ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Immuno-Hematological changes among HIV and TB/HIV co-infected patients taking Highly Active Antiretroviral Therapy for at least six months in Menelik II Referral Hospital, Addis Ababa, Ethiopia.

By: Esuyawkal Kasawmar

Advisor: Aster Tsegaye (PhD)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology)

June 2020
Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Esuyawkal Kasawmar, entitled: Assessment of Immuno-Hematological Changes among HIV and TB/HIV co-infected patients taking HAART for at least six months in Menelik II Referral Hospital, Addis Ababa, 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.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor _____Signature _____ Date _____

_____

Chairman of the Department or Graduate Program Coordinator

ACKNOWLEDGMENT

First of all, I would like to thank my God for giving me the strength and support to do this work.

I would like to extend my appreciation to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences for giving me a chance to conduct a thesis on this topic.

I want to express my great thanks to my advisor Aster Tsegaye (PhD) for advising me and giving valuable comments and information in thesis writing up.

And also I wish to acknowledge the support of the management and staff of Menelik II Referral Hospital for their assistance in this thesis work. All study participants are gratefully acknowledged for kindly participating in this study and allowing me to use their medical record.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Absolute neutrophil count
ART	Antiretroviral Therapy
ARV	antiretroviral
ARVDs	Antiretroviral drugs
AZT/ZDV	Zidovudine
BMI	Body mass index
CBC	Complete blood count
CD_4	Cluster of Differentiation 4
CSA	Central Statistics Agency
d4T	Stavudine
EDHS	Ethiopian demographic and health survey
EFV	Efavirenz
EPHI	Ethiopian Public Health Institute
EQA	External Quality Assurance
FMOH	Federal Ministry of Health
HAART	Highly Active Anti-Retroviral Therapy
HCT	Hematocrit
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NVP	Nevirapine
PCV	Packed cell volume
PLHIV	People living with HIV/AIDS
PLT	Platelets
RBC	Red blood cell
RDW	Red cell distribution width
RTV	Rotanavir

SD	Standard deviation
SPSS	Statistical Package for Social Science
TB	Tuberculosis
TB/HIV	The Intersecting Epidemics of TB and HIV
TDF	Tenofovir
TLC	Total lymphocyte count
UNAIDS	Joint United Nations Program on HIV/AIDS
WBC	White blood cells
WHO	World Health Organization

ABSTRACT

Background: Cytopenias are one of the most common complications in HIV and TB/HIV co-infected patients. Assessing immuno-hematological outcome is very essential for the management and care of people living with HIV and TB/HIV co-infection.

Objective: To determine the Immuno-Hematological changes among HIV and TB/HIV co-infected patients taking HAART for at least six months at Menelik II Referral Hospital.

Methods: This cross-sectional study was conducted on 338 HIV infected patients who have been taking HAART for at least six months. Socio-demographic, clinical, CD4+ T cells and hematological data at baseline and after at least 6 months on HAART were extracted from February to March 2019. Student t-test was used to compute the difference between baseline and current hematological and CD4+ T cells values. SPSS version 20 was used for data entry and analysis. $P<0.05$ was considered as statistically significant.

Results: Of the total 338 individuals, 255 (75.4%) were infected with HIV only and 83 (24.6%) were TB/HIV co-infected. There was significant increase ($p<0.05$) in the mean CD4 (164.6 vs 317.1 cells/μl), WBC (5.89 vs 6.49×10^3/μl), Hgb (13.07 vs 13.76g/dl), platelets (245 vs 265×10^3/μl), MCV (89.92 vs 96.13fl), MCH (30.25 vs 32.69pg) and a decrease in RBC (4.35 vs 4.25×10^6/μl) after six months of HAART. Compared to baseline, there was also significant decrease in the rate of Immunosuppression (CD4<350; 98.5 vs 88.5), anemia (39.9 vs 14.5%), leucopenia (22.2 vs 17.8%), neutropenia (11.5 vs 9.2%) and thrombocytopenia (16.9 vs 9.8%) after 6 months of HAART. When data was analyzed for HIV/TB co-infected patients separately, the effect of TB co-infection on HAART outcome was evident. The respective baseline and current values in this group were: CD4, 149.58 vs 294.72, WBC 5.83 vs 6.62, Hgb 12.25 vs 12.91, PLT 264.1 vs 262.9, RBC 4.22 vs 4.14, MCV 89.5 vs 92.6, MCH 29.54 vs 31.17 ($P<0.05$ for WBC, PLT, CD4, MCV). Rates of Immunosuppression was 98.8 vs 92.8%, anemia 51.8 vs 39.8%, leucopenia 21.7 vs 18.1%, neutropenia 10.8 vs 12.1%, thrombocytopenia 14.5 vs 12.0%. Besides, patients taking AZT based drugs had significantly higher MCV and MCH values ($P<0.01$) than those who did not.

Conclusion: It is evident from this study that has shown significant improvements in immuno-hematological profiles after taking HAART for at least 6 months. The changes were more affected with TB co-infection suggesting further investigation considering HAART adherence.

Keywords: HAART, AZT, TB/HIV co-infection, CD4 T cells, Anemia, Thrombocytopenia, Leucopenia, Neutropenia.

1. INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes Acquired immunodeficiency syndrome (AIDS). As a systemic disorder, it is characterized by causing severe impairment and progressive damage of both cellular and humoral immune responses (1-2). AIDS was first recognized in the United States in the summer of 1981 and by 1984, HIV was demonstrated clearly to be the causative agent of AIDS (3).

Opportunistic infections (OIs) cause significant morbidity/mortality in HIV infected individuals globally. Tuberculosis (TB) is one of OIs leading preventable cause of death among people living with HIV (4-5). TB and HIV/AIDS constitute the main burden of infectious disease in resource-limited countries. In the individual host, the two pathogens, Mycobacterium tuberculosis (MTB) and HIV, potentiate each other, accelerating the deterioration of immunological functions and resulting in premature death if untreated (6-7).

HIV causes functional and numeric depletion of TB-specific lymphocytes, leading to impairment of both cell-mediated immune response, and granuloma formulation and maintenance (8). Protective immunity to TB in humans relies upon monocytes and T lymphocytes that play a very important role in the control of infection. Platelets are also from inflammatory cells and are activated when they pass through the blood vessels (6).

HIV testing is the critical first step in identifying and linking people living with HIV (PLHIV) to the treatment cascade and also provides an important opportunity to reinforce HIV prevention among negatives (9). Antiretroviral drugs (ARVDs) are medicines taken by PLWHA to suppress viral replication in their body (10). Antiretroviral treatment (ART) has dramatically reduced HIV-associated morbidity and mortality and has transformed HIV infection into a manageable chronic condition (11). ART is recommended for all HIV infected TB patients regardless of CD4 (Cluster of differentiation 4) count or World Health Organization (WHO) clinical stage. WHO currently recommends routine screening for TB prior to ART initiation (12-13).

Every patient with HIV entering into care should have a complete medical history, physical examination, and laboratory evaluation and should be counseled regarding the implications of HIV infection. The following laboratory tests which are performed during initial patient visits can be used to stage HIV disease and to assist in the selection of ARV drug regimens:

- HIV antibody (Ab) testing (if prior documentation is not available or if HIV RNA is below the assay's limit of detection);
- CD4 T lymphocyte cell count (CD4 count);
- Plasma HIV RNA (viral load);
- Complete blood count, chemistry profile, transaminase levels, blood urea nitrogen (BUN), and creatinine, urinalysis, and serologies for hepatitis A, B, and C viruses;
- Fasting blood glucose and serum lipids; and
- Genotypic resistance testing (11,14-15).

HIV infection is a multisystem disease, with hematological abnormalities amongst the most common clinic-pathological manifestations (16). Several laboratory tests are important for initial evaluation of patients with HIV upon entry into care, and some tests should be performed before and after initiation or modification of ART to assess the virologic and immunologic efficacy of ART and to monitor for laboratory abnormalities that may be associated with ARVDs (11,17).

Complete blood count (CBC) is one of the most common blood test that is used to diagnose hematological abnormalities and that provides important information about the kinds and numbers of cells in the blood, for making decision of treatment (15,18). Cytopenias are one of the most common complications of HIV and may be broadly classified as being due either to a bone marrow production defect or to increased peripheral loss or destruction of blood cells (19).

Hematological complications such as mild-to-severe anemia are associated with HIV disease progression and subsequent reduced survival (20-21). HIV-infected T cells directly suppress growth of bone marrow progenitors, thus suppressing hemopoiesis. CD4, the cell-surface receptor target of HIV, is carried by T-helper lymphocytes, monocytes and microvascular endothelial cells which are prevalent in marrow (19).

Opportunistic infections such as MTB can cause cytopenias by infiltrating the bone marrow and causing reactive granuloma formation (12). Most of the TB patients showed low hemoglobin and

different types of anemia. There are four possible relationships of TB to hematologic diseases; drugs may cause idiosyncratic reactions, malabsorbtion, interference with iron metabolism, and hemolysis in patients with red blood cell enzyme deficiencies (22-23).

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 (24-25). The poor hematopoietic tolerance of the therapies often necessitates dose reductions, alterations of drug regimens, or interruption of therapies (25). Anemia has been shown to be associated with worsening of health outcomes in HIV infection such as faster HIV disease progression, increased morbidity as well as mortality (26)

Neutropenia is frequently observed in advanced stages of HIV infection after development of AIDS, and has been associated with certain types of antiretroviral medications used to treat HIV infection (27). Neutropenia, as in the other peripheral blood cytopenias in the setting of HIV infection has multiple etiologies, which may be present either singly or in combination. Thus, decreased colony growth of the progenitor cells leads to decreased production of both granulocyte and monocytes produced by the infected cells known to suppress neutrophil production (28-29).

Thrombocytopenia could result from increased platelets destruction and decreased platelet production by the HIV-infected megakaryocytic cells (21, 29).

Cytopenias caused by drug therapy are summarized below.

Drug	**Indication**	**Cytopenias**
Zidovudine (AZT)	ARV: Reverse transcriptase inhibitor	Anemia, neutropenia
Stavudine (d4T)	ARV: Reverse transcriptase inhibitor	Neutropenia, thrombocytopenia
Isoniazid, Rifampicin and Rifabutin	Tuberculosis and other mycobacteriae	Anemia, neutropenia, thrombocytopenia

The magnitude of CD4 recovery is directly correlated with CD4 count at ART initiation (30). The CD4 cell count and HIV RNA viral load are important measures of the efficacy and

effectiveness of ART among HIV participants enrolled in HIV care and treatment programs. The CD4 count at ART initiation determines the degree of immunologic and virologic ART response as well as subsequent risk of morbidity and mortality (11, 31-32).

Different studies were conducted to assess the hematological parameters of HIV positive patients. Limited studies in Ethiopia are published that assessed the Immuno-hematological profiles receiving Highly Active Antiretroviral therapy (HAART), particularly in the study area to provide updated information for the management of HIV and TB/HIV co-infected patients. Therefore, the present study is designed to provide information by assessing the Immuno-hematological profiles of HIV positive and TB/HIV co-infected patients starting HAART at Menelik II Referral Hospital ART unit in Addis Ababa, Ethiopia.

1.2 Statement of the Problem

The HIV pandemic presents a significant challenge to global TB control. HIV infection and TB not only constitute an unresolved public health challenge in sub-Saharan Africa but also in the entire world (11, 13, 33-34).

Ethiopia is one of the sub-Sahara African countries which shared the burden of HIV epidemics (35). An estimated 730,000 Ethiopians are currently living with HIV and all of them require antiretroviral treatment (ART); however, only 426,000 are taking ARV (36-37).

In 2018, global TB/HIV co-infection statistics revealed that the respective deaths from HIV/ TB co-infection, TB alone, and HIV alone are 300,000, 1,300,000 and 826,000 (38). TB/HIV co-infection is associated with special diagnostic and therapeutic challenges and constitutes an immense burden on healthcare systems of heavily infected countries like Ethiopia (37-39).

Cytopenias are the most common disorders including anemia, leucopenia, and thrombocytopenia. They are multifactorial in nature and are associated to an advanced stage of HIV (40). The presence of a high viral load, ART usage failure, the presence of acute and chronic opportunistic infections and infiltrative conditions have been reported as a significant predictor of progression to AIDS or death (39). Anemia is the most common cytopenia and occurs in up to 95% of HIV patients during their disease course (16, 41).

The consequences of hematological problems have major morbidity in themselves, adversely altering the patient's quality of life and hindering the treatment of both the primary viral infection and the secondary infections and neoplastic complications (41-42).

Hematological abnormalities are very important, because specific interventions other than antiretroviral treatment may be indicated for its correction. There are common manifestations of HIV infection and AIDS, which may have considerable impact on patients' well-being, treatment and care. But few studies on Immuno-hematological outcome in HIV and TB/HIV co-infected persons on HAART have been undertaken in sub-Saharan Africa especially in Ethiopia. This study tries to fill information gap in the study area to provide updated information to clinicians for improving the care of HIV and TB/HIV co-infected patents.

1.3 Significance of the Study

Assessing the impact of immuno-hematological aspects is very essential for the management and care of people living with HIV/AIDS and TB/HIV co-infected patients. This is because specific interventions other than antiretroviral treatment may be indicated for its correction. This will also help hospital management to plan appropriately regarding supplies and drugs needed for the diagnosis and treatment of additional comorbidities.

Moreover, this study can serve as a reference material for further researches with regards to immuno-hematological profiles in HIV infected and TB/HIV co-infected patients.

2. LITERATURE REVIEW

Several researches have been conducted about Hematological and Immunological parameters of HIV and TB/HIV co-infected patients in different parts of the world in general and Ethiopia in particular.

Study conducted by Santiago-Rodriguez and colleagues in USA showed that among a total of 1202 HIV positive patients included in a cohort study, the prevalence of pancytopenia was 8.7%. These patients with pancytopenia had lower body mass index (BMI) and lower CD4 count (40).

A cohort study of HIV infected patients starting HAART in Europe and North America showed that the prevalence of anemia at the baseline was 35 %. When analyzed by severity, 25% had mild, 9% had moderate and 1% had severe anemia. After 6 months of HAART initiation, the prevalence declined to 26 %. The prevalence of anemia was higher in women than in men, higher in older patients than in younger patients and increased with decreasing CD4+ T-cell count (43).

The study in Chinese adult HIV ART-naïve patients showed that the prevalence of thrombocytopenia was 3.8%, 7.1%, 3.3%, and 2.8% among patients with CD4 cell counts of <50, 50–199, 200–349, and >350 cells/mm3, respectively (44).

A cross-sectional study was conducted on 100 confirmed HIV positive patients in Iran. According to this study all included patients were at AIDS stage with CD4+ lymphocyte counts ≤ 200cells /µl. The respective mean values for hemoglobin was 11.9 ± 2.6 vs 12.3 ± 2.6g/dl and RBC vale was $4.6\pm3.1\times10^6$/µl and $3.9\pm0.8\times10^6$/µl at baseline and after at least six months of receiving antiretroviral therapy (45).

