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# Comparative cross-sectional study on Prevalence and Associated Factors of Anemia in HAART Naïve and HAART Experienced Adult HIV Patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

By: Rahel Alemu (BSc)

Advisors:

Aster Tsegaye ( MSc,PhD.)

Jemal Alemu (BSc, Msc)

Collaborator: Dr. Aster Shewamare (MD)

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This is to certify that the thesis prepared by Rahel Alemu, entitled:

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Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

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## Table of Contents Page

Acknowledgement ..... II

List of Tables ..... VI

List of Figures ..... VII

List of Abbreviations ..... VIII

Abstract ..... X

1. Introduction ..... 1

1.1. Background ..... 1

1.1.1. Definition of Anemia ..... 1

1.1.2. Etiology of HIV-Related Anemia ..... 1

1.1.3. Effect of Highly Active antiretroviral therapy (HAART) on anemia ..... 2

1.1.4. Consequences of anemia in HIV patients ..... 3

1.2. Statement of the Problem ..... 4

1.3. Significance of the study ..... 6

1.4. Literature Review ..... 7

1.4.1. Anemia And Associated Factors in HAART Experienced Patients ..... 7

1.4.2. Anemia and Associated Factors in HAART-Naïve Patients ..... 9

2. Objectives and Hypothesis ..... 12

2.1. General objective ..... 12

2.2. Specific objectives ..... 12

2.3. Hypothesis ..... 12

3. Materials and Methods ..... 13

3.1. Study area ..... 13

3.2. Study Design ..... 13

3.3. Study Period ..... 13

3.4. Population ..... 13

3.4.1. Source population ..... 13
3.4.2. Study population ..... 13
3.5. Eligibility ..... 13
3.5.1. Inclusion Criteria ..... 13
3.5.2. Exclusion Criteria ..... 13
3.6. Operational Definitions ..... 14
3.7. Study variables ..... 14
3.7.1. Dependent variables ..... 14
3.7.2. Independent variables ..... 14
3.8. Sample size calculation and sampling technique ..... 15
3.8.1. Sample size calculation ..... 15
3.8.2. Sampling Technique ..... 16
3.9. Study Procedure and Data collection ..... 16
3.9.1. Principle of Cell-Dyn 1800 Hematology analyzer ..... 18
3.9.2. Thick Blood Film for Malaria Detection ..... 18
3.9.3. Direct Wet Mount Preparation of Fresh Stool specimen ..... 18
3.9.4. Modified Zheil Neelsen Technique ..... 18
3.9.5. McMaster Concentration Technique ..... 19
3.10. Data Quality Assurance ..... 19
3.10.1. Pre-Analytical Data Quality Assurance ..... 19
3.10.2. Analytical Quality Assurance ..... 19
3.10.3. Post Analytical Data Quality Assurance ..... 19
3.11. Statistical Analysis ..... 20
3.12. Ethical consideration ..... 20
4. Result ..... 21
4.1. Socio-demographic and Clinical Characteristics of the Participants ..... 21
4.2. Prevalence of anemia in HIV patients ..... 24
4.3. Associated Factors of anemia in HAART Naïve adult HIV patients ..... 27

4.4. Associated Factors of anemia in HAART Experienced adult HIV patients ..... 29
5. Discussion ..... 30
6. Strength and Limitations of the Study ..... 35
6.1. Strength ..... 35
6.2. Limitations of the Study ..... 35
7. Conclusion and Recommendations ..... 36
7.1. Conclusion ..... 36
7.2. Recommendation ..... 36
References ..... 37
Annexes ..... 43
Annex 1:- Laboratory Methods ..... 43
2. Procedure and reagents of Cell-Dyn 1800 Hematology Analyzer ..... 50
Annex III- Subject information sheet (for participants, Amharic version) ..... 61
Annex-IV- Consent Form (for participants, English version) ..... 64
Annex-V - Consent form (for participants, Amharic Version) ..... 66
ANNEX VI: Data Extraction Form ..... 68

## List of Tables

**Table 1:- Socio-demographic and Clinical Parameters of HAART Naïve and HAART Experienced adult HIV Patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia, 2015. ..... 22**

**Table 2:- Comparision of Mean values Among HAART Naïve And HAART Experienced Adult HIV Patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November,2015 ..... 24**

**Table 3:- Frequency of Anemia among Adult HIV Patients attending ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015 ..... 25**

**Table 4:- Severity Of Anemia Among HAART Naïve And HAART Experienced Adult HIV Patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November,2015 ..... 26**

**Table 5:- Possible Risk factors of anemia in HAART Naïve adult HIV patients at Art Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia From April-November,2015 ..... 28**

**Table 6:- Possible Risk factors of anemia in HAART Experienced adult HIV patients at Art Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015. ..... 29**

**List of Figures**

**Fig.1:- Study Procedure and Eligibility Criteria for Study Participants At ART Clinic Of Zewditu Memorial Hospital, Addis Ababa, Ethiopia, April- November, 2015. .....17**

**Fig. 2:- Frequency of Intestinal Parasites among Study participants at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015.....23**

**Fig. 3:- Types of Anemia among HAART Naïve and HAART Experienced Adult Anemic HIV Patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015.....26**

**Figure 4:- Relationship of hemoglobin with risk factors of anemia in HAART Naïve Adult HIV patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia, 2015. .....27**

