ mm^3 resulting in a mean change of 227.14 cells/ µl and p value of 0.00001.

The rise in the mean values of WBC, Hgb, MCV, and CD4 after at least 6 months of HAART initiation were statistically significant ($p < 0.05$) with p value for :-WBC (0.0001) , Hgb (0.0001), MCV(0.0001) and CD4(0.0001) and insignificant for MCHC (0.706) and platelet (0.0001). In general the study has revealed there is a significant change in hematological parameters after use of HAART for at least six month of initiation of HAART with P- value less than 0.05 with exception of MCHC (Table 3).

Table 3 : Hematological and Immunological Parameters of Adult HIV-Positive Individuals before and after initiation of HAAART at Yabelo General Hospital, south Ethiopia, 2020

Parameters	Before Initiation of HAART (n= 333) Mean ± SD	After Six Months of HAART Initiation (n= 333) Mean ± SD	t-value (95% *CI)	*P-value
WBC ($\times 10^9$ /l)	4.106 ±0.970	4.995 ±1.34	-12.186(-1.03277-(-0.74567))	0.0001
LC ($\times 10^9$ /l)	1.097 ±0.589	1.588 ±0.70	-11.637 (-574007-(-40801))	0.0001
ANC ($\times 10^9$/l)	2.180±1.212	1.540 ±0.86	-10.536 (0.52105-0.76027)	0.0001
RBC ($\times 10^{12}$/l)	3.929±0.835	4.482±0.65	-10.805 (-0.65432-(-45277))	0.0001
Hgb (g/dl)	12.38±1.77	13.22±1.81	-6.926 (-1.09739-(-0.61192))	0.0001
HCT (%)	36.372±5.60	39.92±6.32	-8.775 (-4.34832-(-2.7557))	0.0001
MCV (fl)	87.57±11.63	89.99±8.87	-4.054 (-3.59256-(-1.24522))	0.0001
MCH (pg)	29.24±3.67	31.33±4.89	-6.16 (-2.74833 –(-1.41791))	0.001
MCHC (g/l)	31.99±2.84	32.06±2.22	-0.378 (-43077-0.29191))	0.706
PLT ($\times 10^9$ /l)	243.3±52.59	292.18±60.539	-14.597 (-55.467-(-42.293))	0.0001
CD4 (Cells/mm3)	356.56±212.32	583.7±238.25	-16.613(-254.04-(-200.247))	0.0001

*P values using paired student t-test for mea * CI confidence interval

5.5 Hematological Abnormalities in HIV Patients at Yabelo General Hospital, Borana Southeast Ethiopia, 2020.

According to WHO classification of anemia based on Hgb concentration, 158(47.4%) were anemic at baseline and the magnitude decreased to 77 (23.1%) after at least 6 months of HAART initiation. When data is analyzed by severity, at base line of the 158 anemic study participants majority of them had mild 142(89.9%) anemia, while 10(6.3%) moderate, and 6(3.8%) severe. But after taking HAART for at least six months, while the prevalence of anemia decreased from 47.4 % to 23.1% the severity remains almost same where 71 (92.2%), 2(2.6%) ,4(5.2%) had mild, moderate , and severe anemia, respectively.

Other hematological abnormalities like leucopenia (WBC<4000cells/µl),Neutropenia (Neutrophil<1500cells/µl; <40%), lymphopenia (lymphocyte<1000cells/µl; <20%) and thrombocytopenia (PLT<150,000cells/µl) has shown changes in prevalence between baseline and at least 6 months after therapy. Accordingly rates of leukopenia is reduced from 244(73.3%) to 121(36.3%); lymphopenia from 256 (76.9%) to 119(35.7%) and thrombocytopenia from 11(3.3%) to 8(2.4%). Nutropenia rates on the contrary increased from 78(23.4%) to 194(58.3%). Of the study participants, 207(62.2%) had immune suppression (decrements in CD4 count< 350 cell/µl) at initiation of HAART of which was 95(45.9%) where with severe immune suppression as defined by CD4<200 cells/µl; there is also increment in CD4 count after initiation of HAART which revealed with decreased immune suppression 69(20.7%). Severe immunodeficiency decreased from 45.9% to 20.7% (Table 4).

Table 4 :-Prevalence of immuno-hematological Abnormalities of Adult HIV patients taking HAART for at least 6 months at Yabelo General Hospital Borana, Ethiopia, 2020

Hematological Disorder	Before Initiation of HAART Number (%)	After 6 Months of Initiation of HAART Number (%)
Anemia	**158(47.4)**	**77 (23.1)**
Mild	**142(89.9)**	**71(92.2)**
Moderate	**10(6.3)**	**2(2.6)**
Severe	**6(3.8)**	**4(5.2)**
Anemia		
Male	**78(50)**	**32(43.7)**
Female	**80(50)**	**45(56.3)**
Leucopenia	**244(73.3)**	**121(36.3)**
Male	**132(54.1)**	**55(45.5)**
Female	**112(45.9)**	**66(54.5)**
Neutropenia	**78(23.4)**	**194(58.3)**
Male	**43(55.1)**	**103(53.1)**
Female	**35(44.9)**	**91(46.9)**
Lymphopenia	**256(76.9)**	**119(35.7)**
Male	**136(53.1)**	**58(48.7)**
Female	**120(46.9)**	**61(37.4)**
Thrombocytopenia	**11(3.3)**	**8(2.4)**
Male	**4(36.4)**	**5(62.5)**
Female	**7(63.6)**	**3(37.5)**
Immunodeficiency	**207(62.2%)**	**69(20.7%)**
Severe	**95(45.9%)**	**18(26.1%)**
Mild	**112(54.1%)**	**51(73.9%)**

5.6 Baseline Immunosuppression level by age category in HIV Patients at Yabelo General Hospital, Borana Southeast Ethiopia, 2020.