The study at Dr. Ram Manohar Lohia Hospital, in New Delhi India, showed that Anemia was prevalent in 40.1%, with slightly higher prevalence in those not receiving ART. High frequency of anemia was observed in patients with immunological (CD4<200 cells µl; 42.05%) and clinical AIDS (70.58%) compared with those who had an asymptomatic HIV infection with CD4 > 200/µl (28.57%). Thrombocytopenia was seen in 3.74% patients, Leucopenia was observed in 5.88% in ART and Pancytopenia was found in 1.6% patients (46).

Another study conducted in India showed that the prevalence of thrombocytopenia before initiation of Zidovudine was 16.6% which rises to 30% after initiation of Zidovidine. Immune mediated destruction of both platelets and megakaryocytes occurs in Zidovudine therapy. Moreover, the prevalence of anemia before Zidovudine therapy was 11.7% and after initiation of Zidovidine it was increased to 29.7%. Pancytopenia was treatable in 70% of the patients, who fully recovered from cytopenia. Death occurred in 20% of the cases, which was due to severe pancytopenia and overwhelming infections (47).

The study in South Africa included 15,646 adult patients with a median follow-up time of 710 and 624 days when restricting analyses to time under viral suppression. Median CD4 cell count at ART initiation was 98 cells/µl. Patients presenting with TB at ART start gained about five CD4 cells more per six months than patients without TB (32).

In a cross sectional study done in Ghana, Owiredu *et al* studied the prevalence of anemia and immunological markers among Ghanaian HAART-naïve HIV-patients (n=276) and those on HAART (n=166). The prevalence of anemia in HAART naïve and HAART receiving patients were 63% and 46%, respectively. The likelihood of developing microcytic hypochromic anemia in HAART-naive patients was five times more compared to those on HAART (48).

Different studies from Nigeria demonstrated the effect of HAART of Hematological parameters. For example, the study conducted in Ido-Ekiti, Nigeria by Ayodele *et al* between August 2013 and February 2014 showed that Neu (%) before and after HAART of mean± SD were 41.53± 12.87 and 51.52± 15.49, respectively (2). The study in Northern Nigeria on the other hand showed that 6.0% of study participants had platelet count of less than 100.0×10^9/L "thrombocytopenia, 6.5% patients had total WBC of less than 3.0×10^9/L ("leukopenia"). They also reported a median absolute lymphocyte count of 1.92×10^9/L (49). Moreover, another study from the same country showed that HAART treatment has the capability of increasing the concentration of hemoglobin. In this study by Amegor *et al*, subjects on HAART showed an increase in hemoglobin concentration from 10.30 ± 1.17g/dl to 10.44 ± 0.17g/dl (50).

On the other hand, in a study from Nigeria which investigated TB/HIV co-infected patients, the majority (72.75%) were on zidovudine (AZT) based first line ART regimens. The median absolute CD4 count at ART initiation was 300 cells/µL with 30.72 % having absolute count less

than 200 cells/µL. Those with TB/HIV co-infection tend to have statistically significant shorter time to failing first line ART regimen compared to those with HIV infection alone. This study signifies the effect of TB co-infection in enhancing antiretroviral treatment failure for first line drugs (6).

The study conducted in Cameroon by Wankah *et al* revealed that anemia was the most frequent hematological abnormalities occurring in 62.9% of HAART naïve HIV infected patients (41). More or less similar trend in the prevalence of anemia was reported by a study conducted in Kinshasa, DR Congo. The study showed that anemia was a regular finding in 69% of patients. This anemia was mostly moderate (92.2%) and with iron deficiency pattern accounting 48% (51).

In Dar es Salaam, Tanzania, overall, 750 females and 1693 males participated of whom respectively 49% and 24% were co-infected with HIV-1. Hemoglobin levels were significantly lower in females than in males and in HIV-positive than in HIV-negative TB positive participants. HIV co-infection in this antiretroviral-naïve TB positive population was also associated with severe anemia (hemoglobin < 85 g/l) in both women (prevalence ratio [PR] = 2.07, 95%CI 1.65–2.59) and men (PR 3.45, 95%CI 2.66–4.47) (52).

Munyazesa *et al*. in their study in Rwandan women showed that, neutropenia observed in HIV-infected women was of higher prevalence in women with CD4 lymphocyte count <200 cells/µl. Similarly, baseline neutropenia, defined as <2000 cells/mm3, was noted in 44% of women participants and a longitudinal analysis found that worsening of HIV disease was associated with subsequent neutropenia (21).

A study conducted by Taremwa *et al* assessed the HIV related thrombocytopenia among clients at Mbarara regional referral hospital, in southwestern Uganda. The study reported that the prevalence of thrombocytopenia was 17.8% among antiretroviral HAART-naive and was 13.0% for clients who were on ART for up to 6 months (53).

The study in Coastal Region of Kenya showed that overall 210/500 (42%) of the suspects had Mycobacterial disease and 78/210 (37.1%) were HIV infected. Most of the participants 53.8% were females and 46.2% males. Age (OR=2.06; 95% CI: 1.1-3.00; p<0.003), baseline CD4 count

(OR=3.23; 95% CI: 1.23-8.34; p<0.0001) and WHO clinical stage at presentation (OR=3.84; 95% CI: 1.45-7.63; p<0.003) remained significant predictors after adjustment for confounding factors. Increase of CD4+T cell counts was observed for all patients under HAART and TB treatment (54).

Available literatures from Ethiopia also indicated improvement of HIV associated cytopenias after HAART intake. A retrospective study was conducted by collecting data from antiretroviral clinic of University Hospital of Gondar, Ethiopia in 2013. The result indicated that the mean hemoglobin value before HAART initiation was 13.65 ± 2.14 g/dl and after HAART initiation was 14.04 ± 1.75 g/dl (42).

The study conducted at Gondar, Ethiopia reported that the prevalence of thrombocytopenia was 4.1% in patients on HAART and 9% in HAART naive patients. The hemoglobin concentration after six months of HAART initiation was higher compared to the HAART naïve counterparts (14 ± 1.6 g/dl vs 13 ± 2.1g/dl). The prevalence of normocytic normochromic anemia before HAART initiation was 48.9% and after six months of HAART initiation, it was 29.4% (55).

The hematological profiles of TB patients in Northwest Ethiopia showed statistically significant difference in hematocrit (38.5 % versus 35.7 %), hemoglobin (12.7 g/l versus 11.8 g/l) and platelet ($268 \times 10^3/\mu l$ versus $239 \times 10^3/\mu l$) values of patients before initiation of treatment and after completion of the intensive phase of tuberculosis treatment, respectively ($P < 0.05$) (56).

In a separate study at Menelik II Addis Ababa Ethiopia involving 230 HIV infected adults, the prevalence of anemia at baseline was 52.6% before and after ART (37.4%) was significantly decreased ($p < 0.05$) (57). Other studies also reported a different prevalence rate of anemia. A study from Hawassa, Ethiopia reported anemia prevalence of 23.4% before ART and 12.0% after ART (58)

The study conducted at Jimma, Ethiopia reported that the mean RBC count was $4.95 \pm 0.57 \times 10^6/\mu l$ for HAART naïve HIV patients and $4.14 \pm 0.07 \times 10^6/\mu l$ for HIV patients on HAART; the prevalence of anemia was 29.9% before and 16.2% after HAART (59).

The Study in Addis Ababa, Ethiopia reported that prevalence of anemia at the baseline was 42.9 % with 79%, 15.6% and 5.3% having mild, moderate and severe anemia, respectively. The study

also added that at 12 months of HAART initiation the prevalence of anemia was reduced to 14.3 % (60).

The study done at Menelik II Hospital, Ethiopia showed that mean CD4 cell count of study subjects were 112 ± 67 cells/μl at baseline and significantly increased after ART and found to be 211 ± 120 cells/μl ($p < 0.05$) (57). Retrospective study was conducted at Zewditu memorial hospital, Ethiopia to assess immunological response among HIV-infected individuals receiving highly active antiretroviral therapy (HAART). The result indicated that there was a good immune recovery at the 6th month of therapy from the baseline mean CD4+ T cell count of 81 cells/μl to 191.65 cells /μl, which was statistically significant and other study done in Zewditu Memorial hospital showed CD4+ T cell count of 116 ± 69.4 to 236 ± 120 cells /μl at baseline and after initiation of ART (61, 64).

In sum, as reviewed above different studies revealed varying rates of cytopenias in HIV as well as TB/HIV co-infected patients, which improved after HAART. Generating more data from different settings would help understand the effect of HAART with and without co-infections so as to improve patient management. As far as my literature search goes no such published study is available in Menelik II Referral Hospital addressing co-infection with TB and this study tried to provide updated data on this.

3. OBJECTIVES

3.1 General Objective

- To assess the Immuno-Hematological changes among HIV and TB/HIV co-infected patients taking HAART for at least six months in Menelik II Referral Hospital, Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To determine the changes that occurs in the Immuno-Hematological parameters among HIV patients taking HAART for at least 6 months.
- To describe the Immuno-Hematological parameters among TB/HIV co-infected patients taking HAART for at least 6 months.
- To assess the effect of clinical conditions on Immuno-Hematological parameters among HIV patients taking HAART for at least 6 months.

4. HYPOTHESIS

H1: There is a significant difference in Immuno-hematological profiles before and after six months of HAART initiation in HIV and TB/HIV co-infected patients as other studies done in Ethiopia (61).

5. MATERIALS AND METHODS

5.1 Study Area

The study was conducted at Menelik II Referral Hospital, ART clinic. The hospital is a governmental Hospital found in the capital city of Ethiopia, Addis Ababa, which is located at Yeka sub city. It is the first modern-run hospital which was built by Emperor Menelik II in 1906 with only 30 beds and named Menelik II hospital. It is managed under Addis Ababa City Administration Health Bureau. Menelik II Referral Hospital has various professionals that included 59 physicians, 203 nurses, 123 other health professionals, and 250 administrative staffs, making a total of 635 staffs, at the time of this study, to provide care to approximately 150,000 people each year. The Hospital receives referrals from around the country and is under the guidance of the Ethiopian Federal Ministry of Health. In 2007, Menelik II referral Hospital added Medical College which is called Menelik II Medical College. This institution was selected based on the availability of patients from all parts of the country as it is referral and specialized teaching hospital in Ethiopia as well as the ease of access to standardized facilities and sufficient availability of the data. No published updated data is available from this hospital to facilitate evidence based decision. Its ART Clinic has two ART Physicians, seven nurses, two druggists, two data clerks, two case managers and 2 laboratories to give full service for attending 50 – 60 patients per day. There were 2802 ART patients obtained from the hospital medical record at the time of the study.

5.2 Study Design and Period

A prospective and retrospective combined cross sectional health facility based study was conducted to assess hematological and immunological profile in HIV and TB/HIV co-infected patients. The data of volunteering HIV infected and TB/HIV co-infected patients attending the ART unit of Menelik II Referral Hospital before and after 6 months on HAART was collected from February to March 2019.

5.3 Population

5.3.1 Source Population

All patients and data from sero-positive HIV infected patients who visited Menelik II referral hospital medical college, Addis Ababa Ethiopia were the source population.

5.3.2 Study Population

All patients and data from sero-positive HIV infected patients who visited ART unit of Menelik II Referral Hospital Medical College for ART service and fulfilling the eligibility criteria.

5.4 Inclusion and Exclusion Criteria

5.4.1 Inclusion Criteria

- HIV positive adults greater than 18 years old.
- Those who were on ART for at least six months and had follow up.
- Patients having complete CBC and CD4 count at the Baseline (Pre-HAART).

5.4.1 Exclusion Criteria

- Those who were diagnosed as having hematological diseases of any identified cause (including thrombocytopenia and leukemia) before ART initiation and during HAART.
- Patients who were Pregnant at the time of the tudy.

5.5 Study Variables

5.5.1 Dependent Variables

✓ Immuno-Hematological parameters (WBC with differential, RBC, HB, HCT, RBC indices, PLT and $CD4^+$ T Cells)

5.5.2 Independent Variables

✓ Age,
✓ sex,
✓ BMI,
✓ Functional status
✓ regimen type,
✓ WHO staging,
✓ Prophylaxis

5.6 Measurement and Data Collection

5.6.1 Sample Size Calculation and Sampling Method

The sample size (n) was determined using the following statistical formula for single proportion:

$n = \frac{Z^2 P(1-P)}{d^2}$, where n = sample size

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

Z = 95% confident interval (1.96) P = Prevalence rate (0.5)

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = 384$$

Since our study population (N = 2802 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) $= \frac{n}{1+\frac{n}{N}} = \frac{384}{1+\frac{384}{2802}} = 338$

Thus, a total of 338 HIV infected individuals who were enrolled at Menelik II Referral Hospital ART unit were included in the study using convenient sampling method.

5.6.2 Data collection procedure

A WHO standardized data extraction format was used to extract socio-demographic characteristics, clinical information and hematological parameters of the study participants from Menelik II Referral Hospital ART log book and patient follow up cards at baseline. When patients came for their routine follow up, the current hematological parameters and CD4 levels were determined on EDTA whole blood. About 3 ml whole blood was collected in EDTA tubes using vacutainer system.

The two data collectors were trained for one day 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. Data on demographic, clinical including ART regimen type and hematological (WBC, CD4+ T cells, RBC count, Hgb, MCV, MCH, MCHC, and Platelets) values were carefully extracted from ART log book and patient follow up cards by using standardized data extraction format which was prepared based on the information.

5.6.3 Laboratory Analysis

Hematological Analysis using Diru BCC 3000B

The analyzer performs the tests using two different measuring principles such as the electric impedance method for determining WBC, RBC and Platelet data while the colorimetric method for determining the Hemoglobin concentration.

In brief the aspirated sample is diluted by conductive solution and taken to testing unit. The testing unit has a test hole. A pair of positive and negative electrodes exists near the hole. As the cells have the characteristic of a poor conductor, when the cell go through the hole under constant negative pressure, the DC resistant between the electrodes will change, resulting in the formation of a pulse change proportional to the cell volume. A series of electrical pulse is produced when the cell continuously goes through the hole. The number of pulses is equivalent to the cell number through the hole. The pulse amplitude is proportional to the cell volume.

The hemoglobin concentration was determined by Colorimetric Method. In the WBC counting pool, after adding hemolytic agent, RBC dissolve, releasing hemoglobin, which when combined with the hemolytic to form hemoglobin complex which is read at a wavelength of 540nm monochromatic LED in one end of WBC counting pool.

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 PE-Cy™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.