**Figure 5:- Relationship of hemoglobin with risk factors of anemia in HAART Experienced Adult HIV patientsat Zewditu Memorial Hospital, Addis Ababa, Ethiopia, 2015……..29**

## List of Abbreviations

| | |
|---|---|
| 3TC | Lamivudine |
| ACD | Anemia of chronic disease |
| AIDS | Acquired Immune Deficiency Syndrome |
| ART | Antiretroviral Therapy |
| ATT | Anti-Tuberculosis Therapy |
| AZT | Azidothymidine |
| BMI | Body Mass Index |
| CBC | Complete Blood Count |
| CD | Cluster of Differentiation |
| D4T | Stavudine |
| DRERC | Departmental Research Ethical Review Committee |
| EDHS | Ethiopian Demographic Health Survey |
| EDTA | Ethylenediaminetetraacetic Acid |
| EFV | Efavirenz |
| EQA | External Quality Assurance |
| FACS | Fluorescence Activated Cell Sorting |
| fl | femtoliter |
| FMOH | Federal Ministry of Health |
| HAART | Highly Active Antiretroviral Therapy |
| HIV | Human Immunodeficiency Virus |
| Hg | Hemoglobin |
| IP | Intestinal Parasite |
| MCH | Mean Cell Hemoglobin |
| MCHC | Mean Cell Hemoglobin Concentration |
| MCV | Mean Cell Volume |
| mm | millimeter |
| NCNC | Normocytic Normochromic |
| NNRTI | Non-nucleoside Reverse Transcriptase Inhibitor |
| NVP | Nevirapine |
| OI | Opportunistic Infection |

| | |
|---|---|
| PCV | Packed Cell Volume |
| PI | principal investigator |
| Pg | Pico gram |
| PLWHA | People Living with HIV/AIDS |
| RBC | Red Blood Cells |
| RNA | Ribonucleic Acid |
| SD | Standard Deviation |
| SOP | standard Operating Procedure |
| SPSS | software package for social sciences |
| TB | Tuberculosis |
| TLC | Total leukocyte Count |
| USA | United States of America |
| WBC | White Blood Cells |
| WHO | World Health Organization |
| ZDV | Zidovudine |
| ZMH | Zewditu Memorial Hospital |

## Abstract

**Background:** Hematological abnormalities are manifested in human immunodefieicincy virus patients as a result of progressive damage to the body's immune system. They are documented to be the second most common causes and strong independent predictor of mortality and morbidity in people living with HIV/AIDS.

**Objective**: To determine prevalence and associated factors of anemia in highly active antiretroviral therapy (HAART) naïve and HAART experienced adult HIV positive individuals.

**Methodology**: A hospital based comparative cross sectional study was conducted from April to November, 2015 at Zewditu Memorial Hospital, Addis Ababa, Ethiopia. A total of 340 HIV infected adult individuals, 170 HAART naïve and 170 HAART experienced, were enrolled in the study. Participant's socio-demographic and clinical information were collected using pre-tested structured questionnaire. Blood and stool specimen were collected from each participant. Blood was examined for full blood count, CD4 and hemoparasite. Stool samples were screened to detect intestinal parasites. Descriptive statistics were used to express the socio-demographic characteristics of the participants. Binary and multiple logistic regressions were computed to assess association between variables using SPSS version 20. P value less than 0.05 was taken as statistically significant.

**Result**: - The overall all prevalence of anemia was 15.44%. The prevalence of anemia in HAART Naïve and HAART experienced patients was 24.71% and 7.1% respectively. Advanced WHO clinical stage (AOR= 9.63, 95% CI= 1.07-86.95), low CD4+ T cells count (<200) (AOR= 7.57; 95% CI= 2.17-27.01), and body mass index <18.5 (AOR= 7.56; 95% CI= 2.12-27.01) were found to be predictors of anemia in HAART naïve patients. On the other hand, none of the variables was found to be independently associated with anemia in HAART Experienced patients.

**Conclusion**: -Anemia is the common manifestation both in HAART naïve and HAART experienced patients. However, Prevalence of anemia is higher in HAART naïve HIV individuals than those on HAART. Therefore; further longitudinal study should be conducted to map every possible correlates of anemia and compare its burden in HAART Naïve and HAART experienced adult HIV patients.

**Keywords: Anemia, HAART-naïve, HAART- experienced, Risk Factors**

# 1. Introduction

## 1.1. Background

### 1.1.1. Definition of Anemia

Hematological abnormalities are manifested in human immunodefieicincy virus (HIV) patients as a result of adverse effects of drug therapy, the secondary effects of opportunistic infections or malignancies or other preexisting or coexisting medical problems. They are documented to be the second most common cause and strong independent predictor of mortality and morbidity in people living with HIV/AIDS (PLWHA). They are also generally marked with cytopenias such as thrombocytopenia, neutropenia and anemia [1, 2]

Anemia is a condition in which there is reduction in the number of circulating red blood cells (RBC) or hemoglobin concentration of the peripheral blood below the expected level for age, sex, residential altitude of an individual and pregnancy status for woman in reproductive age. The World Health Organization (WHO) classifies persons living at sea level as anemic with hemoglobin value below 13 g/dl in men 15 and above years of age, below 12 g/dl in non pregnant women over 15 years old, and below 11 g/dl in pregnant women [3].

### 1.1.2. Etiology of HIV-Related Anemia

The causes of anemia in HIV sero-positive population are multi-factorial in origin with opportunistic infections, nutritional deficiencies, certain medications (including antibiotics and antiretroviral agents), and infiltrative diseases of the bone marrow being among the leading causes [2, 4]. The pathophysiology of HIV associated anemia may generally involve three basic mechanisms: decreased RBC production, increased RBC destruction, and ineffective RBC production [5, 6].