As shown in Figure 1, the majority of the study participants were having CD4<350 cells/µl regardless of age categories. Specifically, 12.9%, 11.4%, 6.9% of the participants in the age groups 29-38, 18-28, and 39-48 years, respectively, were in the CD4 count 200-349 cells/µl category. Severe immunodeficiency (CD4<200 cells/ µl) was predominant in the older age groups 49-58 and >59 years Figure 1.

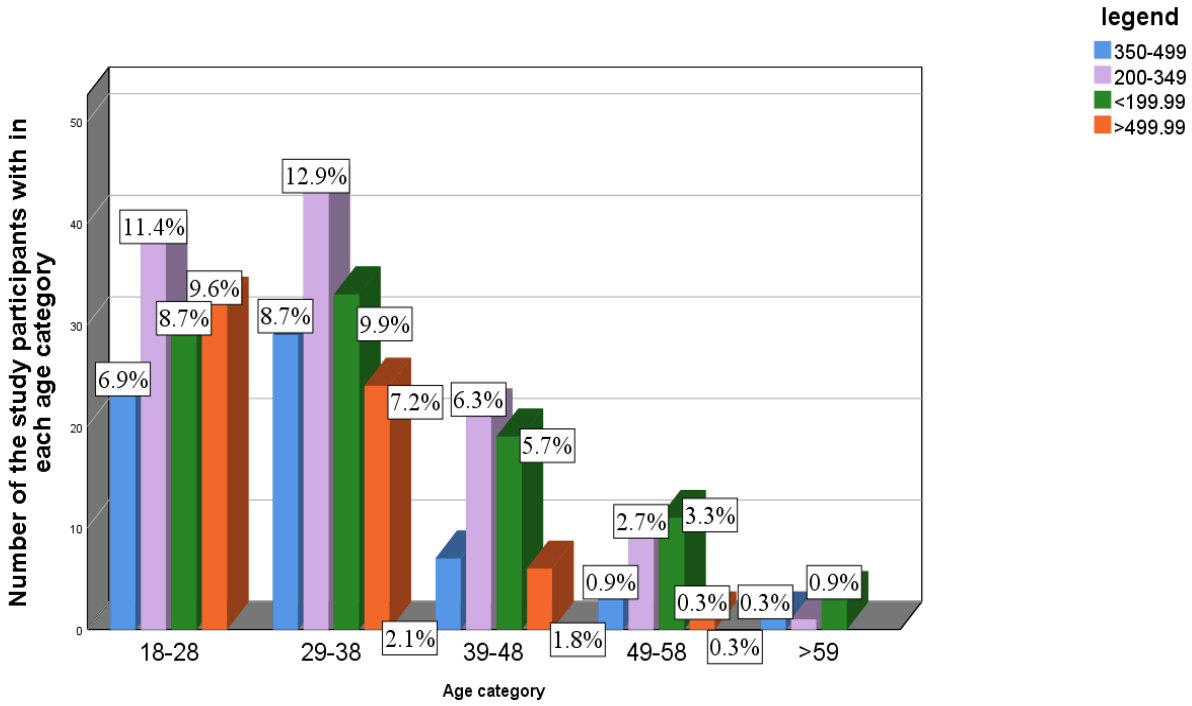

Figure 1:Prevalence of baseline immune-suppression within age category of Adult HIV patients at Yabelo General Hospital Borana, Ethiopia, 2020

5.7 Immunological Abnormalities in HIV Patients after taking HAART for at least at Yabelo General Hospital, Borana Southeast Ethiopia, 2020.

As opposed to the baseline data, the majority of the participants after taking HAART for at least 6 months were found in the CD4>500 cells/µl category irrespective of age category although the highest proportion is seen in the age group 18-28 years where 71.3% are in the CD4>500 cells/µl category. The proportion of patients with severe immunosuppression of CD4<200 cells/µl increased with increasing age category which is 12.3%, 21.7%, 24.5%, 33.3% and 40.0% for the age groups 18-28, 29-38, 39-48, 49-58 and >59 years, respectively (Figure 2).