5.7 Data Quality Assurance

To ensure the quality of instrument performance, the hospital laboratory routinely runs controls and preventive maintenance was performed according to standard operating procedures. The hematological and CD4 analyses were done by senior laboratory technician and technologists. Based on an annual onsite assessment performed in 2008 E.C. and 2009 E.C. as per WHO AFRO checklist, Menelik II Referral Hospital Laboratory (MRHL) scored 2 stars, which indicated that the laboratory had a good performance. MRHL also participates in External Quality Assurance (EQA) program which all in all earned it the two stars.

Data quality was ensured through proper training before the start of data collection with an intensive supervision during data collection and exportation into excel by the principal investigator. The quality of data was controlled at different levels for completeness and consistency; first by data collectors at the end of each day, then by principal investigator during data entry every day. Moreover, extracted data from ART log book and patients' follow up card was double checked and whenever errors detected at any level, the principal investigator trace and correct it. Data exportation quality have been maintained by consulting the data manager into Microsoft excel and then to SPSS version 20 software and verify its quality against the collected hard copy data during entry.

5.8 Data Analysis and Interpretation

The data was coded, cleaned, entered and analyzed using SPSS version 20 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. The mean values of hematological parameters of the subjects on AZT based and non AZT based HAART regimen was compared using independent sample t-test. One-way analysis of variance (ANOVA) was used to check the presence of significant difference in hematological values of the subjects across different WHO stage of the disease. 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 significance.

5.9 Ethical Considerations

The study was 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 Addis Ababa Health Bureau from the Department of Medical Laboratory Sciences, the Institutional Review Board of the Bureau also approved the study. Then a letter informing Menelik II Referral 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 current data; no additional sample was collected for the purpose of this study.

5.10 Dissemination of the Result

The result of this study will be submitted to Department of Medical Laboratory Science and presented on thesis defense. The findings will also be submitted to Addis Ababa Health Bureau and Menelik II Hospital. The findings will be presented at national and international conferences and will be sent for publication on peer reviewed journals.

5.11 Operational Definitions

Anemia: hemoglobin concentration of <13g/dl for male and <12g/dl for female.

Antiretroviral Therapy (ART): therapy designed to suppress the progression of HIV/AIDS consisting of double or triple combination.

Co-Infection: a person living with more than one infection at a time. In the present study TB is a coinfection.

Immuno-Hematological Parameters: Include White blood cells, Red blood cells, Hemoglobin, Hematocrit, Red blood cell indices, Platelets and CD4+ T cells

Immunodeficiency (Immunosuppression): in this study refers to CD4+T lymphocyte counts < 350 cell/µl

Leucopenia: total WBC count of less than 4000cells/µl.

Lymphopenia: Total lymphocyte count of less than 1000cells/µl. (<20%)

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.

Immunosuppression severity was defined based on the CD4 count as per the WHO guideline. Accordingly a CD4 count <200, 200-349 and 350-499cells/ µl were considered as severe immunosuppression, advanced immunosuppression and mild immunosuppression respectively.

Normocytic anemia: anemia with MCV value of 80-100fl.

Microcytic anemia: anemia with MCV value of <80fl.

Macrocytic anemia: anemia with MCV value of >100fl.

Normochromic anemia: anemia with MCHC value of 27-31g/dl

Hypochromic anemia: anemia with MCHC value of <27g/dl

Neutropenia: Absolute neutrophil count less than 1500cells/µl (<40%)

Thrombocytopenia: A platelet counts of less than 150,000cells/µl

6. RESULTS

6.1. Sociodemographic Characteristics

A total of 338 HIV infected individuals who were at least 6 months on HAART were included in the present study. Their ages ranged from 18 to 75 years with a mean age of 38.58 ± 10.07 years and a median age of 37 years. Majority (42%) of the age groups were between 30 to 39 years. Out of the 338 patients, 59.2 % were females giving a female to male ratio of 2 to 1.38.

The result of body mass index (BMI) of the patients at the time of study indicated that 71.6% of them had 18.5 – 24.9Kg/m^2 and 3.8 % of them had a high BMI value (≥25kg/m2). The nutritional status of the patients as recorded in their follow up card indicated that 253 (74.9%) were stated as normal and 1(0.3%) is recorded as having severe malnutrition. The functional status of the patients during the study time were 299(88.5%) working and 5(1.5%) bedridden (Table1).

Table 1: Sociodemographic characteristics of HIV patients taking HAART for at least 6 months at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

Variables		Frequency	Frequency (%)
Age(years)	<20 (18-20)	4	1.2
	20-29	50	14.8
	30-39	142	42.0
	40-49	93	27.5
	50-59	42	12.4
	≥60	7	2.1
Sex	Male	138	40.8
	Female	200	59.2
Nutritional status	Normal	253	74.9
	Mild	63	18.6
	Moderate malnutrition	8	2.4
	Severe malnutrition	1	.3
	Over weight	13	3.8
BMI (Kg/m^2)	<18.5	84	24.9
	18.5-24.9	241	71.3
	≥25	13	3.8
Functional status	Ambulatory	34	10.1
	Bedridden	5	1.5
	Working	299	88.5

6.2. Clinical Characteristics of the Study participants

Of the total of 338 HIV infected individuals included in the present study, 83 (24.6) patients were co-infected with TB. From TB/HIV co-infected patients 46(55.4%) were females and 37(44.6%) were males.

Based on WHO clinical staging at the study time, more than half of them were in stage I, 192 (56.8%), stage III were 66 (19.5%), stage II, 60 (17.8%) and stage IV 20(5.9%). Regarding the eligible reasons to start ART, all HIV seropositive patients who were voluntary to start were eligible. The most widely used ART regimen were 1e, TDF-3TC-EFV, 170(50.3%) followed by 1c, AZT-3TC-NVP 54 (16%) and 1f, TDF-3TC-EFV, 54 (16%). According to the duration of use of HAART, majority of the participants (60.9%) took the treatment for more than 3 years. At the time of the study, 293(86.7%) of them have taken cotrimoxazole as a prophylaxis (Table 2).

Table 2: Clinical characteristics of HIV and TB/HIV co-infected patients taking HAART for at least 6 months at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

Variables		**Frequency**		**Frequency (%)**	
Status	HIV only	255	F=154	75.4	60.4
			M=101		39.6
	TB/HIV	83	F=46	24.6	55.4
			M=37		44.6
WHO Staging	Stage I	192		56.8	
	Stage II	60		17.8	
	Stage III	66		19.5	
	Stage IV	20		5.9	
ART Regimen	1c	54		16.0	
	1d	34		10.1	
	1e	170		50.3	
	1f	54		16.0	
	Others	26		7.7	
Duration of use of HAART(In Months)	6-11	22		6.5	
	12-17	13		3.8	
	18-23	25		7.4	
	24-29	29		8.6	
	30-35	43		12.7	
	≥36	206		60.9	
Prophylaxis	cotrimoxazole	293		86.7	
	no prophylaxis	45		13.3	

Key: 1c= AZT-3TC-NVP, 1d= AZT-3TC-EFV, 1e=TDF-3TC-EFV, 1f=TDF-3TC-NVP
F-Female, M-Male

6.3 Immuno-Hematological parameters of the study participants

6.3.1 Immunological parameters

The study participants had a baseline CD4 count ranging from 10-781 cells/ µl and median (IQR) of 144(85.75- 218.75) cells / µl. The mean CD4 count at the baseline was 164.6 ± 114.6 cells/ µl. After at least 6 months of HAART, the CD4 count showed an increment, to a mean (±SD) of 317.1 ± 175.22 cells / µl ($p<0.001$) and a median (IQR) of 287 (199 – 392.25) cells / µl. Figure 1 displays the box plot of CD4 count at baseline and after at least 6 months of HAART initiation (Detail is shown in Table 3).

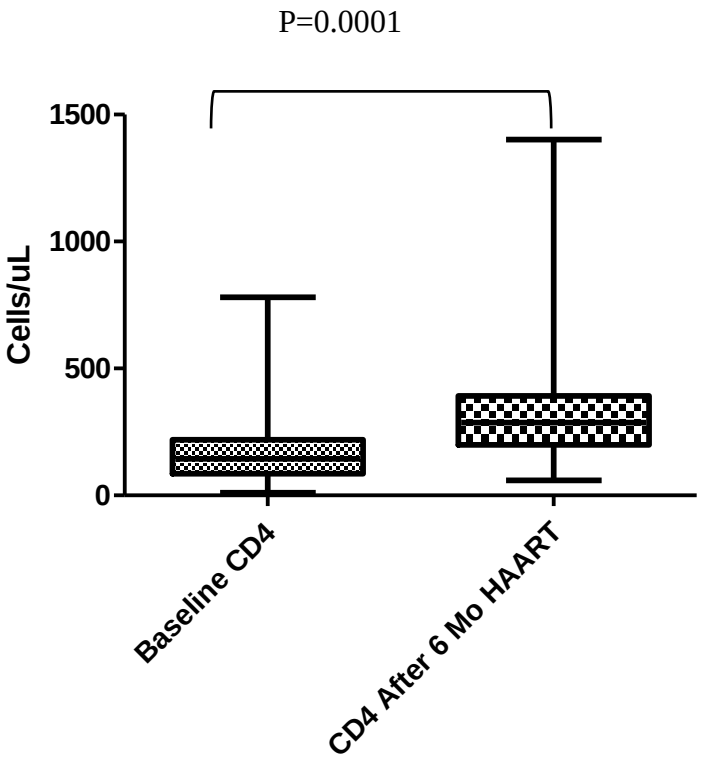

Figure 1. CD4 count at baseline and at least 6 months after initiation of HAART at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019 (n=338).

6.3.2 Hematological Values before and after initiation of HAART

As shown in table 3, the mean WBC count, % Neutrophil (RNC) (%Neu) and % Lymphocytes (RLC) (% Lym) were slightly increased and found to be 5.89± 2.81 $x10^3$ /µl, 58.12 ± 14.66% and 30.91 ± 12.86%, respectively at the baseline. These WBC parameters were 6.49 ± 2.95 $x10^3$ /µl, 58.28 ± 14.15% and 30.93 ± 13.19%, respectively, after at least 6 months of receiving HAART. The median (IQR) of WBC, RLC and RNC were 5.37 (4.09 – 7.01) $x10^3$ /µl, 59.8 (48.6 – 66.85) % and 29.55 (22.9 – 38.7) %, respectively at the baseline and 5.9(4.22 – 7.87) $x10^3$ /µl, 57.7 (47.88 – 68.43) % and 31.1 (21.0 – 38.33) %, respectively, after at least 6 months of HAART initiation (Table 3). The increments in the mean values of WBC, RNC and RLC after at least 6 months of HAART initiation were statistically significant ($p < 0.001$).

Mean RBC count were 4.35 ± 0.80×10^6/µl at the baseline and 4.25 ± 0.73×10^6/µl after at least 6 months of receiving HAART. The mean Hgb concentration and hematocrit were increased and found to be 13.07 ±2.48 g/dl and 38.87 ±6.86% at the base line and 13.76 ± 2.36g/dl and 40.47 ±6.57 after six months of HAART. The changes were statistically significant ($P<0.001$)

Regarding the RBC indices, baseline mean values of MCV, MCH and MCHC were 89.91 ± 10.51fl, 30.25 ±4.05pg and 33.76 ± 2.47g/dl, respectively. After 6 months of HAART initiation, the values were 96.13 ± 10.37fl, 32.69 ± 4.27pg and 33.88 ± 2.29g/dl, respectively. Hgb, MCV, MCH and MCHC were higher after 6 months of HAART initiation compared to the baseline while RBC count showed decrement in their mean values. All were significant with p value < 0.001 except MCHC which was $p>0.05$.

The mean platelet count of the study participants were 245.01 ± 101.19×10^3/µl at the baseline and 265.17 ± 105.63×10^3/µl after 6 months of HAART initiation. The increment of platelet count was significant with p value < 0.001 (Table 3).

Table 3: Immuno-Hematological values before and after initiation of HAART for at least 6 months at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

Parameters	Before initiation of HAART (n= 338)		After six months of HAART initiation (n= 338)		*p-value
	Mean ± SD	Median(IQR)	Mean ± SD	Median(IQR)	
WBC($\times 10^3/\mu l$)	5.89±2.81	5.34(4.09-7.03)	6.49±2.95	5.87(4.23-7.86)	0.001
NEU(%)	58.12±14.66	59.65(48.6-66.8)	58.28±14.15	57.7(47.9-68.4)	0.001
LYMP(%)	30.91±12.86	29.55(22.8-38.67)	30.93±13.19	31.1(21.05-38.3)	0.001
MIXED(%)	11.12±5.86	10.05(7.14-13.93)	10.99±5.12	10.19(7.38-13.83)	0.001
RBC($\times 10^6/\mu l$)	4.35±0.80	4.31(3.85-4.84)	4.25±0.73	4.27(3.8-4.71)	0.001
HB(g/dl)	13.07±2.48	13.08(11.59-14.76)	13.76±2.36	13.96(12.467-15.18)	0.001
HCT(%)	38.87±6.86	38.65(35.17-43.2)	40.47±6.57	41.07(37-44.6)	0.001
MCV(fl)	89.91±10.51	88.93(82.27-95.6)	96.13±10.37	95.47(89.6-102.68)	0.001
MCH(pg)	30.25±4.049	29.97(27.9-32.33)	32.69±4.27	32.3(29.99-35.46)	0.001
MCHC(g/dl)	33.76±2.47	33.9(32.43-35.03)	33.88±2.29	34.07(32.66-35.45)	0.330
PLT($\times 10^3/\mu l$)	245.01±101.19	237.5(183.3-301)	265.18±105.63	247.67(197.14-318)	0.001
CD4(Cells/μl)	164.57±114.61	144.5(86-219.5)	317.1±175.22	287.14(199-392)	0.001

*P values using paired student t-test for mean

6.3.3 Immuno-hematological values with respect to clinical characteristics

Immuno-Hematological values by AZT based therapy status

Independent t-test was used to compare the mean hematological values based on type of ART. As shown in table 4, patients on AZT based therapy showed lower mean values of WBC, RNC, RBC, MCHC, platelets and CD4 counts compared to patients who were on non AZT based therapy while HGB, HCT, MCV, MCH and RLC showed decrement in their mean values among patients on non AZT based therapy compared to those on AZT based therapy. The difference was statistically significant for MCV and MCH only where individuals on AZT based therapy had higher values (Table 4).

Table 4. Mean Immuno-Hematological values by type of HAART regimens in HIV infected individuals attending ART clinic of Menelik II Referral Hospital, Addis Ababa, Ethiopia, 2019.