Decreased RBC production may result from bone marrow infiltration, use of myelosuppressive medications such as zidovudine (ZDV), release of inflammatory cytokines, and decreased production of endogenous hematopoietic growth factors. Additionally, it may be directly attributable to the virus or may be caused by opportunistic infections, hypogonadism or a blunted response to erythropoietin [2, 6-8)]. Increased intravascular hemolysis or destruction of premature RBCs in reticuloendothelial system may occur in patients with HIV infection. Hemolytic anemia may be a consequence of RBC autoantibodies, hemophygosytic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, or glucose-6-phosphate dehydrogenase deficiency [6].

Anemia may also result from micronutrient deficiencies; most commonly deficiency is dietary iron coupled with malabsorption or impaired recycling of iron, as well as vitamin B12 or folic acid deficiencies [5, 6]. The most common explanation for poor vitamin B12 is a low intake of animal-source foods and malabsorption due to probably as a result of an array of infections or other conditions that affects the gastric mucosa in HIV- infected patients. Whereas, the primary cause of folate deficiency is low intake of sources rich in the vitamin such as green leafy vegetables. Other situations that impose risk of folate deficiency include lactation and alcoholism [6, 9].

### 1.1.3. Effect of Highly Active antiretroviral therapy (HAART) on anemia

In order to combat the outcome of the virus, use of a combination ART is more effective than just one medicine (monotherapy). It consists of 2 nucleoside analogues (reverse transcriptase inhibitors) [either zidovudine (AZT) or stavudine (d4T) along with lamivudine (3TC)] and 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) [either nevirapine (NVP) or efavirenz (EFV)] [10, 11)]. Therefore, the introduction of highly active antiretroviral therapy (HAART) is accepted as the current standard of care for treating patients who live with HIV/AIDS. This is due to its capacity to profoundly suppress viral replication; it increases CD4+ T cell count and delays disease progression and subsequent death [10, 12].

Based on recent estimates from the World Health Organization (WHO), highly active antiretroviral therapy (HAART) was accessible to 15.8 million people globally in 2015 of which 41% of them were all adult HIV patients. In 2014, 10.7 million HIV/AIDS patients living in Sub-Saharan Africa were able to access HAART [13]. Despite of the recent increases in access to HAART and its high potential for disease management, patients commonly suffer from side effects of the drug and need to change the HAART regimen owing to its toxicity [12,14]. The adverse reactions are experienced by 80% of HIV infected patients within the first twelve months of therapy [15].

Studies indicated that some antiretroviral regimens containing nucleoside analogue reverse transcriptase inhibitors, especially zidovudine may cause anemia or worsen pre-existing anemia as it is a potent myelosuppressive medication. For this reason, zidovudine is usually excluded from the antiretroviral regimen of severely anemic patients [10, 16]. However, there is a huge variation in adverse effects of HAART that occur in developed countries and those occurring in developing countries for several reasons. One of the many reasons of being economic constraints is that it limits the repertoire of accessible antiretroviral medications, making a handful of drugs responsible for most

toxicities in developing countries. Prohibitory laboratory monitoring costs may occasionally delay the diagnosis of specific toxicities, thereby increasing their severity. Co-morbid conditions that are more prevalent in resource-limited regions, such as anemia and malnutrition; initial presentation with advanced immunosuppression; use of concomitant anti-tuberculosis therapy (ATT); and use of herbal medications may influence the incidence of adverse effects as well [17].

### 1.1.4. Consequences of anemia in HIV patients

The consequence of untreated anemia may lead to multisystem disabling symptoms and fatigue, exhaustion, increased requirement for erythropoietin therapy, increased risk of HIV dementia, poor quality of life and possibly even exacerbated poverty in communities with a high HIV prevalence [18].

Therefore, regular follow up and monitoring the patient by assessing the level of CD4 count, hemoglobin and body mass index (BMI) may improve the quality of life in HIV infected patients [ 10, 19]. It is also important to identify risk factors that can be both environmental or host factors in order to prevent or retard the occurrence of anemia in HIV infected population and ameliorate its severity [5].

## 1.2.Statement of the Problem

Anemia is among extensively spread global public health problems affecting both resource-limited and well-developed countries. It has a major contribution for human ill-health as well as influencing socio-economic development. Despite the lack of accurate data and widely differing estimates of the prevalence of anemia, significantly elevated proportion of young and women of childbearing age are found to be anemic. Although it has been recognized as a major debilitating health problem for many decades, little advancement has been achieved and the global prevalence of anemia remains unacceptably high. [20, 21]

According to a 2008 WHO report, anemia affected nearly 2 billion people, which corresponds to 24.8% of the global population. The highest prevalence was shown to be in pre-school age children (42.4%), and the lowest prevalence was in men (12.7%). The population group with the greatest number of individuals affected was non-Pregnant women [21].

Prevalence of anemia in the lower income regions of sub-Saharan Africa increased from 16.4 % in 1990 to 23.9 % in 2010. Over the same time period, higher –income regions of the world recorded the lowest prevalence estimates, all less than 25%. Iron deficiency anemia, hookworm infection, sickle cell disorders, thalessemias, schistosomiasis and malaria are the known top 6 causes of anemia worldwide. However, in eastern sub-Saharan Africa, iron deficiency and schistosomiasis play a major role for the highest prevalence of anemia in the subcontinent. [22].