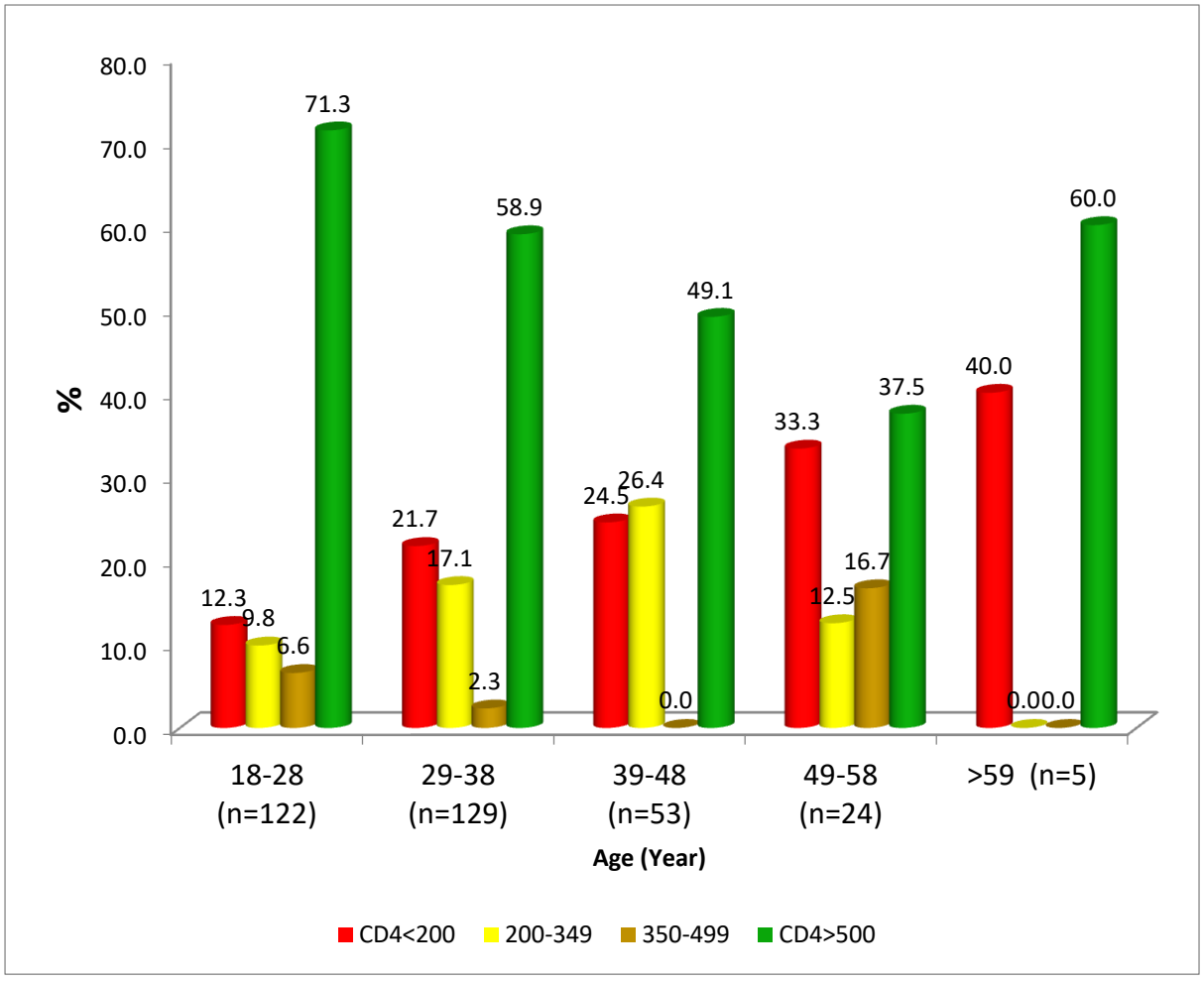

Figure 2: **Prevalence of immunosuppressant with age category of Adult HIV patients taking HAART for at least 6 months at Yabelo General Hospital Borana, Ethiopia, 2020**

5.8 Factors associated with Anemia

To determine factors associated with current anemia among adults living with HIV in bivariate analysis P-value 0.20 and below was used as a cut of value to determine candidate variable for multivariate logistic regression. Based on the above assumption; treatment interruption, types of opportunistic infection, BMI, gender, WHO clinical staging, residence, and educational status were candidate variables for multivariate analysis.

Among the variables included in final model, those patients with treatment interruption and Presence of extra pulmonary tuberculosis are factor associated with Current Anemia; those individuals with BMI greater less 18.49 are more likely to develop anemia (5.4 times)[OR=5.428(95%CI,**1.425-20.674**] when compared to those with normal BMI; and female patients are more likely to have anemia when compared to male patients [OR=2.02 (95% CI, 1.164-3.503)].

Table 5:- Factors associated with Anemia after taking HAART for at least six month.

Variables	Anemia		COR (95%CI)	P –value	AOR(95%CI)	P-value
	Yes N(%)	**NO N(%)**				
Treatment interruption						
Yes	19(16.7)	95(83.3)	**0.555(0.312-0.988)**	**0.046***	**0.545(0.298-0.997)**	**0.049***
No	58(26.5)	161(73.5)	**1**		**1**	
Types of opportunistic						
Severe bacterial pneumonia	5(27.8)	13(72.2)	1.270(0.436-3.699)	0.661	1.454(0.478-4.424)	0.510
Pulmonary tuberculosis	3(9.7)	28(90.3)	0.354(0.104-1.202)	0.096*	0.356(0.101-1.247)	0.106
Extra pulmonary tuberculosis	6(46.2)	7(53.8)	2.830(0.918-8.728)	**0.070***	**2.988(0.937-9.533)**	**0.064***
No opportunistic infection	63(23.2)	208(76.8)	1		1	
Current BMI						
18.5-24.9	61(22.7)	208(77.3)	1		1	
<18.49	5(50)	5(50)	3.410(0.956-12.166)	**0.059***	**5.428(1.425-20.674)**	**0.013***
>24.99	11(20.4)	43(79.6)	0.872(0.424-1.794)	0.710	0.955(0.452-2.030)	0.905
Age						
18-28	26(21.3)	96(78.7)	1			
29-38	31(24)	98(76)	0.856(0.473-1.548)	0.607		
39-48	11(14.3)	42(79.2)	1.034(0.468-2.285)	0.934		
>49	9(31)	20(69)	.602(0.245-1.478)	0.268		
Sex						
Male	32(18.8)	138(81.2)	1		1	
Female	45(27.6)	118(72.4)	1.646(0.982-2.754)	**0.059***	**2.02(1.164-3.503)**	**0.012***