Hematological parameters	**Types of HAART regimen**		
	AZT based (n=114)	**Non AZT based (n=224)**	**P- value**
WBC($\times 10^3/\mu l$)	6.09±3.15	6.69± 2.82	0.929
RNC (%)	55.73± 13.85	59.57± 14.15	0.901
RLC (%)	32.75± 13.32	30.0±13.06	0.647
RBC ($\times 10^6/\mu l$)	4.16±0.73	4.30±0.73	0.714
Hgb (g/dl)	13.78±2.31	13.75±2.38	0.437
HCT (%)	40.59±6.55	40.41±6.59	0.893
MCV (fl)	98.39±11.92	94.98±9.31	0.001*
MCH (pg)	33.41±4.85	32.33±3.90	0.004*
MCHC (g/dl)	33.86± 2.43	33.9±2.22	0.872
PLT ($\times 10^3/\mu l$)	257.66±101.20	269.0± 107.83	0.281
CD4(Cells/µl)	297.87±152.34	326.88±185.34	0.289

Key: AZT= Zidovudine, ART = Antiretroviral therapy

*P values using Independent t-test

Immuno-Hematological values by current WHO clinical staging status

One-way ANOVA was used to compare the mean values of Immuno-Hematological parameters among the different stage of HIV/AIDS. As shown in Table 5a, as the disease progresses to the advanced stage (Stage I to stage IV); the mean values of each Immuno-Hematological parameter were decreasing. The observed difference, however, was statistically significant for CD4 counts ($p<0.05$) only. The study also tried to investigate what the baseline Immuno-Hematological parameters of these participants look like in the four stages. As shown in Table 5b, the pattern was similar except that the difference in RBC decline across the stages was significant in addition to CD4 count ($P<0.05$).

Table 5a. Mean Immuno-Hematological values by WHO staging at the time of the study among HIV infected individuals attending ART clinic of Menelik II Referral Hospital, Addis Ababa, Ethiopia, 2019.

Hematological parameters	**WHO stages**				**F test, P - value**
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	
WBC($\times 10^3/\mu l$)	6.71 ±3.0	6.31 ±3.16	6.12 ±2.62	6.06 ±2.81	0.916, 0.433
RNC (%)	58.91 ±15.01	56.94 ±12.71	57.64 ±12.08	58.29 ±13.88	0.349, 0.790
RLC (%)	30.32 ±13.52	32.13 ±12.25	31.56 ±13.05	31.09 ±13.89	0.354, 0.786
Mixed (%)	10.4 ±5.14	12.09 ±5.14	11.88 ±5.03	10.46 ±4.5	2.562, 0.055
RBC ($\times 10^6/\mu l$)	4.3±0.74	4.25±0.65	4.21 ±0.82	3.99 ±0.5	1.228, 0.300
Hgb (g/dl)	13.84 ±2.4	13.81 ±2.2	13.67 ±2.5	13.19 ±1.8	0.510, 0.676
HCT (%)	40.7 ±6.85	40.91 ±6.11	40.14 ±6.69	38.35 ±7.45	0.920, 0.431
MCV (fl)	95.4 ±10.31	96.86 ±10.8	97.47 ±10.48	96.5 ±9.29	0.796, 0.497
MCH (pg)	32.42 ±3.87	32.9 ±4.64	33.11 ±4.9	33.27 ±4.6	0.642, 0.588
MCHC (g/dl)	33.96 ±2.1	33.69 ±2.5	33.72 ±2.61	34.27 ±2.33	0.505, 0.679
PLT ($\times 10^3/\mu l$)	265.15 ±109.7	273.5 ±106.5	265.86 ±97.5	238.2 ±90.56	0.558, 0.643
CD4(Cells/μl)	338.76 ±191.6	303.76 ±133.5	290.13 ±168.8	238.05 ±90.2	3.025, 0.030

Table 5b. Mean Immuno-Hematological values at baseline by current WHO staging among HIV infected individuals attending ART clinic of Menelik II Referral Hospital, Addis Ababa, Ethiopia, 2019.

Hematological parameters	**WHO stages**				
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**F test, P - value**
WBC($\times 10^3/\mu l$)	5.98 ±3.1	5.92 ±2.74	5.77 ±1.99	5.24 ±2.46	0.467, 0.705
RNC(%)	57.48 ±14.75	57.8 ±13.48	59.0 ±15.67	62.27 ±13.88	0.745, 0.526
RLC(%)	31.94 ±13.48	30.21 ±10.45	29.6 ±12.84	27.41 ±13.09	1.201, 0.309
Mixed(%)	10.8 ±5.5	12.05 ±6.7	11.47 ±6.3	10.35 ±4.5	0.894, 0.444
RBC ($\times 10^6/\mu l$)	4.44 ±0.83	4.43±0.8	4.25 ±0.67	3.94 ±0.9	2.595, 0.045*
Hgb (g/dl)	13.25 ±2.4	13.19 ±2.6	12.76 ±2.4	12.09 ±2.88	1.779, 0.151
HCT (%)	39.31 ±6.6	39.23 ±7.7	38.27 ±6.3	35.55 ±7.75	2.071, 0.104
MCV (fl)	90.31 ±10.4	87.9 ±10.24	90.2 ±11.8	91.12 ±11.8	0.930, 0.426
MCH (pg)	30.33 ±3.9	29.67 ±4.2	30.39 ±4.28	30.80 ±4.05	0.584, 0.626
MCHC (g/dl)	33.8 ±2.97	33.68 ±2.17	33.48 ±2.97	34.5 ±2.17	0.911, 0.436
PLT ($\times 10^3/\mu l$)	240.95 ±99.85	243.52 ±85.95	260.62 ±113.3	236.9 ±11575	0.672, 0.570
CD4(Cells/μl)	176.45 ±114.8	162.83 ±83.4	148.88 ±139.4	107.45 ±81.3	2.805, 0.040*

6.3.4 Immuno-Hematological values before and after initiation of HAART in TB/HIV co-infected patients

Out of the total participants, 83 of them (24.6%) had TB/HIV co-infection. The female proportion accounted 46(55.4%. When data was analyzed for this group separately, none of the RBC parameters showed significant change except MCV which increased at least 6 months after HAART. 89.58±9.87 vs 92.64±8.63fl (P=0.035). The mean WBC count and RLC were slightly increased while the platelets were slightly decreased, though it reached a statistically significant level (P<0.05). As it is shown in table 6, the mean WBC count and %RLC were 5.83± 3.1 $x10^3$ /µl and 27.34 ± 12.96%, respectively at the baseline and 6.62± 3.15 $x10^3$ /µl and 29.84 ± 14.19%, respectively after at least 6 months of receiving HAART. The mean values of %RNC and %Mixed were instead decreased and found to be 61.64 ± 15.65% and 11.17 ± 7.17%, respectively at the baseline and 59.92 ± 15.65% and 10.16 ± 5.13% respectively after at least 6 months of receiving HAART.

The mean CD4 count of the study participants were 149.58 ± 97.43×10^3/µl at the baseline and 294.72 ± 158.38×10^3/µl after 6 months of HAART initiation. The increment of CD4 count was significant with p value < 0.001 (Table 6).

Table 6: Mean Immuno-Hematological Values before and after initiation of HAART in TB/HIV co-infected patients at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

Parameters	**Before initiation of HAART (n= 83) Mean ± SD**	**After six months of HAART initiation (n= 83) Mean ± SD**	**p-value**
WBC($\times 10^3/\mu l$)	5.83±3.1	6.62±3.15	0.023
NEU (%)	61.64±15.65	59.92±16.72	0.001
LYMP (%)	27.34±12.96	29.84±14.19	0.001
MIXED (%)	11.17±7.17	10.16±5.13	0.019
RBC($\times 10^6/\mu l$)	4.22±1.05	4.14±0.81	0.155
HB(g/dl)	12.25±2.75	12.91±2.57	0.180
HCT(%)	37.24±7.69	38.22±7.44	0.201
MCV(fl)	89.58±9.87	92.64±8.63	0.035
MCH(pg)	29.54±3.77	31.17±3.71	0.131
MCHC(g/dl)	33.13±2.36	33.74±2.21	0.270
PLT($\times 10^3/\mu l$)	264.13±118.46	262.89±103.6	0.001
CD4(Cells/μl)	149.58±97.43	294.72±158.38	0.001

*Mean P values using paired student t-test

6.4 Prevalence of immuno-hematological abnormalities by infection status

6.4.1 Prevalence of immuno-hematological abnormalities in the study participants

Immuno-hematological abnormalities were assessed both before and after treatment with HAART. Common abnormalities found in this study were presented in Figure 2. Accordingly, the prevalence of Anemia significantly decreased from 39.9% to 14.5%, Thrombocytopenia from 16.9% to 9.8% and Leucopenia from 22.2% to 17.8% after at least 6 months on HAART. Immunosuppression (CD4<350 cells/µl) was also decreased from 98.5% to 88.5%. On the other hand, lymphopenia increased from 31.4% to 35.2% following HAART (Figure 2).

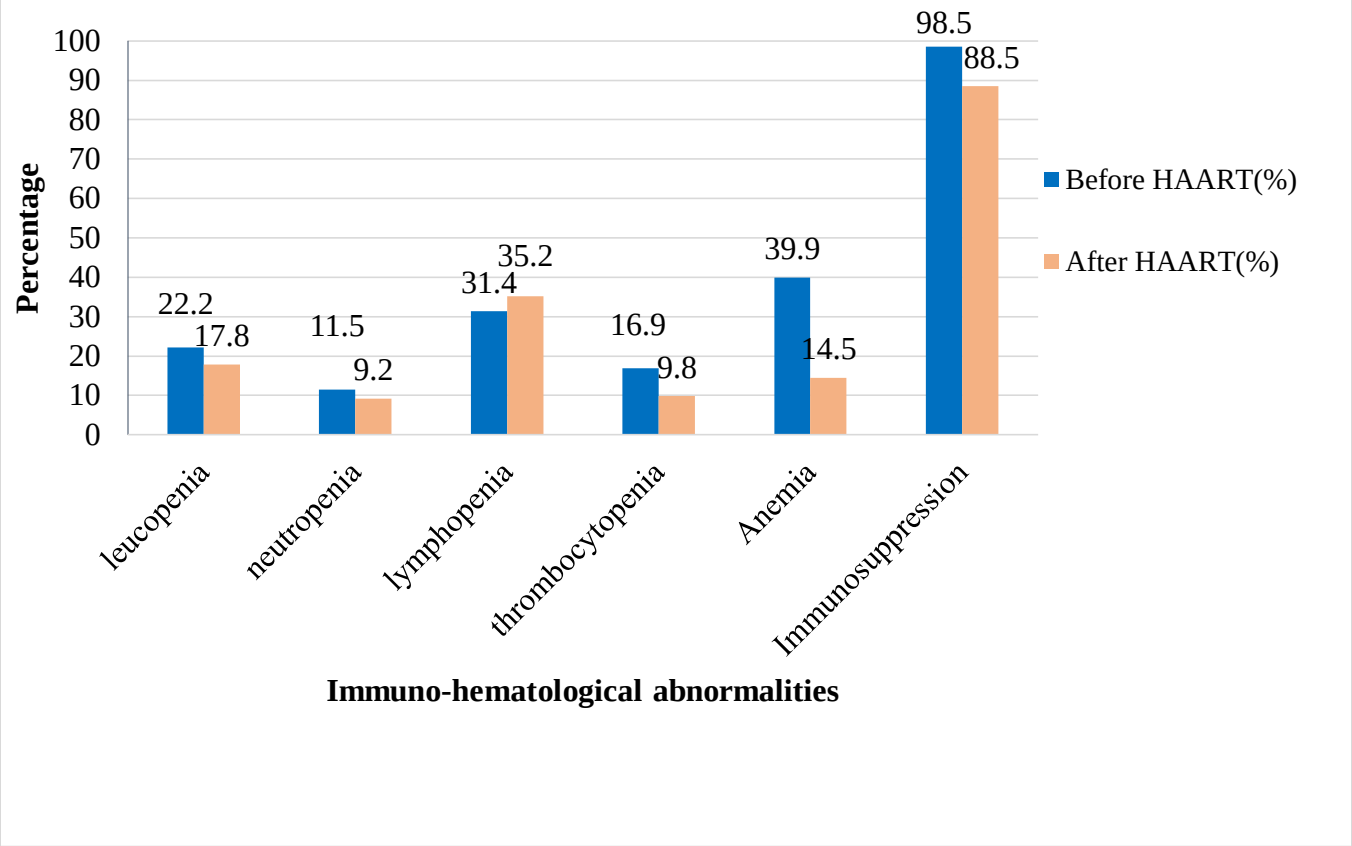

Figure 2: Immuno-hematological abnormalities at baseline and after 6 months of HAART at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019 (n=338).

Anemia was further characterized by severity at baseline and at the last visit. Among the anemic cases, the majority had mild anemia at the baseline which accounts 55.6%. This study also found moderate anemia in 37.8% of the anemic cases and severe anemia in 6.8% of the cases at baseline and the proportion remained almost same after 6 months of HAART as depicted in Figure 3.

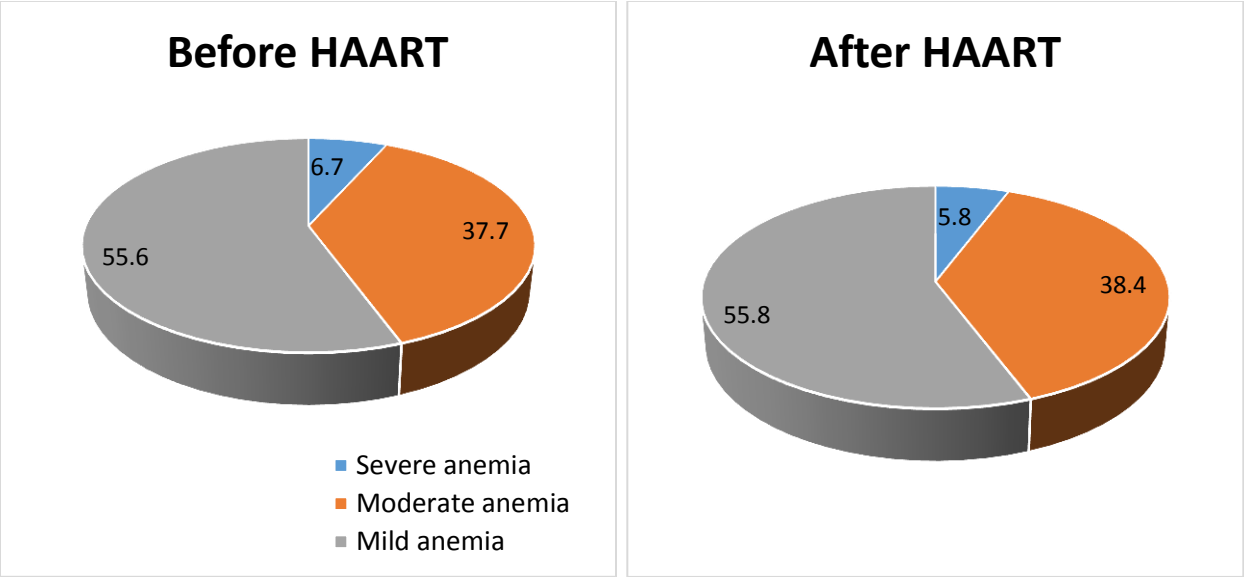

Figure 3: Anemia by Severity at baseline (n=135) and after 6 months (n=49) of HAART at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

Based on the red cell indices anemia was characterized as shown in Figure 4. From the total anemic HIV infected individuals at the baseline, 55.6% had normocytic-normochromic anemia followed by microcytic-normochromic 20.7% and normocytic-hypochromic anemia 11.1%. After six months of HAART, normocytic-normochromic anemia was present in 48.8% of the cases. Of interest, macrocytic-normochromic anemia increased from 8.9% at baseline to 18.6% at least 6 months on HAART (Figure 4).