Detection of hematological disorders in HIV patients followed shortly after the first cluster of AIDS cases was identified [23]. Worldwide, the prevalence of anemia in persons with HIV infection has been determined in many studies with values that range between 16.2% and 70.1% [24,25]. It is also shown to be developed in close to 58% of HIV infected patients before the initiation of HAART [16]. There is persistent anemia negatively affecting prognosis in up to 75.8% of HAART experienced patients regardless of anemia response to combination antiretroviral therapy. This makes it more common than thrombocytopenia or leucopenia in patients with AIDS [25,26]. Beside its commonness in people having HIV, there has been recognized linkage between anemia and decreased survival in this population. Many studies have found an association between anemia during established infection and a faster progression to AIDS and death [27, 23,28].

Even though there is no a comprehensive ballpark figure illustrating the burden of anemia in HIV/AIDS infected population living in sub-Saharan Africa, there are a lot of related studies conducted in individual countries of the region to determine the prevalence and establish the possible correlates of anemia. In addition, the studies revealed anemia as the biggest challenge for having a quality life and survival of people living with HIV/AIDS (PLWHA). For instance, a study from South Africa reported that overall prevalence of anemia at ART initiation was 25.8% [7]. In Nigeria, a study stated that 57.5% of HAART-naive participants were 3 times at risk of anemia compared with 24.3% of HAART-experienced participants [16].

According to a report launched by the Federal Ministry of Health (FMOH) in 2013, there were an estimated 793,700 (716,300-893,200) people living with HIV including 200,300 (172,400 – 232,400) children in Ethiopia. There were approximately 45,200 (36,500-55,200) AIDS related deaths in 2013 and about 898,400 (770,700 – 1,048,500) AIDS orphans in the same year. HIV adult prevalence is estimated at 1.5% in 2011. By the end of June 2013, the number of people ever enrolled in chronic care reached 728,874 while the number ever started ART was 439,301 and 317,443 were currently receiving ART. Only 70.3% of individuals who ever started ART are currently on treatment indicating challenges in patients' retention [29].

The prevalence of anemia in HIV infected Ethiopians was predicted by previous studies in different study settings .The overall prevalence of anemia was rated between 23% -70.1% [2, 25]. In Addis Ababa, only few studies have been conducted to show the burden of anemia in PLWHA. For instance, a retrospective cohort study from 2011 to 2012 at ZMH estimated a 42.9% of prevalent anemia at baseline. However, the prevalence significantly decreased to 20.9% at 6 months and to 14.3% at 12 months after HAART initiation [30].

Based on the findings of the studies, higher prevalence of anemia was found among females, patients having low CD4+ T cell counts, rural resident and HAART naïve patients. Besides, HAART regimens containing ZDV, HAART duration and opportunistic infections were also determined as predictors for the presence of anemia [2].

## 1.3.Significance of the study

HIV infected individuals are at higher risk of developing severe hematological complications like anemia. Thus, early diagnosis and treatment of anemia is essential to improve the quality of life of the patients [10].

The existence of co-morbid infections, malnutrition, the effect of ART as well as other therapeutic drugs and additional potentially predisposing or worsening conditions increase the probability of anemia in this population. Even though area specific studies are mandatory to profile HIV-associated anemia, only few studies were carried out in different regions of Ethiopia to establish the prevalence and to show possible associated factors of anemia focusing on HIV infected adult individuals.

Therefore, this study determined the prevalence of sever, moderate and mild anemia and their predictors. Moreover, we identified the morphological types of anemia and compared their severity between HAART naïve group of patients and those on HAART.

The finding of this study is believed to provide useful information to policy makers so that they can set prevention programs and update treatment protocols for proper management, follow-up and care of HIV patients. Evidence based planning for diagnosis and management of anemia in this population group is an added significance for the hospital management. Also, it can strengthen knowledge about the subject revealed by previously conducted similar studies.

## 1.4.Literature Review

### 1.4.1. Anemia and Associated Factors in HAART Experienced Patients

Zidovudine is the drug of choice in the first line antiretroviral regimen because it suppresses viral replication, plays a crucial role in reconstitution of immunity, and decreases AIDS related opportunistic infections. However, it is associated with life threatening hematological adverse effects like anemia. A study conducted in India by Agrawal *et al*. reported that out of 1256 patients on ZDV regimen, 203 (16.2%) (143 females) developed anemia having hemoglobin values of <8 g/dl. Severe anemia (<6.5 g % of Hb) was recorded in 100 (7.9%) patients. The study also revealed that women were more prone to develop anemia. Therefore, the study highlighted the need to regular monitoring of patients; particularly women on ZDV based antiretroviral regimens since there was high incidence of ZDV- induced anemia [24]. Another similar study was also conducted in Guwhati, India in 2013 by Reddy *et al* to assess adverse drug reaction in HIV patients on ART. Of 300 patients under study, 8.13% patients developed anemia [31].

Prevalence and predictors of anemia at the initiation of combined antiretroviral therapy were retrospectively assessed in across-sectional study conducted by Mijiti *et al*. in China. Out of 2252 patients, the prevalence of mild, moderate, and severe anemia at the initiation of combined antiretroviral therapy were 19.2%, 17.1%, and 2.6%, respectively. Overall, 38.9% of the patients were anemic at the initiation of combined antiretroviral therapy. Following the multivariate logistic regression analysis, female gender, lower CD4 count, lower body mass index value, self-reported TB infection, and oral candidiasis were associated with a higher prevalence of anemia [32].