WHO stage Current				
Stage 1	75(23)	251(76.9)	0.747(0.142-3.929)	0.731
Stage 2	02(28.6)	5(71.4)	1	
CD4 at current				
350-499	**12(18.2)**	**54(81.8)**	1	
<349	**17(25.8)**	**49(74.2)**	1.561(0.678-3.595)	0.295
>500	**48(23.9)**	**153(76.1)**	1.106(0.583-2.097)	0.758
Residence				
Urban	**48(24.6)**	**147(75.4)**	1	
Rural	**29(21)**	**109(79)**	1.227(0.727-2.071)	0.443
Educational Status				
No formal education	**30(26.8)**	**82(73.2)**	0.911(0.33-2.512)	0.857
Can read and write	**11(22.4)**	**38(77.6)**	1.152(0.368-3.607)	0.809
Primary education	**14(16.1)**	**73(83.9)**	1.738(0.586-5.152)	0.319
Secondary	**16(26.2)**	**45(73.8)**	0.938(0.317-2.777)	0.907
Higher education	**6(25)**	**18(75)**	1	
Occupation				
House wife	**17(20)**	**68(80)**	0.8(0.16-3.996)	0.786
Employee	**15(22.4)**	**39(72.2)**	1	
Student	**7(13)**	**47(87)**	1.343(0.242-7.449)	0.736
Merchant	**20(26.3)**	**56(73.7)**	0.56(0.113-2.779)	0.478
Daily labor	**10(27.8)**	**26(72.2)**	0.52(0.097-2.802)	0.238*
Farmer	**8(28.6)**	**20(71.4)**	0.333(0.054-2.067)	0.038*

6. Discussion

Immunohematological parameters are changed in HIV-infected patients with which causes morbidity, mortality, and poor quality of life. The discovery of HIV drugs (ART) in recent year dramatically improved the clinical and hematological changes of patients with HIV infection making it a manageable disease.

The current study investigated the immune-hematological changes among HIV-positive adults after initiation of HAART for more than six months at Yabelo General Hospital. The study revealed there is a significant change in hematological parameters after use of HAART for at least six month with exception of MCHC. In addition, there was an average increase of 227.14 CD4 cells/µl following treatment. Most common abnormality includes anaemia, thrombocytopenia, leucopoenia, lymphopenia, Immunosuppression and neutropenia.

Various researches conducted on patients living with HIV around the world concerning hematological and immunological changes, has shown anemia, leucopenia, lymphopenia, neutropenia, and thrombocytopenia are the most prevalent hematological abnormalities. Low CD4 count is the most immunological abnormality. These studies have revealed that initiation of HAART has decreased the prevalence of these abnormalities like anemia, lymphopenia, neutropenia, thrombocytopenia and increased others including leucopenia (6, 21-24).

The present study found that the prevalence of anemia at the baseline was 47.4% with 89.9% mild, 6.3 % moderate and 3.8% severe anemia. After 6 months of HAART initiation, the prevalence of anemia was reduced to 23.1 %(92.2%, 2.6%, 5.2% mild, moderate and severe anemia, respectively). The finding of anemia at baseline is in line with a study conducted in Black Lion hospital (41.9%), Arba Minchi Public Health facilities (52.3%) Zewditu Memorial Hospital (42.9%), (31, 33, 34), indicating the effect of HIV infection regardless of geographic location and population differences.

The prevalence of anemia before HAART initiation in this study is higher than studies conducted in Gondar (29.7%), Hawassa University Referral Hospital (23.4%), Debre Tabor Comprehensive specialized Hospital (31.8%), Jimma university referral hospital(29.9%), Ras Desta memorial hospital(24.1 %) and Goba referral Hospital (37.1%), (5, 35, 36, 37, 38, 39).

This discrepancy might be due to the difference life style of ART patients, the presence of opportunistic infections and nutritional status of the study participant in the other studies.

The baseline prevalence of anemia in this study is lower than the prevalence reported in Nigeria (57.5%), Ghana 63% before HAART and 46% after HAART, and India (40-42). The discrepancy might be attributed to the difference in the life style, study population, sample size, socio-demographic characteristics of study subjects, and variability in the definition of anemia.

Concerning the prevalence of anemia after taking HAART for at list six months it was 23.1%. The result was in line with study conducted in Debre tabor (17.4%), Nigeria (24.3%), and Zewditu Memorial Hospital (20.9%) (34, 36, 40).

This prevalence is higher than those reported from Gondar (11.7%), Black Lion Hospital (11.4%), and Goba Referral Hospital (14.6%) (5, 39, 31). This difference in prevalence might be attributed due to life style, altitude, study population, sample size, socio-demographic characteristics of study subjects.