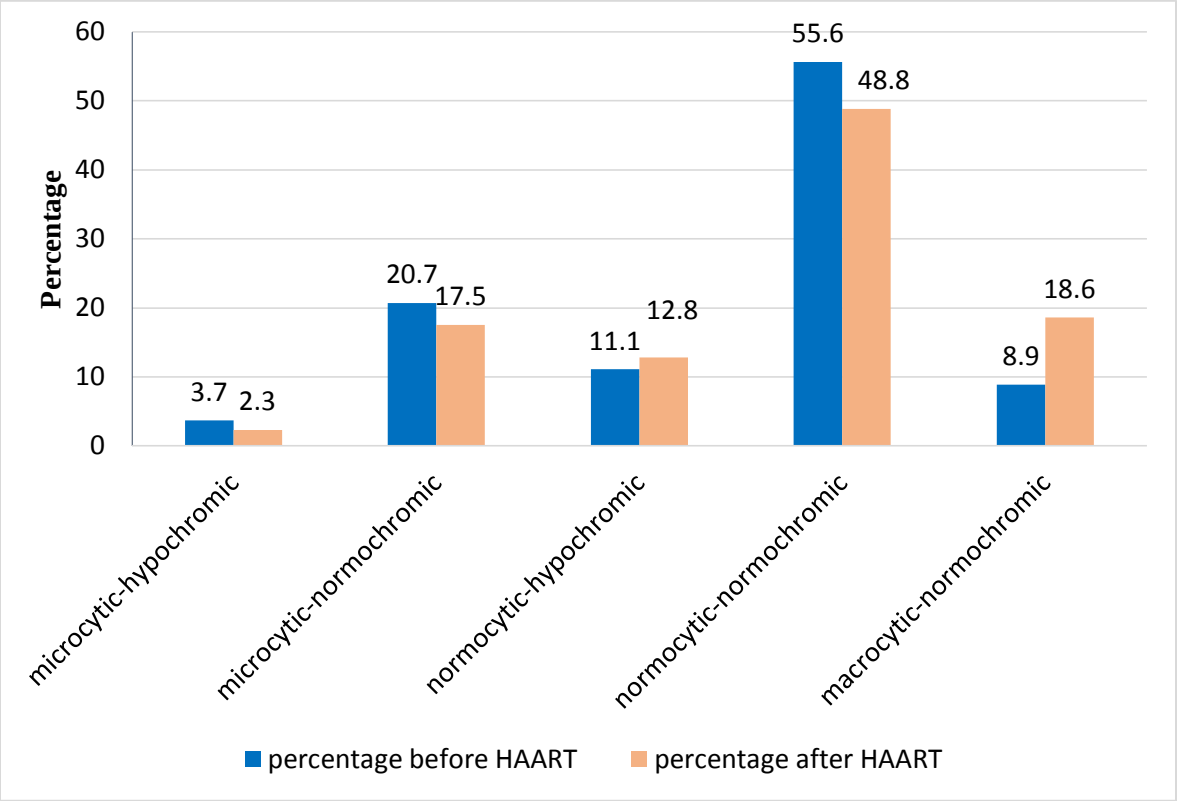

Figure 4: Summary of the types of anemia at baseline (n=135) and after 6 months of HAART (n=49) at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

When degree of Immunosuppression was assessed, majority of the individuals at the baseline were severely immunosuppressed as defined by CD4<200 cells/ µl (69.95%). Whereas 47.4% of the individuals had advanced immunosuppression (CD4 count 200-349 cells/ µl) after HAART initiation. After at least 6 months of HAART initiation, the degree of severe immunosuppression was decreased from 69.95% to 28.81%; the observed difference was statistically significant (p value < 0.01) (Figure 5).

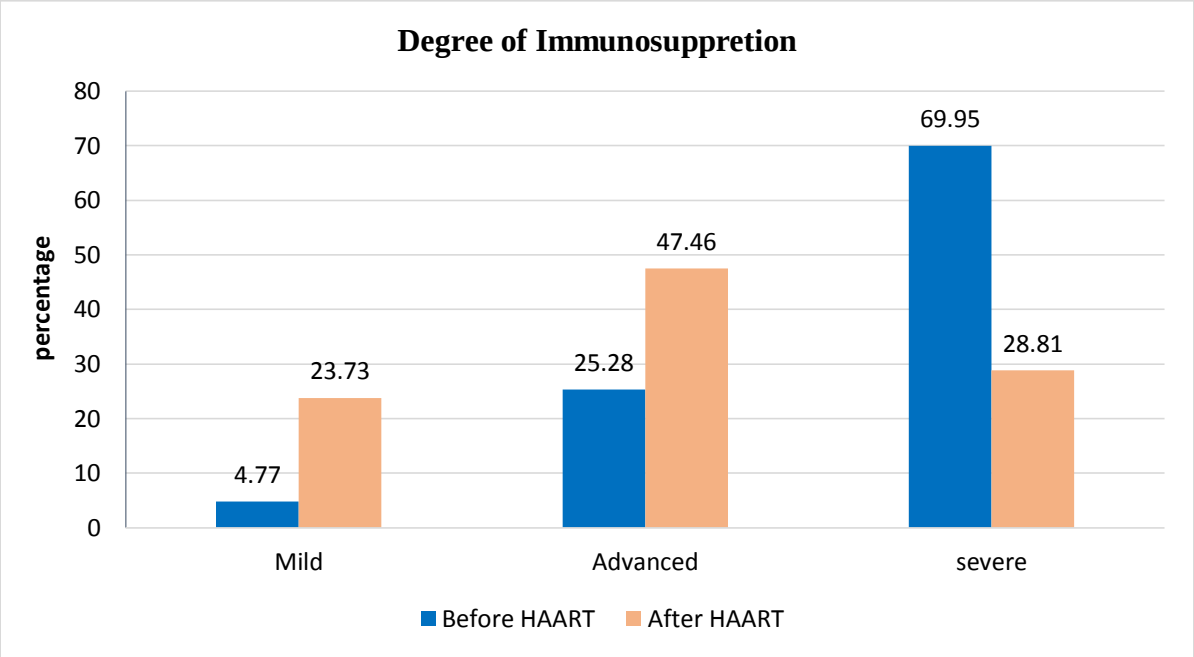

Figure 5: Degree of immunosuppression at baseline and after 6 months of HAART at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

6.4.2 Magnitude of immuno-hematological abnormalities in TB/HIV co-infected patients

Common Immuno-hematological abnormalities found in this study in TB/HIV co-infected patients were presented in Figure 6. Accordingly; leucopenia was seen in 21.7% and 18.1%, neutropenia 10.8% and 12.1%, lymphopenia in 42.2% and 37.3% and thrombocytopenia in 14.5% and 12.0% of the subjects before and after initiation of HAART on TB/HIV co-infected individuals, respectively.

Anemia was found in 51.8% of subjects at the baseline and 39.8% of the subjects after at least 6 months of HAART in TB/HIV co-infected individuals.

The most common abnormality observed in this study was immunosuppression (CD4 count < 500 cells/mm3) which is found in 98.8% of the individuals at the baseline and 92.8% of the individuals after HAART initiation.

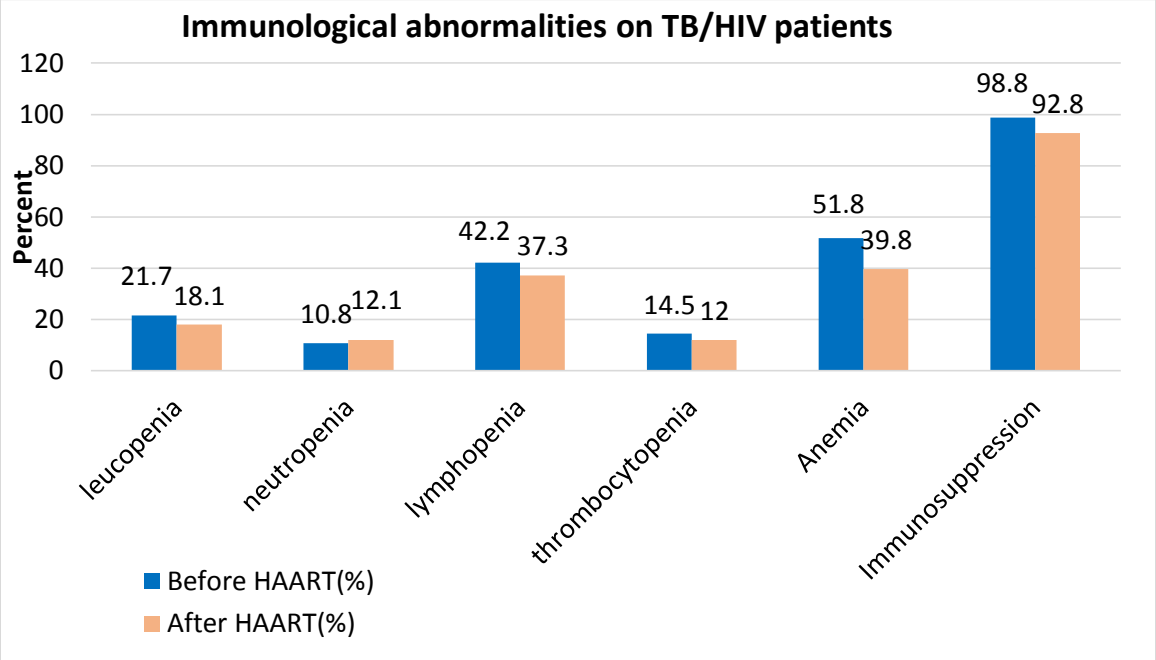

Figure 6: Magnitude of Immuno-Hematological abnormalities in TB/HIV co-infected individuals (n=83) at baseline and after 6 months of HAART at Menelik II Referral Hospital ART unit, Addis Ababa, Ethiopia, 2019.

7 DISCUSSION

This study investigated the Immuno-Hematological profiles of 338 adult HIV positive individuals taking HAART for at least six months at ART clinic of Menelik II Referral Hospital in Addis Ababa. The study will have contribution towards understanding the effect of the drugs on different hematological parameters.

The study has found that six months of treatment with HAART resulted in a significant and favorable change in Immuno-hematological profiles of HIV positive subjects. The result showed that the mean values of each Immuo-Hematological parameter were decreasing when individuals are in the advanced stage (WHO Stage I to stage IV). The observed difference, however, was statistically significant for CD4 counts ($p<0.05$). In this study, even after taking HAART for at least 6 months, as the disease status progresses to the advanced stage (WHO Stage I to stage IV), the mean values of each blood cell counts were lower than the early stage of the disease. Similarly, different studies (40) have reported a lower blood cell counts in the advanced HIV/AIDS stage. This might be due to increase in the frequency of bone marrow abnormalities as the disease progresses.

The present study showed that patients on AZT based HAART regimen had a lower blood cell counts compared to those taking non AZT based HAART regimen. Whereas, MCV and MCH were significantly higher as AZT induces macrocytosis. In agreement with this finding, several studies (47,55) reported that AZT was associated with significant reduction of blood cell counts. This might be due to bone marrow suppression by AZT and also it inhibits the proliferation of blood cell progenitor cell. The effect of AZT is modest when taken as HAART than administered as a single dose (60). Macrocytosis might be due to AZT induced marrow erythroid hypoplasia, aplasia and megaloblastic maturation.

The current finding was corroborated by a finding from St Paul's Hospital in Addis Ababa by Tamir *et al* (62) who demonstrated anemia of Macrocytic-normochromic type in 7.3% at baseline 36.4% at 6 months and 52.6% at 12 months of follow up in those participants taking AZT containing drug. Whereas, in the non-AZT group the respective figures were 3.5%, 13.9%, and 12% at the three time points, signifying the role of AZT in inducing Macrocytosis.

In the current study the mean CD4 count increased from a baseline count of 164.6 ± 114.6 cells/ μl to 317.1 ± 175.22 cells / μl after at least 6 months of HAART. In line with this, statistically significant increment in CD4 count was also reported by a retrospective study from Zewditu Memorial hospital, Ethiopia which assessed immune recovery at the 6th month of therapy from the baseline mean CD4+ T cell count of 81cells/μl to 191.65 cells /μl. The majority of their HIV patients had CD4 cell counts < 200 cells/μl since the CD4 cutoff criteria for antiretroviral treatment eligibility in those days was 200 cells/μl (61).

On the other hand, mean values of CD4 count among TB/HIV co-infected individuals taking HAART at the baseline and after 6 months of HAART was slightly lower than the total participants' values though the improvement was statistically significant (149.58 vs 294.72 cells/ μl). The finding agrees with a study in Coastal Region of Kenya which showed a significant increase of CD4+T cell counts for all patients under HAART and TB treatment (54).

This study showed that 83 of the 338 individuals (24.6%) had TB/HIV co-infection, females accounting 46(55.4%). In agreement with this finding, the study conducted between June 2006 and January 2014 in Amhara Region, Ethiopia showed that from 571 participants, 158(27.7%) had TB/HIV co-infection. The female part accounted 107(69%) compared to male participants 48(31%) (39). A study in Coastal Region of Kenya indicated that in total, 210/500 (42%) of the suspects had Mycobacterial disease and 78/210 (37.1%) were HIV infected and majority 53.8% were females (54). According to a Systematic Review and Meta-Analysis the prevalence of TB/HIV co-infection was 31.25% in African countries (63). The slight difference may be because of method and sociodemographic settings in the systematic review and meta-analysis study.

7.1. Changes in Leucocytes after HAART initiation in HIV and TB/HIV co-infected patients

7.1.1 Changes in Leucocytes after HAART initiation in HIV patients

The increments in WBC count after six months of HAART initiation shown in the current study implies boosting of the immune system of the body (5.89 ± 2.81 vs 6.49 ± 2.95×10^3/μl). In line with the current finding, a retrospective study done at Zewditu Memorial Hospital Addis Ababa, Ethiopia, indicated that during initiation of HAART, the mean ± SD WBC count of HIV infected patients was 4.77 ± 1.82 ×10^3/μl and after 6-month initiation of HAART significantly increased

to 6.41 ± 1.99×10^3/μl (61). In addition, a study done in Nigeria also showed an increment of mean WBC count after HAART initiation (65). The finding was also supported, by another study in Markurdi, Benue state of Nigeria which indicated that ART brings statistically significant increment in WBC from 4.07 ± 0.25 ×10^3/μl at baseline to 4.76 ± 0.15×10^3/μl after ART (50). Increase in WBC count after HAART initiation might be due to increase in hematopoietic progenitor cell growth, decreasing the HIV viral load and decreasing marrow suppression by opportunistic infections (66).