Anemia with microcytosis plus hypochromia is more frequently seen in patients with advanced AIDS disease rather than in HIV infection alone. Jam et al. evaluated the morphology of red blood cells in 642 HIV infected patients in Iran. As a result, Macrocytosis was seen in 11%, normocytic normochromic (NCNC) RBC in 41.1% and microcytic hypochromic anemia in 47.9% of the study population. The overall prevalence of anemia in the patients was 10.3%. In addition, their study showed positive association between anemia and drug history such as use of anti-TB drugs and ART regimens containing Zidovudine (AZT), lamivudine (3TC) and stavudine (d4T). Presence of opportunistic infection, clinical stage of AIDS and female gender were also associated with anemia [33].

A total of 9690 HIV infected patients participated in a multicentre cross sectional study conducted in USA by Mildivan *et al*. overall prevalence of anemia and marked anemia was found out to be 36% and 5% among the study population respectively. Out of the total population, 7252 were on HAART and35.5% of them subsequently being anemic. The study revealed that anemia is most prevalent among men. In contrary, marked anemia was common in women. Besides, no ART regimen was associated with increased risk for marked anemia [34].

In 2008, Denue *et al* had done a study in Nigeria among 534 HIV patients to assess the impact of HAART in resolving immunological and hematological complications. They reported that among the HIV patients studied, HAART-naïve participants were 3 times at risk of anemia compared with HAART- experienced participants. Additionally, the mean total leukocyte count (TLC) in HAART-experienced participants was significantly higher than the mean TLC in HAART-naïve participants. Consequently, HAART-Naïve participants had 4 times increased risk of developing leucopenia.This study revealed that HAART has the capability of reducing the incidence of anemia, other deranged hematological and immunological parameters associated with disease progression, and death in HIV-infected patients [16].

Among the several factors associated with anemia, co- infection with HIV and Malaria is very common in most tropical countries since malarial infection is endemic in most countries of the region. Therefore, it is highly probable for HIV patients living in these countries to be infected by plasmodium species at one time or the other during the course of their infection.A cross-sectional study at Benin Teaching Hospital, Benin City, Nigeria was conducted to assess the prevalence of *Plasmodium falciparum* infection in HIV-infected persons on HAART. Of the 285 recruited patients, 129 (45.26%) had anemia. All six patients that had malaria parasitemia were anemic (4.65%).Anemia was significantly associated with asymptomatic malaria infection among HIV patients on HAART with an odd ratio of 16.47. The authors concluded that measures must be taken to reduce malaria infection and anemia among HIV patients on HAART [35].

Drug induced anemia is commonly associated with prescription of AZT. It causes suppression of bone marrow and induces megaloblastic anemia. In 2013, a study conducted at Gonder university hospital, Ethiopia by Tadele *et al* aimed to determine the prevalence of zidovudine induced megaloblastic anemia in HIV/AIDS patients. The prevalence of AZT induced anemia among the study popultion was36 (11.3%). among the anemic patients, 50% (18/36) showed mild type of anemia.

Macrocytic anemia (Mean Cell Volume (MCV) >100 fl) was developed in 29 (80.6 %) of the patients at the 6th months of initiation of AZT. The authors highlighted the need for pharmacological studies on AZT to elucidate its mechanism of action in inducing megaloblastic anemia [36].

### 1.4.2. Anemia and Associated Factors in HAART-Naïve Patients

HIV-related anemia may also emanate from underlying chronic diseases like hepatitis co- infection. Due to the effect of inflammation, iron will be diverted from the circulatory system to reticuloendotelial systems and other iron storage sites. This renders iron to be unavailable for hematopoiesis. In addition, iron maldistribution increases susceptibility for opportunistic infections thereby accelerating disease progression. Wisaksan *et al* conducted a three-year cohort study in Indonesia on HIV infected patients to determine the prevalence of anemia and iron homeostasis. The study enrolled 611 ART naïve and 258 ART experienced patients. In ART- naïve stratified group, mild, moderate and severe anemia was present in 30.4%, 14.1% and 4.6%, respectively. In this same group, anemia was mostly normocytic normochromic and characterized by normal or low reticulocyte index suggesting 'anemia of chronic diseases' [37].

A cross-sectional analytical study was done by Mata-Marin *et al* in Mexico to establish risk factors and correlates for anemia in HIV infected treatment naïve patients without co-infection or opportunistic infections. A total of 54 men and 9 women participated in the study. The overall rate of anemia was 20.3% and only one patient (1.58%) had a severe anemia. Microsytosis was found in 2 patients (1.5%) and was associated with anemia. Nobody had macrocytosis (MCV> 100 fl) while 85% of the study population had normocytic normochromic anemia. WBC < 4000 cells/ $mm^3$, platelets < 200,000 cells/ $mm^3$, CD4 + cells < 200 cells/ $mm^3$ and HIV RNA viral load > 100,000 copies/ ml were associated with increased risk of anemia [38].

Similarly, another institution based cross sectional study was done in China on newly diagnosed adult HIV patients during 2009 and 2010 to establish the prevalence of anemia among 1948 patients. The overall prevalence of anemia was 51 %. (51.5% among men and 53.2% among women). The prevalence of mild, moderate, and severe anemia was 32.4%, 17.0%, and 2.5% respectively. The study stated that the prevalence of anemia increased with increasing age and with decreased CD4+ T cell count. It also indicated the importance of routinely screening anemia for timely and adequate clinical management [39].