Anemia after taking HAART finding of this study is lower than the finding from Ghana (46%) (41); this discrepancy might be due to difference in sample size, and geographical location.

In the current study the prevalence of anemia after taking of HAART for at least six month was significantly associated with presence of treatment interruption, sex and with BMI greater than 24.99. Female patients are less likely (49.3%) to have anemia when compared to male patients which is supported by study done in Dessie (42).

The result of this study has shown that leucopenia was the most common hematological abnormality with higher prevalence at before Starting of HAART than after taking HAART for at least 6 months (73.3% vs. 36.35%), this finding is consistent with the reports of various studies (25, 36, 43-45).

In current study the rise in mean WBC count after six months of HAART initiation indicates boosting of the immune system of the body (4.106 ±0.970 to 4.995 ±1.34). The finding was concurrent with a retrospective study done at Zewditu Memorial Hospital and Goba Referral Hospital (35, 39). The finding was also supported, by study in Markurdi, Benue state of Nigeria which indicated that HAART brings statistically significant increment in WBC from 4.07 ± 0.25 ×103 /µl at baseline to 4.76 ± 0.15×103 /µl after ART (46). Increase in WBC

count after HAART initiation might be due to increase in hematopoietic progenitor cell growth, following decreasing of the HIV viral load (35).

In contrast with the current finding there are reports showing decrease in WBC count after taking HAART (47, 48).This difference may be attributed by short duration of HAART intake in the other studies as in the initial stage of ART initiations, WBC count will be low which gradually adjusts itself over a period of time (49).

The prevalence of Neutropenia in the present study was 23.4% before HAART initiation and 58.3 % after taking HAART for at least six months. This finding contradicts with reports from Gondar (14.8%) and South Korea (10%) at base line and 28.3% in Gondar (18) and 8.9 % in South Korea (14) after initiation of HAART. The higher prevalence of nutropenia might be attributed to o due 0pportunistic and other infection in the current study.

The present study also revealed differences in mean CD4+ T cells count before and after HAART initiation (356.56±212.32 vs. 583.7±238.25cells/µl) with an average increase of 227.14 CD4 cells/µl following treatment. In contrast studies conducted at Zewditu Memorial hospital reported limited increment after HAART (116 ± 69.4 vs. 112 ± 67 cells/µl) (6). This can be explained by the policy change in ART initiation where treatment is given regardless of CD4 count in recent years so that the participants of the current study had better initial CD4 counts compared to the earlier studies.

The lower CD4 count at the baseline is due to HIV infection which decreases production of CD4+ T cells by averting maturation of lymphoid precursors. Inhibition of viral replication by HAART is, therefore, associated with improved production of CD4+ T cells (4, 17, 19-20). Highly active antiviral treatment decrease in plasma viral load after the taking of HAART for at least six month which then intern raise in CD4 count (5).

The prevalence of thrombocytopenia in present study is high at base line when compared to patients on HAART for at least six month. Thrombocytopenia was reduced from 3.3 to 2.4% after at least six months of HAART. The result is consistent with the finding of the study in India (4.76% to 3.74%) (26). The improvement in thrombocytopenia might be attributed to the effect of treatment in which after HAART initiation abnormalities of hematopoietic, opportunistic infections and immune causes related to HIV leading to low platelets count could be reverted (27,29).

Also the research in Gondar (9% to 4.1%) and Bale (Goba Referral Hospital) (11.4% to 4.5%) has revealed decrease in thrombocytopenia after they taking for at least six months of HAART, but the results were slightly higher than the current finding (5,39). The variation in results seen from this study attributed due to the variation in the definition of thrombocytopenia, size of the study population and study design used.

In summary, this study was conducted having an objective of identifying changes in Immuno-hematological parameter among adult HIV positive patients taking HAART at least for 6 month in Yabelo General Hospital. As shown in these study participants at baseline have significantly higher rates of anemia, thrombocytopenia, and CD4+ T lymphocytopenia which improved dramatically after initiation of HAART.

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7. Conclusion

In conclusion, the current study found that Anemia, thrombocytopenia, leucopenia, lymphopenia, neutropenia, and thrombocytopenia were the most prevalent hematologic abnormalities (outcomes) in HIV/AIDS patients. Immunohematological profile of the patients has improved after initiation of HAART. Factors like treatment interruption, BMI less than 18.49, presence of extra pulmonary TB and sex are among factor associated with anemia after initiation HAART. Those who have ever interrupted treatment were less likely to be anemic while female sex, presence of extra pulmonary TB and BMI <18.49 more likely to be anemic.

8. Strength and Limitation of the Study

8.1 Strength of the study

- The study is carried out in the first and model ART center of the south Oromia.

8.2 Limitation of the study

- The current study did not include viral load findings, treatment Duration, drug regimen and parasitic infection.
- Different hematological and CD4 analyzers were used during baseline data recording due to placement of equipment nationally.
- The present study was not sure about the effect of TB.

9. Recommendation

Early initiation of HAART is helpful in dropping the degree of hematological abnormalities in HIV infected patients in turn improving patient outcome. To reduce Anemia Patients should be advised not to interrupt treatment and nutritional advice to increase BMI should be strengthened among HIV patients so as to reduce the prevalence of anemia.