However, in contrast to the abovementioned findings, a decreased in WBC count for HIV patients on HAART compared to those who were not on HAART was reported (42,67). This difference might be due to a short duration of ART 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 (54).

The prevalence of leucopenia in the current study decreased from 22.2% at baseline to 17.8% after at least six months of HAART. Though the proportion of leucopenia is low, a significant reduction has also been reported by a study conducted in Nigeria which described 6.1% prevalence in HAART naïve patients and 1.2% in patients on HAART (66). This difference might be due to a difference in defining the WBC cutoff value for leucopenia.

In the present study, relative neutrophil count (RNC) also showed slightly increment after 6 months of HAART initiation which was also supported by a study conducted by Ayodele Esan and Co-workers (2). The improvements in neutrophil count after HAART initiation might be due to increased production of Granulocyte Macrophage Colony Stimulating Factor(GM-CSF) and Granulocyte Colony Stimulating Factor(G-CSF), which plays important role in the activation of granulocytes (25).

In this study the prevalence of neutropenia declined after six months of HAART initiation (11.5% vs 9.2%). On the other hand, the prevalence of lymphopenia showed significant increment after six months of HAART (31.5% vs 35.2%). A retrospective study done in South Korea showed that the prevalence of neutropenia and lymphopenia were 10% and 25.7% in HAART naïve subjects and 0.89% and 4.17% in HAART initiated patients, respectively (71).

This difference might be due to a difference in defining value for neutropenia and lymphopenia as well as variation in study populations, clinical conditions and study methods.

The present study revealed a significant increase in CD4+ T cells count after at least 6-month initiation of HAART (164.6± 114.6 vs 317.1 ± 175.2 cells/μl). In agreement with this finding, retrospective study conducted at Zewditu Memorial and Menelik II Hospital reported that an increase of CD4 count from 116 ± 69.4 vs 112 ± 67 cells/μl to 236 ± 120 vs 211± 120 cells/μl before and after initiation of HAART respectively (57, 64). The increase in CD4 count after HAART is mainly attributed to the reduction in plasma viral load after the administration of HAART (11). The lower CD4 count at the baseline is due to HIV infection. HIV infection is generally attributed to the destruction of mature CD4+ T cells in the peripheral lymphoid system. Alternatively, HIV may decrease production of CD4+ T cells by preventing maturation of lymphoid precursors (e.g., multilineage hematopoietic progenitor cells in the bone marrow, lymphoid-restricted progenitors of T cells in the thymus, and/or clonally distributed memory T cells in peripheral lymphoid organs). Inhibition of viral replication by HAART is, therefore, associated with improved production of CD4+ T cells (30).

The present study showed that the rate of immunosuppression is the most commonly encountered problem in HIV infected individuals before HAART although it declines after HAART (98.5% vs 88.5%). Majority of the participants at the baseline were severely immunosuppressed but after at least six months of HAART the percentage of sever immunosuppression was decreased markedly. In agreement with this study, other study conducted in India showed that 89.2% of the cases at the baseline were with CD4 count less than 200 cells/μl (68). Immunosuppression might be due to cytotoxic effect of HIV on T helper lymphocytes, which in line leads to low CD4 count. After HAART initiation the degree of immunosuppression is reduced because it improves immune function by suppressing HIV viral replication and increasing CD4+ T-cell counts (64).

7.1.2 Changes in Leucocytes after HAART initiation in TB/HIV co-infected patients

Changes in leucocyte count was also noted in TB/HIV co-infected participants from 5.83± 3.1 at baseline to 6.62 ± $3.15\times10^3/\mu l$ after at least 6 months 'of HAART initiation. Leukocyte response varied from leukocytosis to leucopenia and normal Leukocyte count was documented in 67% of patients with pulmonary tuberculosis (64). Significant increments from 4.07 ± 0.25 at baseline to 4.76 ± $0.15\times10^3/\mu l$ after 6 months of HAART were also reported from Nigeria (50). Kuppamuthu and his collogues attributed the drop in total WBC count at baseline due to the suppressive effect of HIV infection on the marrow, affecting their production (69).

The present study showed that the prevalence of leucopenia was reduced from the baseline 21.7% prevalence to 18.1% after six months of HAART. The study conducted at University of Gondar Hospital, Northwest Ethiopia demonstrated the effect of TB in lowering the total white cells count (56) in addition to the effect of HIV (69).

In this study the prevalence of neutropenia increased after at least six months of HAART initiation from 10.8% to 12.1% and lymphopenia decreased from 42.2 to 37.3%, as opposed to the whole HIV patient data before segregating the TB co-infected ones. In the current study, it was not possible to rule out TB drug adherence effect as the program is decentralized to nearby health centers and it was difficult to trace patient records. A retrospective study conducted in South Korea also contradicts the current finding in that the prevalence of neutropenia and lymphopenia were 10% and 25.7% in HAART naïve subjects and 0.89% and 4.17% in HAART experienced patients, respectively (71). Possible justifications for those studies which reported improvement in lymphopenia have been documented. Accordingly, during HIV infection, T helper cells constantly stimulate the proliferation of CD8 T cells which is intensified during viral replication. This related to an accumulation of memory CD8 T cells and this explains the high rate of total lymphocytes in peripheral blood in TB/HIV co-infected groups (70).

The present study also revealed differences in CD4+ T cells count both before and after HAART between the whole group and when data is disaggregated for TB co-infected patients separately signifying the effect of TB in inducing immunosuppression. This finding is further strengthened when one notices the rate of decline in immunosuppression before and after 6 months of HAART in the two groups (from 98.5% to 88.5% vs 98.8 to 92.8%, the latter in TB co-infected alone). Despite HAART, 92.8% of the TB co-infected group still had low CD4 count. The

observed improvement agrees with other studies including a study in Coastal Region of Kenya which showed an increase of CD4+T cell counts for all patients under HAART and TB treatment (54).

In contrast, a study in South Africa showed CD4 cell count at ART initiation was 98 cells/µl. Patients presenting with TB at ART start gained about five CD4 cells more per six months than patients without TB. From six months onwards TB patients were doing slightly better than patients without TB (32), underscoring the effect of both TB and HIV treatment on improving CD4 counts.

7.2 Changes in Erythrocyte parameters after HAART initiation

7.2.1 Changes in erythrocyte parameters after HAART initiation in HIV patients

In this study, the mean value of RBC count was lower after HAART initiation than at the baseline (4.35 ± 0.8 vs 4.25 ± 0.73×10^6/µl) which is in agreement with a study done at Jimma, Ethiopia which reported a decline in mean value of RBC count after taking HAART (4.95 ± 0.57 vs 4.14 ± 0.07×10^6/µl) (59). In line with this, a report from Iran also showed a reduction from 4.6±3.1×10^6/µl at the baseline to 3.9±0.8 ×10^6/µl after at least six months of receiving ART (45). A higher mean value of RBC counts before HAART than after the treatment was also reported by other study (55). The depletion of RBC in HAART experienced patients are usually attributed to the adverse effect of Zidovudine, which could also explain the current findings (71). It is claimed that both AZT and d4T induce a metabolic defect in developing RBC precursor. However, AZT, but not d4T, has broader myelosuppressive effects both *in vitro* and *in vivo* (41).

On the other hand, hemoglobin, HCT and red cell indices values (MCV and MCH) value were increased after HAART initiation which is consistent with other studies from Ethiopia (42, 55, 59) and elsewhere (50).

A striking and discrepant observation was showed that circulating RBC counts decreased significantly after the initiation of effective ART, despite increases in the hemoglobin level. This can be explained by the observation that the red cell size as indicated by the MCV and the average amount of Hgb each cell carries as shown by MCH were both increased. This effect was most pronounced for patients receiving zidovudine treatment which may be associated with

defects in the production of erythrocytes from erythroid progenitor cells, leading to the generation of fewer but larger cells as also observed in other studies (59, 62).

The present study revealed that the prevalence of anemia was also reduced from a baseline value of 39.1% to 14.5% after at least 6 months of therapy predominantly being of moderate and mild type. This finding is in agreement with a study in Addis Ababa, Ethiopia reported a reduction in the prevalence of anemia from a baseline of 42.9 % to 14.3% after 12 months of HAART; the anemia was predominantly of mild type (60). The study conducted in Kinshasa, DR Congo also showed that anemia was a regular finding in 69%, which was mostly moderate (92.2%) (51). The study conducted in Cameroon reported that anemia was the most frequent hematological abnormalities occurring in 62.9% of HAART naïve HIV infected patients (41).

A study done from the study site Menelik II Hospital, Addis Ababa, Ethiopia in 2012 involving 230 HIV infected patients, reported a high prevalence (52.6% vs 37%) before and after ART initiation (57). Other studies from Hawassa (58) and Jimma (59) reported the prevalence of anemia was 23.4% vs 29.9% before and 12.0% vs 16.2% after HAART respectively and the baseline prevalence of anemia is lower than the current report. On the other hand, studies from Europe and North America reported a prevalence rate of anemia at baseline was 35% and at 6 months after HAART initiation, the prevalence declined to 26 % (43). Differences observed may be attributed to the difference in socio-economic status, geographic, sample size difference, study time and variability in the definition of anemia.

The decrease in the prevalence of anemia after HAART initiation is attributed to the positive effect of HAART. HIV infection of marrow stromal cells, decrease in serum erythropoietin levels, auto-antibodies to erythropoietin, or marrow suppression by opportunistic infections, may contribute to the anemia commonly observed in HIV-infected persons. HAART may enhance many of these effects in an indirect manner simply by decreasing the HIV viral burden (19).

The characteristics of anemia were also assessed in this study. Most them were normocytic normochromic type which is in agreement with a study from Gondar, although the proportion varies between the two studies (55.6% vs 48.8% in the current study and 48.9% vs 29.4% in Gondar, before and after HAART, respectively) (27). The proportions of microcytic hypochromic anemia cases were lower after at least 6 months of HAART. Findings which are in

agreement with another cross sectional study done in Ghana, which reported a five times more likelihood of developing microcytic hypochromic anemia in HAART-naive patients compared to those on HAART (48). This may reflect the overall nutritional deficiencies (malnutrition and malabsorption) associated with HIV patients. Macrocytic normochromic anemia on the other hand was present in 8.9% of anemic subjects at the baseline whereas after HAART it was increased to 18.6%. In agreement with the current findings, study conducted in Ghana and Gondar, Ethiopia reported that macrocytic normochromic anemia was more common after HAART than before HAART initiation (27, 48). This is probably due to the effect of HAART particularly AZT which is responsible for the development of macrocytosis; after disaggregating the data between AZT and non-AZT groups, the current finding supported this justification as also reported by Tamir *et al* (62).

7.2.2 Changes in erythrocyte parameters after HAART initiation in TB/HIV co-infected patients

Of the RBC parameters unlike in the total HIV infected patients, only MCV was significantly changed after receiving HAART for at least 6 months. The increased in MCV after HAART is usually attributed to the adverse effect of Zidovudine, which could also explain the current findings (41, 62).

The present study showed that the prevalence of anemia at the baseline was 51.8% and 39.8% after six months of HAART which is in agreement with a study in Addis Ababa, Ethiopia that reported a baseline prevalence of 42.9 % which after HAART initiation was reduced to 14.3 % (60). The study conducted in Dar es Salaam, Tanzania showed that the HIV co-infection in antiretroviral-naïve population was associated with severe anemia (hemoglobin < 85 g/l) (52), much lower than the finding in the current study. Differences observed may be attributed to the difference in socio-economic status, geographic, sample size difference and variability in the definition of anemia. The decrease in the prevalence of anemia after HAART initiation is attributed to the positive effect of HAART. HIV infection of marrow suppression by opportunistic infections, may contribute to the anemia commonly observed in HIV-infected patients (19).

7.3 Changes in Thrombocyte levels after HAART initiation in HIV and TB/HIV co-infected patients

7.3.1 Changes in Thrombocyte levels after HAART initiation in HIV patients

In the present study, platelet count was increased in consistent with a study from Gondar (55) and thrombocytopenia was significantly reduced from 16.9% to 9.8% after at least 6 months of treatment. A study conducted in Uganda reported thrombocytopenia in 17.8% of HAART-naive and 13.0% in clients who were on HAART for up to 6 months (53). In another study conducted at Gondar, Ethiopia the prevalence of thrombocytopenia was 9% in HAART naive patients and reduced to 4.1% in patients on HAART (55). The difference in results seen from the present study might be due to the difference in the definition of thrombocytopenia, study design and size of the study population. The improvement in thrombocytopenia might be attributed to the effect of treatment in which after HAART initiation disorders of hematopoiesis, opportunistic infections and immune causes related to HIV leading to low platelets count could be reverted (68). Additionally, the incidence of opportunistic infection that hampers thrombopoietin production will decrease (44).

7.3.2 Changes in thrombocyte levels after HAART initiation in TB/HIV co-infected patients

In the present study the mean platelet count decreased after 6 months of the treatment which were 264.13±118.46×103/μl and 262.89±103.6×103/μl unlike the observed increment in the total HIV population (245 vs 265×10^3/μl) before and after HAART initiation. TB co-infection might have caused the discrepancy. This explanation is supported by a finding from Northwest Ethiopia which showed that the platelet values of patients after completion of the intensive phase of tuberculosis treatment decreased from 268×10^3/μl to 239×10^3/μl (56).

Moreover, the reduction in the prevalence of thrombocytopenia from 14.5% at baseline to 12.0% after six months of HAART initiation in TB/HIV co-infected patients was lower than that of the whole HIV patients (16.9% to 9.8%). This again signifies the role played by TB co-infection in affecting the hematological outcome of HIV patients after undergoing HAART.

8. STRENGTH AND LIMITATION OF THE STUDY

8.1 Strength of the study

- Data was disaggregated for TB and HIV co-infected patients; very limited studies have been carried out during these phase of treatment.