In a- ten- year- cohort study carried out in Puerto Rico, 1486 patients participated. The study showed the prevalence, associated factors and impacts on one year mortality of anemia in HIV infected Hispanics. High prevalence of anemia (41. 5% of 1,486 patients) was observed in the study. Unemployment, CD4 count less than 200 per micro litter, HIV Viral load > 100,000 copies/ ml, white blood cell count < 4,000 cells/ ml and having at least one AIDS defining conditions showed significant association with increased odds of anemia. On the contrary, being overweight or obese favored for decreased odds of anemia when it was compared to having normal BMI. Sex, age, level of education, platelets counts and antiretroviral medication were not associated with the presence of anemia [23].

Hematological parameters in HIV- infected and uninfected Rwandan women were determined by a cross-sectional study done by Munyazesa *et al* in 2011. A total of 710 HIV-infected HAART- naïve and 226 HIV-uninfected women were included in the study. Prevalence of anemia (hemoglobin (Hb<12.0 g/dl) was higher in the HIV positive group (20.5% vs. 6.3%; p<0.001), and increased with lower CD4 counts: ≥350 (7.6%), 200–349 (16%) and <200 cells/mm3 (32.2%). Marked anemia (Hb<10.0 g/dl) was found in 4.2% of HIV positive patients. High prevalence of anemia was more common in the HIV-infected than in uninfected women, especially those with greater disease progression as indicated by lower CD4 cell counts [40].

A retrospective study was also carried out at Gondar University hospital northwest, Ethiopia to assess the prevalence and related factors of anemia in HAART naïve HIV positive patients. The overall prevalence of anemia was 138 (35%) and majority128 (32%) of patients had mild to moderate anemia. Mild to moderate anemia and severe anemia occurred in 10 (3%) patients, respectively. Female HAART naive HIV positive patients had significantly higher prevalence of anemia than males (62% Vs 38%) [27].Another similar hospital based cross-sectional study was conducted by Addis *et al*at same hospital. Anemia was observed among 42.3% of all 189 newly diagnosed adult patients. Severe anemia was found in 6.25% and mild anemia in 93.75% of the study population [41].

In 2012, a total of 234 HIV positive individuals were enrolled in a facility based comparative cross-sectional study conducted by Gedefaw *et al* at Jimma University specialized hospital in south west Ethiopia. Using the Hb level of the participants', the overall prevalence of anemia was found to be 23.1%. Nineteen (16.2%) of HAART experienced and 35 (29.9%) of HAART naïve participants were anemic from their perspective group. From the total anemic individuals; 1 (1.9%), 14 (25.9%), and 39 (72.2%) had severe, moderate and mild anemia respectively. Presence of opportunistic infection, CD4

Count< 200 cells/ μL, and rural residence for HAART naïve participants; HAART regimen and duration of HAART for HAART experienced group were independently and significantly associated with anemia [2].

Intestinal parasites are common infections and also associated with acute and chronic diarrhea and even weight loss in HIV/ AIDS patients. In a comparative cross sectional study conducted by Teklemariam *et al* at Hiwot Fana specialized university hospital, Eastern Ethiopia, 371 HIV- infected individuals (112 ART- naïve and 259 on ART) were recruited. The study aimed to determine the prevalence of parasitic infection in the study participants. Overall, 33.7% of the study population was found to have a parasitic infection. Yet, ART naïve group (45.5%), diarrheic (53%), and those with CD4 less than 200cells/ μL (46%) had significantly higher prevalence. *Entamoeba histolytica/E.dipar* (13.5%), *Giardia lamblia* (8.1%), *Strongyloides stercoralis* (4.0%) and cryptosporidium Species (2.2%) were the most commonly identified parasites [42].

# 2. Objectives and Hypothesis

## 2.1.General objective

- To determine prevalence and associated factors of anemia in highly active antiretroviral therapy naïve and HAART experienced adult HIV positive persons at Zewditu Memorial Hospital from April–November, 2015.

## 2.2.Specific objectives

- To determine the prevalence of Anemia among HAART naïve and HAART experienced HIV positive participants
- To morphologically characterize types of anemia among HAART naive and HAART experienced patients
- To compare prevalence of anemia between HAART naïve and HAART experienced HIV patients
- To determine severity of anemia in HAART naïve and HAART experienced HIV patients
- To identify the associated factors of anemia in HAART naïve and HAART experienced HIV positive persons

## 2.3.Hypothesis

- There is no difference between prevalence of anemia in HAART naïve HIV infected individuals and HAART experienced patients

# 3. Materials and Methods

## 3.1.Study area

The research project was done at Zewditu Memorial Hospital in Addis Ababa, Ethiopia. It is one of the oldest hospitals in Ethiopia and is located in kirkose sub-City. It was built, owned and operated by the Seventh - day Adventist church but was nationalized during the Derg regime in about 1976. It is Ethiopia's leading hospital in the treatment of ART patients and where the first ART program of the country started in 2003. As the largest and model HIV clinic in the country, Zewditu memorial hospital offers comprehensive HIV and integrated treatment. Currently the center treats more than 6,500 HIV patients each month as 200 clients are being seen daily. The ART clinic is run by three physicians and nine nurses, two Health officers, nine data clerks [43].