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11. Annex

Annex I:- Principles of laboratory analysis

Complete blood count: - The complete blood count (CBC) is important because people with HIV may have low blood cell counts (cytopenias) due to chronic HIV infection or as a side effect of medications, particularly drugs that damage the bone marrow. Blood cell counts are typically reported as the number of cells per μl of blood (cells/μl) or as a percentage of all blood cells.

Principles:-CBC is measured by using hematological analyzer using two different measuring principles such as electric impedance method for determining WBC, RBC and Platelet while the colorimetric method used for determining the hemoglobin concentration.

Measurement Principles

The sysmex hematology analyzer XE-2100 can analyze and output the results for 32 hematological parameters of whole blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained.

Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the DIFF scattergram.

Measurement of WBC

RBCs are lysed with the acid haemolytic reagent STROMATOLYSER-FB. This reagent selectively suppresses the degranulation of Basophils, resulting in their separation from other WBC. After this reaction the sample is analyzed by flow cytometry using a semiconductor laser to detect forward and side scattered light information, based on which a WBC/BASO scattergram is obtained. By analyzing this scattergram, WBC and Basophil counts are taken.

WBC 4-part differential

RBCs are lysed with STROMATOLYSER-4DL. At the same time the reagent acts on the WBC membrane to allow dye passage .STROMATOLYSER-4DS (dying solution) is then added to allow the dye to enter WBC at the damages portion of its membrane and stain the DNA and RNA therein. Following this reaction the sample is then analyzed by flow cytometry using the s semiconductor laser to detect forward and side scattered light information, based on which a 4-DIFF scattergram is obtained. By analyzing this scattergram, 4-parameter counts (lymphocytes, monocytes, Eosinophils, Neutrophils and Basophils are taken.

The immature information (IMI) channel of the XE-2100 counts human progenitor cells (HPC). The reagents specifically affect the lipid components of the cell membranes; the membranes of mature cells, with a higher content of lipid are lysed while immature cells retain their membranes. In normal samples no intact cells are seen in the IMI area. The IMI channel is used to discriminate between immature and mature white blood cells. It utilizes the RF/DC detection method.

Measurement of RET and optical measurement of PLT

Blood is diluted with RET SEARCH (II) to preset concentration. RET SEARCH (II) staining solution is also added to stain WBC DNA and RNA and RET RNA. The sample is then analyzed by flow cytometry using semiconductor laser to detect forward scattering light and side fluorescence information, based on which an RET scattergram is obtained. By analyzing this scattergram, RET counts, RET ratios for individual fluorescence intensity zones (LFR, MFR, HFR), immature reticulocyte fraction (IRF) and PLT-O are determined.

Platelets are also measured with sheath flow DC detection method. By measuring larger number of platelets this method offers increased accuracy. However, in cases where a large number of RBC fragments or large Platelets are present, accuracy tends to decrease. For such samples, the PLT-o measurement ensures higher accuracy than the DC detection method. XE-2100 presents the platelet counts of high accuracy by adopting the results from either method that seems more accurate judging from the cell distributions and the scattergrams obtained.

Measurement of NRBC

Nucleated red blood cells (NRBC) occur in very low concentrations; however are found in high concentrations in haemolytic diseases of new born, sickle cell anaemia and thalassaemia major particularly following splenectomy. In NRNC channel the cell membranes of NRBC are lysed with the STROMATOLYZER_NR diluent to expose the nuclei of NRBC, the white

cells are slightly perforated to allow quick influx of the dye, but remain intact. Nuclear material is stained with a polymethine based fluorescent dye and cells and nuclei are hydrodynamically focused. Fluorescence intensity and forward scatter light intensity of each cell are electronically analyzed. This allows the clear separation of leucocytes and NRBC.

CD4 tests: - HIV primarily targets CD4 cells. As HIV disease progresses, CD4 cell counts decline, typically by about 30-100 cells/µl per year (depending on viral load), leaving a person increasingly vulnerable to infections and cancers. .

Principle of CD4 count using FACSPresto

The CD4+T cell count was done by FACSPresto (Beckton Dickson, San Jose, CA) POC analyzer. The multicolor (3-color) fluorescent photo-microscopy and light absorbance detection were used to measure CD4 and Hemoglobin respectively.

The added whole blood sample to an inlet of the cartridge which contains CD3 APC, CD4 PECy™5, CD14 PE, and CD45RA APC is stained at the incubation of 18 minutes. Then the sample is illuminated from two LEDs the excitation sources. Then the resulting images are captured through a microscope lens system by a digital camera assembly. Finally, the captured images are analyzed by software and results are reported to the operator.

ANNEX II: - Participants Information sheet

Title of the Research Project: Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at least Six Month in Yabelo Hospital, Boerne, Ethiopia**.**

Principal Investigator: Girma Ashenafi (BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors and physician .Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to asses Assessment of Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at least Six Month in Yabelo Hospital, Borena, Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process).The purpose of this proposed study is to asses Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at least Six Month in Yabelo Hospital, Borena, Ethiopia. You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to final finding. Thus, result from this study is anticipated to improve the health status and information of the physician and other stake holder at large in Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by trained nurse. Then, you are requested to give your consent to the sample collector. After consent, a

sample will be taken from for arm vein. Moreover, there will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 5 minutes. You will be asked to provide blood sample (4-5 ml) venous blood. We will conduct laboratory examination to determine different hematological and immunological parameters.