8.2 Limitation of the study

- Unable to determine the effect of anti-TB drugs as the TB treatment service is decentralized to patient's nearby health facility.
- The data were not accessible and fully computerized; however, quality of data was ensured by double checking of medical records and laboratory log books at Menelik II Hospital.
- The present study was not sure about the effect of TB than other OIs.
- Different hematological and CD4 machines were used at the baseline record.
- The study focused on comparisons of hematological and immunological parameters but does not addresses socio-demographic effects due to financial and time constraint

9. CONCLUSION

Based on the results of the current study, the following conclusions were made:

- There was significant increase in CD4+ T cell count, WBC count, neutrophil, lymphocyte, hemoglobin, HCT, MCV, MCH, and platelets after six months of HAART while there was a decrease in mixed % and count of red blood cells (RBCs) compared to baseline in HIV patients.
- Anemia, immunosuppression, leucopenia, neutropenia, lymphopenia and thrombocytopenia were commonly prevalent hematologic abnormality encountered before HAART initiation but the prevalence of these abnormalities significantly reduced after initiation of HAART.
- This study has established significant changes in the hematological variables studied in TB/HIV co-infected patients.
- The rate of improvement in the most common hematological abnormalities mainly Anemia and immunosuppression were smaller among TB/HIV co-infected patients taking HAART compared to the improvement in the total population underscoring the role of TB in affecting hematological outcomes after HAART.

10.RECOMMENDATION

Recommendation for this study:

- Prospective longitudinal studies are needed to confirm protective immunological thresholds for TB with an adequate assessment of HAART.
- Immuno-hematological profiles should be regularly checked during HAART and tuberculosis treatment.
- Further research is needed on hematological parameters including Reticulocyte count, monocytes and coagulation tests.
- Further investigation needs to be done to identify the associated risk factors for each hematological abnormality.
- The Effect of use of HAART duration with TB treatment on hematological parameters needs to be confirmed by large scale longitudinal cohort studies.
- The varied hematological abnormalities observed in HIV and TB/HIV co-infected patients after HAART suggests the need for continuous monitoring and evaluation of patients for adverse hematological abnormalities during HARRT.
- HIV and TB services and programs should be more integrated to improve efficiency of the services delivered.

11.REFERENCES

1. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med. Feb 18 2011; 62:141-155.(http://www.ncbi.nlm.nih.gov/DOI: 10.1146/)
2. Ayodele E, Omisakin C, Kolawole F, Titilayo O, Oyegue K, Boluwatitife O et.al. Assessment of some hematological and immunological parameters in HIV-infected patients on HAART and HAART naïve in Ekiti state. British journal of advance academic research. 2015;4(1):54-61 (https://www.researchgate.net/publication/286914150)
3. Barre-Sinoussi F. HIV as the cause of AIDS. Lancet. 1996;348: 31-35.

   (http://www.ph.ucla.edu)
4. Iroezindu MO. Disparities in the magnitude of human immunodeficiency virus-related opportunistic infections between high and low/middle-income countries: Is highly active antiretroviral therapy changing the trend?. Ann Med Health Sci Res 2016; 6:4-18. (www.amhsr.org/10.4103/2141-9248.180234)
5. Tesfaye B, Alebel A, Gebrie A, Zegeye A, Tesema C, Kassie B. The twin epidemics: Prevalenceof TB/HIV co-infection and its associated factors in Ethiopia; A systematic review and meta-analysis. PLoS ONE 2018; 13(10):e0203986. (https://doi.org/10.1371/journal.pone.0203986)
6. Musa BM, Musa B, Muhammed H, Ibrahim N, Musa AG. Incidence of tuberculosis and immunological profile of TB/ HIV co-infected patients in Nigeria. Ann Thorac Med 2015; 10:185-92. (http://www.thoracicmedicine.org)
7. Judith B, Margarida CN, Gunilla K. Tuberculosis and HIV Co-infection: Cold Spring Harb Perspect Med. 2015; 5:7 (https://dx.doi.org/10.1101/cshperspect.a017871)
8. Karo B, Krause G, Castell S, Kollan C, Hamouda O, Haas W. Immunological recovery in tuberculosis/HIV co-infected patients on antiretroviral therapy: implication for tuberculosis preventive therapy. ClinSurv HIV Study Group: BMC Infect Dis. 2017; 17: 517. (https://dx.doi.org/10.1186/s12879-017-2627-y)
9. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents, ADS info, Oct 2018. (https://aidsinfo.nih.gov/guidelines)
10. Eluwa GI, Badru T, Akpoigbe KJ. Adverse drug reactions to antiretroviral therapy (ARVs): incidence, type and risk factors in Nigeria. Biomedical Central Clinical Pharmacology, 2012, 12:7 (https://dx.doi.org/10.1186/1472-6904-12-7)
11. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. 2018.(http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf)
12. WHO guidelines on the public health response to pretreatment HIV drug resistance. Geneva: World Health Organization; 2017

(http://www.who.int/hiv/pub/guidelines/hivdr-guidelines-2017/en), Accessed 6 July 2018
13. WHO, WHO Three I's Meeting. Report of a Joint WHO HIV/AIDS and TB Department Meeting, World Health Organization, Geneva, Switzerland, 2008. (http://www.who.int/hiv/pub/meetingreports/WHO 3Is meeting report.pdf)
14. Centers for Disease Control and Prevention. HIV among Youth. Division of HIV/AIDS Prevention; April 2017. (https://www.cdc.gov/hiv/pdf/group/age/youth/cdc-hiv-youth.pdf)
15. Madhu V, Teena M. Current practices in laboratory monitoring of HIV infection. Indian J Med Res, 2011;134:801-822(http://www.ijmr.org.197.156.95.19)
16. Jessica O. Haematological complications of HIV infection. S Afr Med J 2012; 102:6:465-468.(http://www.ncbi.nlm.nih.gov/pubmed/123831254)
17. Aberg JA, Gallant JE, Ghanem KG, Emmanuel P, Zingman BS, Horberg MA. Primary care guidelines for the management of persons infected with HIV: Clin Infect Dis. 2014; 58(1):1-34. (http://www.ncbi.nlm.nih.gov/pubmed/24235263)
18. Mohamed E, Amera A, Nada Y, Ahmed ME, Exany S. Hematological parameters among tuberculosis patients under treatment attending Kosti and Kenana teaching hospitals in White Nile state, Sudan. American Journal of Research Communication, 2017; 5(6): 58-94. (www.usa-journals.com/2325-4076)
19. Van Schalkwyk WA, Opie J, Novitzky N. The diagnostic utility of bone marrow biopsies performed for the investigation of fever and/or cytopenias in HIV-infected adults at Groote Schuur Hospital, Western Cape, South Africa. Int J Lab Hematol 2011; 33(3):258-266. (http://dx.doi.org/10.1111%2Fj.1751553X.2010.01280.x)
20. Woldeamanuel GG, Wondimu D. Prevalence of anemia before and after initiation of antiretroviral therapy among HIV infected patients at Black Lion Specialized Hospital, Addis Ababa, Ethiopia: a cross sectional study BMC Hematology. 2018;18:7 (https://doi.org/10.1186/s12878-018-0099-y77)
21. Munyazesa E, Emile I, Mutimura E, Hoover DR, Shi Q, McGinn AP et al. Assessment of haematological parameters in HIV-infected and uninfected Rwandan women: a cross-sectional study. BMJ Open 2012; 12: e001600. (http://bmjopen.bmj.com/.doi:10.1136/bmjopen-2012-001600)
22. Lawn SD, Harries AD, Williams BG, Chaisson RE, Losina E, De Cock KM et al. Antiretroviral therapy and the control of HIV-associated tuberculosis. Will ART do it? Int J Tuberc Lung Dis.2011;15(5):571-581. (http://www.ncbi.nlm.nih.gov/pubmed/21756508)
23. Danel C, Moh R, Gabillard D, Badje A, Carrou JL, Ouassa T et al. A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. N Engl J Med. Aug 27 2015; 373(9):808-822. (http://www.ncbi.nlm.nih.gov/pubmed/26193126)
24. Centers for Disease Control and Prevention, Association of Public Health Laboratories. Laboratory testing for the diagnosis of HIV infection: Updated recommendations. 2014. (https://stacks.cdc.gov/view/cdc/23447) Accessed: October 4, 2018.

25. Pretorius E, Klinker H, Rosenkranz B. The role of therapeutic drug monitoring in the management of patients with human immunodeficiency virus infection. Ther Drug Monit. 2011; 33(3):265-274.(http://www.ncbi.nlm.nih.gov/pubmed/21566505)
26. Agatha ND, Agatha EW, Elizabeth A'D, Olufemi A. Prevalence of Anaemia and the Relationship between Haemoglobin Concentration and CD4 Count in HIV Positive Children on Highly Active Antiretroviral Therapy (HAART) in Lagos, Nigeria. Curr Pediatr Res 2016; 20 (1&2): 29-36 (https://www.researchgate.net/publication/30910409)
27. Meseret A, Zelalem A, Mulugeta M, Enawgaw B. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study. BMC Hematology. 2014; 14(8):2-7. (http://biomedcentral.com/2052-1839/14/8)
28. Mata-Marín JA, Gaytán-Martínez JE, Martínez-Martín RE, Arroy-Anduiza CI, Fuentes-Allen JL, Casarrubias-Ramirez M et al. Risk factors and correlates for anemia in HIV treatment-naïve infected patients: a cross-sectional analytical study. BMC Res Notes 2010; 8:2:230-234. (http://www.biomedcentral.com/1756-0500/3/230)
29. Velasco M, Castilla V, Sanz J, Gaspar G, Condes E, Barros C et al. Effect of simultaneous use of highly active antiretroviral therapy on survival of HIV patients with tuberculosis. Journal of acquired immune deficiency syndromes. 2009; 50(2):148-152 (https://www.ncbi.nlm.nih.gov/pubmed/19131895)
30. Le T, Wright EJ, Smith DM, He W, Catano G, Okulicz JF et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. N Engl J Med. Jan 17 2013;368(3):218-230. (http://www.ncbi.nlm.nih.gov/pubmed/23323898)
31. Palella FJJ, Armon C, Chmiel JS, Brooks JT, Hart R, Lichtenstein K et al. CD4 cell count at initiation of ART, long-term likelihood of achieving CD4 >750 cells/mm3 and mortality risk. Journal of antimicrobial chemotherapy. 2016; 71(9):2654-2662. (https://www.ncbi.nlm.nih.gov/pubmed/27330061)
32. Michael S, Matthias E, Mhairi M, Andrew B. Immune recovery after starting ART in HIV-infected patients presenting and not presenting with tuberculosis in South Africa. J Acquir Immune Defic Syndr.2013; 63(1): 142–145. (https://dx.doi.org/10.1097/qai.0b013e318288b39d)
33. Yihunie L, Susan B, Demewoz H. Social determinants of HIV infection, hotspot areas and subpopulation groups in Ethiopia: evidence from the National Demographic and Health Survey in 2011. BMJ Open 2015;5: e00866 (http://bmjopen.bmj.com/BMJ Open10.1136/bmjopen-2015-008669)
34. Basel K, Walter H, Christian K, Barbara GB, Osamah H, Lena F. Tuberculosis among people living with HIV/AIDS in the German ClinSurv HIV Cohort: long-term incidence and risk factors. BMC Infect Dis. 2014; 14: 148. (https://dx.doi.org/10.1186/1471-2334-14-148)

35. Tadele G, Abebaw W, Abdulsemed W. Trend of HIV/AIDS for the last 26 years and predicting achievement of the 90–90-90 HIV prevention targets by 2020 in Ethiopia: a time series analysis. BMC Infectious Diseases. 2018; 18:320 (https://doi.org/10.1186/s12879-018-3214-6)
36. Ethiopian Public Health Institute (EPHI). HIV and AIDS Estimation and Projection for Ethiopia Based on SPECTRUM Modeling. Addis Ababa, Ethiopia: EPHI 2017.
37. UNAIDS. UNAIDS report on the global AIDS epidemic. Geneva: UNAIDS, 2018
38. World Health Organization: Global Tuberculosis Report 2018. Geneva, Switzerland: WHO; 2018. (www.ijastnet.com/2221-1004)
39. Aweke AM, Zelalem GD, Essey KM, Demeke LW. Prevalence and associated factors of TB/HIV co-infection among HIV Infected patients in Amhara region, Ethiopia Afr Health Sci. 2016; 16(2): 588–595.(https//dx.doi.org/10.4314/ahs.v16i2.29)
40. Santiago-Rodríguez EJ, Mayor AM, Fernández-Santos DM, Hunter-Mellado RF. Profile of HIV-Infected Hispanics with Pancytopenia. International journal of environmental research and public health, 2015; 13(1):38-45.
41. Wankah PN, Tagny CT, Mbanya DNS. Profile of blood cell abnormalities among antiretroviral therapy naïve HIV patients attending the Yaounde University Teaching Hospital, Cameroon. BMC hematology, 2014; 14(1):1-6. (https://www.ncbi.nlm.nih.gov/pubmed/156789)
42. Tesfaye Z, Enawgaw B. Prevalence of anemia before and after initiation of highly active antiretroviral therapy among HIV positive patients in Northwest Ethiopia: a retrospective study. *BMC research notes*, 2014; 7(1): 1-5.
43. Harris RJ, Sterne JA, Abgrall S, Dabis F, Reiss P, Saag M. Prognostic importance of anaemia in HIV-1 infected patients starting antiretroviral therapy: collaborative analysis of prospective cohort studies in industrialized countries. *Antiviral therapy*, 2008; 13(8): 959-967. (http://www.ncbi.nlm.nih.gov/pubmed)
44. Fan HW, Guo FP, Li YJ, Li N, Li TS. Prevalence of Thrombocytopenia among Chinese Adult Antiretroviral-naïve HIV positive Patients. Chin Med J 2015; 128:459-64. (www.cmj.org/10.4103/0366-6999.151078)
45. Rezaei E, Ebrahim-Saraie HS, Heidari H, Ghane P,Rezaei Kh, Manochehri J. Impact of vitamin supplements on HAART related hematological abnormalities in HIV- infected

patients. *Medical Journal of the Islamic Republic of Iran (MJIRI)*, 2016; 30(1): 350-355. (http://mjiri.jums.ac.ir)

46. Mathews S, Srivastava D, Balayadav R, Sharma A. Association of Hematological profile of HIV positive patients with clinicoimmunologic stages of the disease. Journal of laboratory physician, 2013; 5(7):34 (http://www.researchgate.net/10.4103/0974-2727.15929)
47. Suma K, Sesank R, Vidyasagar NK, Satyanarayana RK. Hematological parameters in HIV subjects on Zidovudine therapy. Perspectives in medical research, 2015; 3(2): 11-14.
48. Owiredu WK, Quaye L, Amidu N, Addai-Mensah O. Prevalence of anaemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. Africa health science, 2011; 11(1): 2–15. (https://www.ncbi.nlm.nih.gov/pubmed)
49. Babadoko AA, Muktar HM, Mamman AI. Immunohematological abnormalities of human immunodeficiency virus-1 infected highly active antiretroviral naïve adults in Zaria, Northern Nigeria. Arch Int Surg 2015; 5:199-205.
(http://www.archintsurg.org/10.4103/2278-9596.174658)
50. Amegor OF, Bigila DA, Oyesola OA, Oyesola TO, Buseni ST. Hematological changes in HIV patients placed on anti-retroviral therapy in Markurdi, Benue State of Nigeria. Asian Journal of Epidemiology, 2009; 2(4): 97-103. (http://scholar.google.com)
51. Mulenga CM, Kayembe JM, Kabengele BO, Bakebe A. Anemia and Hematologic Characteristics in Newly Diagnosed Pulmonary Tuberculosis Patients at Diagnosis in Kinshasa. Journal of Tuberculosis Research, 2017; 5, 243-257.
52. Saathoff E, Villamor E, Mugusi F, Bosch RJ, Urassa W, Fawzi WW. Anemia in adults with tuberculosis is associated with HIV and anthropometric status in Dar es Salaam, Tanzania. Int J Tuberc Lung Dis. 2011 Jul; 15(7): 925–932. (http://dx.doi.org/10.5588/ijtld.10.0477)
53. Taremwa IM, Muyindike WR, Muwanguzi E, Boum Y, Natukunda B. Prevalence of HIV Related thrombocytopenia among clients at Mbarara regional referral hospital, Mbarara, southwestern Uganda. *Journal of blood medicine*, 2015; *6:109*-113.
(http://www.ncbi.nlm.nih.gov/doi:10.2147/JBM.S80857)
54. Shadrack AY, Michael FO, Rekha RS. Predictors, Haematological and Cytokine profiles in Tuberculosis-HIV Coinfection patients during TB treatment and HAART in Coastal Region of Kenya. International Journal of Applied Science and Technology. 2016; 6(3):126-137.

55. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking HAART and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study. *BMC hematology*, 2014;14(1):3-7. (https://doi.org/10.1186/2052-1839-14-8)
56. Kassa E, Enawgaw B, Gelaw A, Gelaw B. Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia. BMC Hematology,2016;6:5,1-11. (https://dx.doi.org/10.1186/s12878-015-0037-1)
57. Adane A, Desta K, Bezabih A, Gashaye A, Kassa D. HIV-associated anaemia before and after initiation of antiretroviral therapy at Art Centre of Minilik II Hospital, Addis Ababa, Ethiopia. *Ethiop Med J*. 2012; 50(1):13-21. (https://www. ncbi.nlm.nhi.gov/ pubmed/22519158)
58. Daka D, Lelissa D, Amsalu A. Prevalence of anaemia before and after the initiation of antiretroviral therapy at ART center of Hawassa University Referral Hospital, Hawassa, South Ethiopia. Sch J Med, 2013; 3(1): 1-6. (http://www.scholar.google.om)
59. Gedefaw L, Yemane T, Sahlemariam Z, Yilma D. Anemia and risk factors in HAART naive and HAART experienced HIV positive persons in south west Ethiopia: a comparative study. *PloS one*, 2013; 8(8): 1-5.(https://www.ncbi.nlm.nih.gov/doi:10.1371/journal.pone.0072202)
60. Assefa M, Abegaz WE, Shewamare A, Medhin G, Belay M. Prevalence and correlates of anemia among HIV infected patients on highly active anti-retroviral therapy at Zewditu Memorial Hospital, Ethiopia. *BMC hematology*, 2015; *15*(1):1-6. (https://www.researchgate.net/DOI:10.1186/s12878-015-0024-6)
61. Derbe M, Monga DP, Daka D. Immunological response among HIV/AIDS Patients before and after ART therapy at Zewuditu Hospital Addis Ababa, Ethiopia.*American Journal of Research Communication*, 2013;1(1): 103-115. (https://www.usa-journals.com)
62. Tamir Z, Alemu J, Tsegaye A. Anemia among HIV Infected Individuals Taking ART with and without Zidovudine at Addis Ababa, Ethiopia. Ethiop J Health Sci. 2018; 28(1):73-82. (http://dx.doi.org/10.4314/ejhs.v28i1.9)
63. Junling G, Pinpin Z, Hua F. Prevalence of TB/HIV Co-Infection in Countries Except China: A Systematic Review and Meta-Analysis, 2013; 8(5): e64915.PMCID: PMC3669088 (https://www.researchgate.net/publication/237061179/10.1371/journal.pone.0064915)

64. Huruy K, Kassu A, Mulu A, Wondie Y. Immune restoration disease and changes in CD4+ T-cell count in HIV-infected patients during highly active antiretroviral therapy at Zewditu memorial hospital, Addis Ababa, Ethiopia. AIDS research and therapy, 2010; 7(1):1-7. (https://www.aidsrestherapy.com/content/7/1/46)
65. Yakubu A, Zama II, Amina A, Mohammed HY. Some hematological profiles of HIV/AIDS patients on HAART in Usmanu Danfodiyo university teaching hospital Sokoto, North western Nigeria *American Journal of Science and Technology*, 2014; *2*(1): 27-32. (https://www.aascit.org/journal/ajst/ISSN: 2375-3846)
66. Bello HS, Denue BA, Abja AU, Bukar AA. Changes in blood profile of human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral therapy in North Eastern Nigeria. Journal of AIDS and HIV Research, 2013; 5(8):284-291. (https://www.semanticscholar.org/DOI:10.5897/JAHR12.005)
67. Wanjari A, Acharya S, Singh AP, Rathi C. Study of Hematological Profile in HIV/AIDS. *International Journal of Health Sciences &Research*, 2013; 3(5):60-75.
(https://www.researchgate.net/publication/274709050)
68. Huang S. Susan G, Jason D, Barbour A, Steven G, Jeannie S. Reversal of Human Immunodeficiency Virus Type 1–Associated Hematosuppression by Effective Antiretroviral Therapy. *Clinical Infectious Diseases*; 2000; 30: 504–510. (https://researchgate.net/publication/12594362)
69. Kuppamuthu R, AlaguV, Rajaiah S , Karuppusamy K, Ponniah T. Hematological parameters in Pulmonary Tuberculosis patients with and without HIV infection, Int J Biol Med Res.2016;7(3):5640-5643. (http://www.biomedscidirect.com)
70. Guiedem E, Pefura YEW, Ikomey G, Gonsu KH, Monamele CG, Fokam J. Intensive Phase of Therapy of Tuberculosis and HIV Co-infection: CD4, CD8, and Certain Hematological Finding Amongst Patients in Yaounde Cameroon. International Journal of Immunology. 2015;3(6):72-77 (https://www.sciencepublishininggroup.com/j/iji)
71. Choi SY, Kim I, Kim NJ, Lee S, Choi Y, Bae J. Hematological manifestations of human immunodeficiency virus infection and the effect of highly active anti-retroviral therapy on cytopenia. Korean J Hematol. 2011 Dec; 46(4): 253–257. (http://www.scholargate.net/doi:10.5045/kjh.2011.46.4.253)

11. ANNEX

Annex I – Information sheet

My name is Esuyawkal Kasawmar, I am MSC student at Addis Ababa university department of Medical Laboratory. I am here to carry out a research on assessment of hematological profiles among HIV positive adult individuals before and after starting highly active antiretroviral therapy in partial fulfillment of my MSC.

The objective of this study is to assess the immuno-hematological profiles among HIV and TB/HIV co-infected patients before and after starting HAART. For this we will take 3ml of venous blood. There is no direct benefit you got from this study, but indirectly the outcome of the result will be used to advance the life of our HIV infected individuals who start ART and management of ART which further lead to improve the survival of HIV infected individuals after initiation of HAART, so your participation is important for this research. The risk of this study is, you feel a mild pain while samples are collected except that there will be no risk to participate in the project.

Coding method will be applied in all the procedures, code will be given to the questioner form and also to blood sample, the same code is use for both blood sample and laboratory result and data is investigated accordingly so your results will be kept confidential, and no one knows your result.

There is nothing you will loss if you are not willing to participate in the study or stop filling the questioner in between, so it is your right to participate or not to participate in the study.

Would you be willing to participate?
Yes, I want to participate in the study. Please go to the next page.
No, I don't want to participate in the study. Thank you very much!
If you need to have extra information or if you have related question on this study, you can contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory or the investigator using the following address: -

Investigator: - Esuyawkal Kasawmar

Phone: - 0913457304 E-mail: - esu.kasa.ekgmail.com

በምርምሩ ለመሳተፍ የስምምነት ማሳወቂያ

ስሜ እሱያውቃል ካሳውማር ሲሆን በአዲስ አበባ ዩኒቨርስቲ የላቦራቶሪ ዲፓርትመንት ተማሪ ነኝ፡፡ የኤች አይቪ ኤድስ መድሀኒት ከመጀመሩ በፊት እና ከተጀመረ በኃላ በደም ህዋሳት ላይ ያለዉ ተፅእኖ በሚል ርዕስ ላይ ለማስተርስ ዲግሪ የመመረቂያ ፅሑፍ እየሰራሁ እገኛለሁ፡፡ የዚህ ጥናት አላማም የኤችአይቪ ኤድስ መድሀኒት ከመጀመሩ በፊት እና ከተጀመረ በኃላ በደም ህዋሳት ላይ ያለዉን ተፅእኖ ማጥናት ነው፡፡ ለዚህም 3 ሚ.ሊ ደም ይወሰዳል፡፡ ከጥናቱ በቀጥታ የሚያገኙት ጥቅም አይኖርም፡፡ ነገር ግን በተዘዋዋሪ የጥናቱ ውጤት የኤችአይቪ ኤድስ ታካሚዎች በህይወት የመቆየት አድልን ለመጨመር አስተዋፅኦ ይኖረዋል፡፡

በተጨማሪም ለቀጣይ ምርምር መነሻ በመሆን ያገለግላል፡፡ ስለዚህ የእርሶ ተሳትፎ ለዚህ ምርምር ጠቃሚ ነው፡፡ በዚህ ምርምር መሳተፍ ምንም አይነት የጎንዮሽ ጉዳት አይኖረውም፡፡ ለሁሉም የአሰራር ቅደም ተከተል ምስጥራዊ ቁጥር አሰራር ዘዴ እንጠቀማለን፡፡ ለመጠይቁም ሆነ ለደም ናሙናው ኮድ እንጠቀማለን፡፡ የደም ናሙናው እና የላብራቶሪ ውጤቱ ተመሳሳይ ኮድ ይኖራቸዋል፡፡ በመሆኑም የእርስዎ ውጤት ምስጥራዊ ነው፡፡ በጥናቱ በፍቃደኝነት አለመሳተፍ ምንም አያሳጣዎትም፡፡ ስለዚህ በጥናቱ መሳተፍም ሆነ አለመሳተፍ የእርሶ መብት ነው፡፡ ከጥናቱ ጋር የተያያዘ ጥያቄ ካለዎት ወይም ተጨማሪ መረጃ ከፈለጉ በሚከተለው አድራሻ እኛን ማግኘት ይችላሉ፡ ፡

የጥናት አድራጊው ስም፡ እሱያውቃል ካሳውማር

ስ.ቁ፡- 0913457304

ኢሜል፡- esu.kasa.ek@gmail.com

Annex II – Consent Form

Code no_____

Information about the study has been described to me by the investigator. I have understood that the objective of this study is to evaluate the immune-hematological profiles among HIV positive patients before and after initiating highly active antiretroviral therapy, and small amount of blood that I will contribute will not hurt my health. It has also been explained to me that I have the right to stop participation at any time in between and there is nothing, I will loss if I refuse to participate.

I agree to participate in the study and hereby approve my agreement with my signature.

Participant signature _____Date_____

Investigator signature_____ Date_____

በምርምር ለመሳተፍ የፍቃደኝነት መዋዋያ ቅፅ

መለያ ቁጥር .....

ስለጥናቱ በቂ መረጃ ተሰጥቶኛል፡፡ የዚህ ጥናት አላማም የኤችአይቪ ኤድስ መድሀኒት ከመጀመሩ በፊት እና ከተጀመረ በኃላ በደም ህዋሳት ላይ ያለዉን ተፅእኖ ማወቅ ነው፡፡ከኔ የሚወሰደዉ ደም ምንም አይነት የጤና ጉዳት የማያስከትል መሆኑን ተረድቻለሁ፡፡በጥናቱ ለመሳተፍ ፈቃደኛ ካለሆንኩ በጥናቱ ለመሳተፍ እንደማልገደድ ነገር ግን በዚህ ጥናት በመሳተፌ ለሳይንሳዊ እዉቀት ጠቃሚ መረጃ ማበርከትና ወደፊት በዚህ ዙሪያ ለሚሰሩ ስራዎች መሰረት የሚሆኑ መረጃዎችን መስጠት እንደምችል ተረድቻለሁ፡፡

በመሆኑም በዚህ ጥናት ላይ ለመሳተፍ የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊ ፊርማ---------- ቀን----------

የጥናት አድራጊ ፊርማ---------- ቀን----------

Annex III – Hematological parameter at the start of ART (Baseline) and after 6 month of therapy

Identification Code:			Date of data collection:	
Data collector name:			Supervisor:	
Immuno-hematological Parameters	HIV patients		TB/HIV Co-infected patients	
	0 Month	After 6 Month	0 Month	After 6 Month
RBC (x10 12/l)				
HB (gm/dl)				
HCT (%)				
MCV(fl)				
MCH(Pg)				
MCHC(gm/dl)				
WBC(X109 /l)				
Neutrophil(X109/l)				
Lymphocyte(X109/l)				
Monocyte(X109 /l)				
Eosinophil(X109 /l)				
Basophil(X109 /l)				
Platelet(X109 /l)				
CD4 count (cells/µl)				

Annex IV– Data extraction form

Identification Code:		**Date of data collection:**	
Data collector name:		**Supervisor:**	
HIV only	☐	**TB/HIV co-infected**	☐
Characteristics	**Tick**	**Characteristics**	**Tick**
Age		**BMI**	
18–20		<18.5	
20 –29		18.5 – 24.9	
30 – 39		≥ 25	
40 – 49		**ART Regimen**	
50 – 59		1c	
≥ 60		1d	
		1e	
		1f	
Sex		Others specify:_____	
M		**Functional status**	
F		Ambulatory	
WHO Staging		Bedridden	
I		Working	
II		**Follow-up status**	
III		On treatment	
IV		Dead	
Nutritional status		Stop ART	
Normal		Lost	
Mild		Restart	
Moderate malnutrition		Transferred out	
Severe malnutrition		**Reason for switch to $2^{nd}/3^{rd}$-line regimen**	
Over weight		Clinical treatment failure	
Duration of use of HAART (in months)		Immunologic failure	
6 – 11		Virologic failure	
12 – 17		**Prophylaxis taken**	
18 – 23		Cotrimoxazole	
24 – 29		No prophylaxis	
30 – 35			
≥36			

Key: 1c= AZT-3TC-NVP, 1d= AZT-3TC-EFV, 1e=TDF-3TC-EFV, 1f=TDF-3TC-NVP

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Esuyawkal Kasawmar (B.Sc.)

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.