## 3.2.Study Design

A hospital based comparative cross sectional study design was employed in this study

## 3.3.Study Period

The study was performed from April to November, 2015 at Zewditu memorial hospital, Addis Ababa, Ethiopia.

## 3.4.Population

### 3.4.1. Source population

HIV positive patients attending at Zewditu memorial hospital during the study period

### 3.4.2. Study population

Adult HIV patients attending ART clinic of Zewditu memorial hospital that fulfilled the inclusion criteria

## 3.5.Eligibility

### 3.5.1. Inclusion Criteria

All adult HIV patients ($\geq$ 18 years old) who visited the ART clinic of Zewditu memorial hospital during the study period

### 3.5.2. Exclusion Criteria

Pregnant women, patients who were on treatment for anemia in the past three months, and HAART experienced persons who took HAART for less than 3 months were excluded.

## 3.6.Operational Definitions

**Severe anemia** is when both non-pregnant women and men aged 18 and above have hemoglobin concentration lower than 8 g/dl.

**Moderate anemia** is hemoglobin concentration between 8-10.9 g/dl in both non- pregnant women and men at 18 years of age and above.

**Mild anemia** is when non-pregnant women ≥18 years old have hemoglobin concentration in the range of 11.0- 11.9 g/dl; and men ,aged 18 and above, having 11.0- 12.9 g/l of hemoglobin concentration

**Working Functional Status**: - Able to perform usual work in or out of the house

**Ambulatory**: - Able to perform activities of daily living

**Bedridden**: - Not able to perform activities of daily living

## 3.7.Study variables

### 3.7.1. Dependent variables

- Prevalence of anemia
- Severity of anemia
- Types of anemia
- Association between factors and anemia

### 3.7.2. Independent variables

-Socio demographic characteristics (sex, age, occupation,)

- Clinical variables
    - Functional Status
    - Body Mass Index,
    - Opportunistic infection,
    - TB infection
    - Intestinal Parasites,
    - hemoparasites,
    - HAART regimen,
    - other therapeutic drugs

- Duration of HAART in months,
- WHO clinical stages of HIV/AIDS
- CD4+ T cells count

## 3.8.Sample size calculation and sampling technique

### 3.8.1. Sample size calculation

The minimum required sample size to determine the two population proportion (i.e. prevalence of anemia in HAART-naïve and HAART experienced adult HIV patients) was calculated using the formula discussed by VK Chadha [44] and we checked its validity using OpenEpi soft ware version 3.03 (http:// www.openepi.com)

$$N=2\times\left[\frac{\left(z_{cv}\sqrt{2\bar{p}(1-\bar{p})}+z_{power}\sqrt{p_1(1-p_1)+p_2(1-p_2)}\right)^2}{(p_1-p_2)^2}\right]$$

$$\bar{p}=\frac{(p_1+p_2)}{2}$$

where;

n is the sample size estimate for one group

Zcv = Z critical value for alpha (.05 alpha has a Zcv of 1.96)

Zpower = Z value for 1-beta (.08 power has a Z of 0.842)

$p_1$ is the prevalence of anemia in HAART naïve HIV patients (52.6%) (Adane et al,2012)[19]

$P_2$ is the prevalence of anemia in HAART experienced patients (37.4)(Adane et al,2012)

$$N1= 2x\left(\frac{(1.96\sqrt{0.495}+0.842\sqrt{0.483})^2}{(0.526-0.374)^2}\right)$$

$$=2x\left(\frac{1.964}{0.0231}\right)$$

=170

Therefore, A total of 340 HIV infected adult patients (i.e. 170 HAART naïve and 170 HAART experienced )were enrolled in the study.

### 3.8.2. Sampling Technique

Consecutive sampling technique was used and the participants were grouped into HAART naïve and HAART experienced HIV patients.

## 3.9.Study Procedure and Data collection

HIV patients who visited the Adult ART clinic of Zewditu Memorial Hospital during the study period were screened for eligibility criteria by trained clinical nurses. Then, after explaining the type, purpose and benefits of the study, participants who gave their written informed consent were sent to the hospital laboratory to give blood and fresh stool specimens. Well- experienced phlebotomists collected venous blood with a volume of 4ml venous blood in ethylenediaminetertacetic acid (EDTA) vacutainer tube from each participant. A stool specimen was also collected in clean, leak proof and wide-mouthed standard caps. All specimens were properly labeled with patient codes. Hematological parameters were determined using Cell-DYN 1800 automated hematology analyzer (Abbot Laboratories Diagnostics Division, USA). CD4+ T cells were counted by BD FACSCalibur machine (Beckton Dickenson and Company, California, USA).

Thick blood films were prepared and stained using 10% Geimsa working solution to detect malarial infection. Direct wet mount, McMaster concentration technique and modified zheihl-Neelson techniques were used to detect intestinal parasites. Participant's weight and height measurements were taken using Seca medical Scale (Seca GmbH & Co.KG, Hamburg, Germany) at ART outpatient department to calculate their body mass index (BMI). Socio-demographic and clinical data of the participants were obtained using pre-tested data extraction format.