Potential risks and Discomforts

There might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory/nurse professionals.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of Acute Leukemia. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

NamePhone:E-mail:

1.Girma Ashenafi 0916189005 girmaashenafi059@gmail.com

ANNEX III :- Participants Information sheet (Ahmaric Version)

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመ
መረቂያጥናትላይእዲሳተፉተጋብዘዋል።እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየ
ሚገኘዉንምንባብበጥሞናያንብቡናግልጽያልሆነልዎትንማንኛዉምሃሳብይጠይቁ።

መግቢያ

የጥናቱርዕስ “Assessment of Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month In Yabelo Hospital, Boerne, Ethiopia”.

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎፈቃደኝነትላይየተመሰረተነዉ።በዚህጥናት
ዉስጥላለመሳተፍወይምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህሆስፒታልየ
ሚሰጠዉማንኛዉምአገልግሎትአይቋረጥም።በጥናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበ
ጹሁፍወይምበጣትፊርማማስቀመጥይጠበቅዎታል።

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል።ከተወ
ሰደዉናሙናላይየሚገኙመረጃዎችከዚህሆስፒታልዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነገ
ርየማይቃወሙመሆኑንመስማማትይጠበቅብዎታል።ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገ
ልጹመረጃዎችንማለትምስም፤አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎቸንአይጨምርም።ይልቁንም
ለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረጋል።በ
ተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠትይኖር
ብዎትአል።

በዚህጥናትመሳተፍየሚያስከትላቸዉቸግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም።ሆኖምግንናሙናዉንለመሰብሰ
ብልምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖር
ም።

የህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥና
ቱአላማብቻነዉ።ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው።ከዚያምበላይ

ስለእርስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲ
ቀመጥይደረጋል።

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅ
ምባይኖርምከጥናቱበሚገኝውውጤትግንተጠቃሚነዎት።የእርሶዎተሳትፎየእርስዎንናየወገንዎትንየደ
ምካንሰርለማወቅናለማከታተልከፍተኛጥቅምይኖረዋል።

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው ?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማቋረ
ጥሙሉመብትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምአ
ይነትየሆስፒታልአገልግሎትአይኖርም።ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄ
የመጠየቅናገለጻየማግኘትመብትአለብዎት።የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ።ነገር
ግንእርስዎበሚሰጡንመረጃየችግሩንስፋትለመከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎትጥ
ያቄቀጥተኛመልስይሰጡንዘንድበታላቅአክብሮትእንጠይቃለን።

ጥያቄካለኝወይምችግርቢያጋጥመኝምንማድረግይገባል?

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይ
ምጥያቄካለዎትበሚመለከተውአድራሻይጠቀሙ።

ሞባይል፡ +251-916189005

ኢሜል፡girmaashenafi059@gmail.com

Annex IV. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is Assessment of Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month In Yabelo Hospital, Borena, Ethiopia.. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of acute leukemia in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and bone marrow sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex V. Informed consent form in Amharic version
የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥርቁጥር ----------

የተሳታፊዉስም ----------

እኔስሜከላይየተጠቀሰውተሳታፊ “Assessment of Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy (On Follow Up) at Least Six Month In Yabelo Hospital, Borena,Ethiopia.”ጥናት ላይ በቂገለጻተ ደርጎልኛል።ለጥናቱምደም(blood sample)ናሙናእንደሚያስፈልግ ተገልጾልኛል።የጥናቱንምአላማዎችምተረድቻለሁ።

በቃለመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል።በጥናቱላ ይያለመሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብ ቴየተጠበቀእንደሆነተገልጾልኛል።

ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝ ነትነው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪያአግኝቻለሁ።የዚህ ጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ። በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ።ስለዚ ህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ።

ፊርማ---------- ቀን ----/---/----------

(የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች)

የአማካሪነርስስም ---------- ፊርማ ----------

ቀን----------

Annex VI:- Questionnaire (English version)

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Dear Respondent,

My name is Girma Ashenafi and I am a postgraduate student at Addis Ababa University. Currently I am conducting my Master's Thesis paper and I kindly invite you to participate in this research study entitled "Assessment of Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month in Yabelo Hospital, Boerne, Ethiopia.

The purpose of this study is to asses Immunological and hematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy by comparing treatment initiation(base line data) with Six Month after initiation in Yabelo Hospital.

Your contribution to the successful outcome of this research is invaluable; please answer all the questions as fully and honestly as possible.

I hope you will take a few minutes to complete this questionnaire. Therefore, I kindly request you to respond to each question item carefully and responsibly.

If you have any questions or concerns about completing the questionnaire or about participating in this study, you may contact me at 09161819005.

"Thank you in advance"

Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month in Yabelo Hospital, Boerne, Ethiopia 2021.

NO.	VARIABLES	CATEGORIES	REMARK
	Unique ART No		
1	**Age** at start of ART	_____.	
2	**Sex**	□ Male Female	
3	**Religion**	□ Protestant □ Orthodox □ Muslim □ Catholic □ Other (specify----------	
4	**Maritalstatus** **Note**: marital status may change; please mark status at enrolment into	□ Single □ Married □ Divorced □ Widowed □ Separated	
5	**Educationalstatus of the patient**	□ No formal education □ Can read and write □ Primary education □ Secondary education □ Higher education	
6	**Occupationalstatus of the patients**	□ non employed □ full time employed □ Part time employed	
7	**Residence**	□ Urban □ Rural	

8	**WHO STAGE**	BEFEORE AFTER ➢ Stage 1 Stage 1 ➢ Stage 2 Stage 2 ➢ Stage 3 Stage 3 ➢ Stage 4 Stage 4	
9	**Laboratory measures at base line (start of HAART)** **NB:- Attach the current immunohematological parameters print out data on each questioner.**	1. CD4 (cell/microlitre)_____ 2. WBC (cell/$mm^{3)}$_____ 3. RBC (cell/$mm^{3)}$_____ 4. Hgb (g/dl)_____ 5. HCT (%)_____ 6. Neutrolphile (%)_____ 7. Lymphocytes (%)_____ 8. MCV_____ 9. MCH_____ 10. MCHC_____ 11. Platelet _____	

10	**Treatment interruption**	1.Yes 2.No	
11	**Current BMI (Kg/m2)**	_____	
	Initial BMI (Kg/m2)	_____	
12	**Functional status at base line start of ART**	□ Working □ Ambulatory □ bedridden	
13	**History of opportunistic infections after six month since start of ART.**	□ YES □ NO	

14	**If YES Que. 13**	□ Pneumocystis CariniPneumo □ severe Bacterial Pneumonia □ CNS Toxoplasmosis □ Cryptococcus Meningitis □ 1.Pulmonary Tuberculosis □ 2.Extra pulmonary tuberculosis □ **O**ther (specify	

ANNEX VII :- Questioners (Afan oromo version)

Yuuniivarsitii AddiIs Ababaatii
Kuuta Laabooratorii

Kabajamoo hirmatoota koo

Maqaa koo Girmaa Ashannafii jedhama yunivarsitii finfine irran dhufee bartaa digiri lamafaa yoonat"u mastarsii ko xumurudhaff gaafi deebi armaan gadii kana fayadamuun qoranoo waan gageesuu fedhuf yoo fedha keesan taee akka hirmataan kabjadhaan issin gafadha. . mat dureen qoranoo kiyaa "Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month in Yabelo Hospital, Borena, Ethiopia" kan jedhudha.

Yoo gafii qabatan lakk:-09161819005 naaf bilbilaa

Galatoomaa

Immunohematological Outcome among HIV Patients Taking Highly Active Antiretroviral Therapy for at Least Six Month in Yabelo Hospital, Borena, Ethiopia 2021.

Lakk .	**jijiramtootaa**	**Ramadii**	**yadaa**
	Lakk galme:-		
1	**umrii**	_____.	
2	**salaa**	□ Dhiraa Dubra	
3	**Amantaa**	1. porostantanti 2. ortodoksii 3. Musilmaa 4. katolikii 5. waqefataa	
4	**Halaa Gayilaa**	a. qeenxee b. Kan fudhe c. Ka adaa bahee d. Kan jal du"e/dutee	
5	**Sadarkaa barnootaa**	❖ Kan hin barane ❖ Baressu fi dubisu kan danda"u ❖ Sadarka tokkoffa ❖ Sadarkaa lamaffa ❖ Sadarkaa oolana	
6	**Occupationalstatus of the patients**	❖ Hadha mana ❖ Kan hojii hinqabnee ❖ Hojeta motumaa ❖ Barataa/tuu ❖ Daldalaa ❖ Hojataa humnaa ❖ Qotee bulaa	

7	**Idddo jireenya**	● Magalaa ● Badiyaa	
8	**WHO STAGE**	Duraa boda ➢ sad 1 sad 1 ➢ Sad 2 sad2 ➢ Sad 3 Sad3 ➢ Sad 4 sad 4	
9	**Firii laboratorii yeroo jalqabatii** **Hub-kan amma asirrati qansisaa.**	CD4 (cell/microlitre)_____ WBC (cell/$mm^{3)}$_____ RBC (cell/$mm^{3)}$_____ Hgb (g/dl)_____ HCT (%)_____ Neutrolphile (%)_____ Lymphocytes (%)_____ MCV_____ MCH_____ MCHC_____ Platelet _____	

10	**Dawwa addan kutani jirtu**	1.eeye 2.lakii	
11	**BMI amaa (Kg/m2)**	_____	
	IBMI duranii (Kg/m2)	_____	
12	**Halaa dhub yeroo duranii gr mana yaala dhufee**	I. Kan demu II. Ampulansiidhan III. Algadhan	
13	**Dhukuba birrotin qabamni jiru.**	1. Eyee 2. lakii	
14	**Eeye yoo jetan kamii**	3. Pneumocystis CariniPneumo 4. severe Bacterial Pneumonia 5. CNS Toxoplasmosis 6. Cryptococcus Meningitis 7. 1.Pulmonary Tuberculosis □ 2.Extra pulmonary tuberculosis □ **O**ther (specify	

Declaration

I the undersigned declare that this thesis is my original work and has not been presented in this or any other university and all sources of material used for this thesis have been acknowledged.

M.Sc. candidate: Girma Ashenafi (BSc)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Melatwork Tibebu

Signature_____

Date: _____

Place: Addis Ababa, Ethiopia.