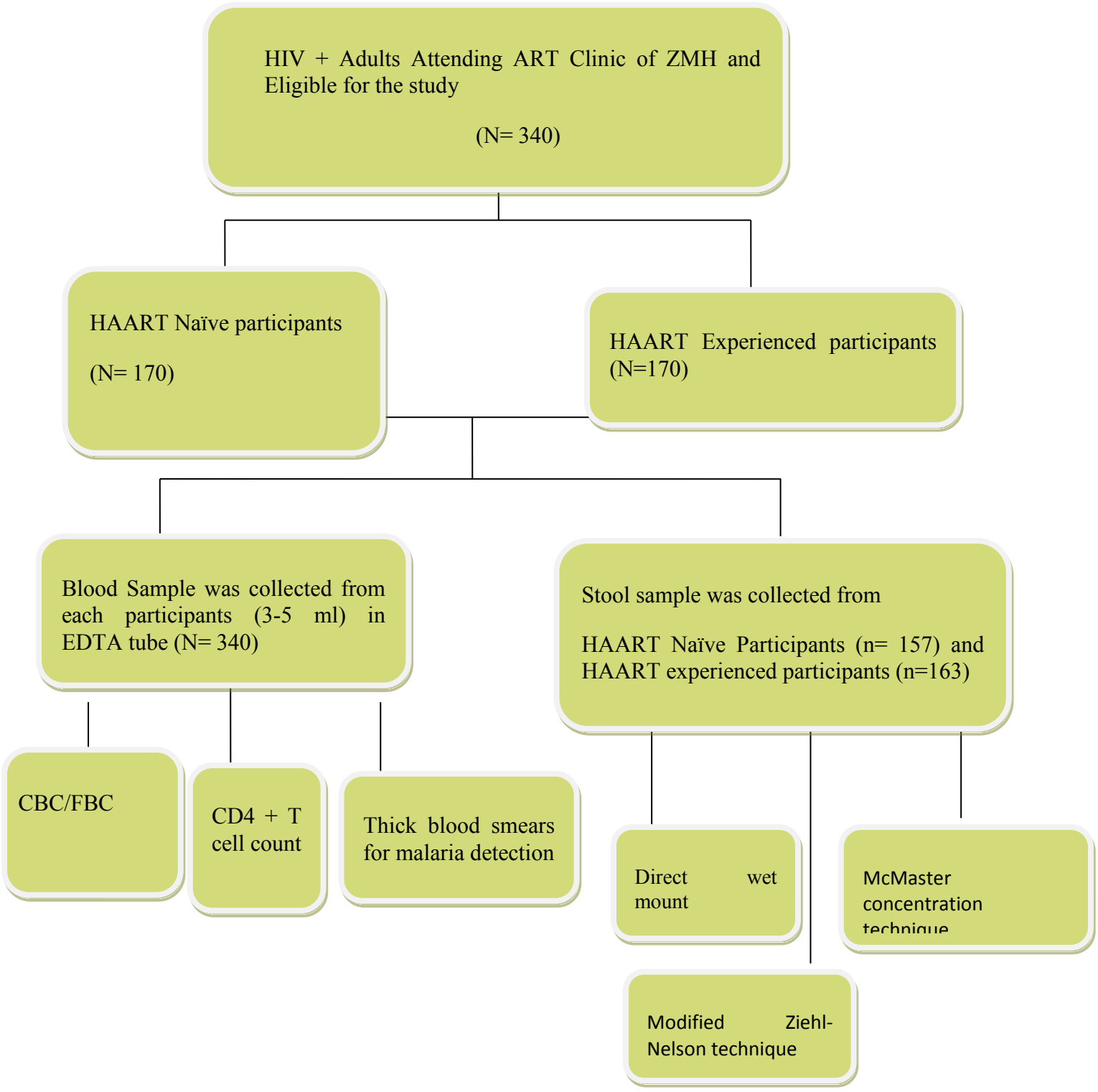


**Fig 1: Study Procedure and Eligibility Criteria for Study Participants At ART Clinic Of Zewditu Memorial Hospital, Addis Ababa, Ethiopia, April- November, 2015.**

### 3.9.1. Principle of Cell-Dyn 1800 Hematology analyzer

Cell Dyn 1800 is a 3-part differential hematology analyzer which uses a combination of electrical impedance, optical scatter, light absorption and fluorescence methods to produce complete red blood cell, platelet and leukocyte analysis. Cells are sized and counted by detecting and measuring changes in electrical resistance when a particle passes through a small aperture. The number of pulses is proportional to the number of cells counted. The size of the voltage pulse is also proportional to the volume or size of the cell

### 3.9.2. Thick Blood Film for Malaria Detection

The definitive diagnosis of malaria infection is based on finding malaria parasites in blood films. With a thick blood film, the red cells are approximately 6-20 layers thick which results in a larger volume of blood being examined. Therefore, examination of a thick blood film is recommended sincemalaria parasites may be missed on a thin blood film when there is a low parasitemia.

### 3.9.3. Direct Wet Mount Preparation of Fresh Stool specimen

The direct wet mount is used primarily to detect motile protozoan trophozoites. These organisms are very pale and transparent, two characteristics that require the use of low light intensity. The direct wet smear is prepared by mixing a small amount of stool (about 2gm) with a drop of 0.85% NaCl. This mixture provides a uniform suspension under a 22 by 22 coverslip .The entire 22 by 22 mm cover slipwas systematically examined with the low power objective (10X) and low intensity; any suspicious objects was then examined with the high dry objective (40X).

### 3.9.4. Modified Zheil Neelsen Technique

The lipid capsule of acid fast oocyts of protozoa takes up carbol fuschin and resists decolorarization with a diluted acid rinse. The lipoid capsule of the organisms is of such high molecular weight that is waxy at room temperature and successful penetration by the aqueous based staining solutions is prevented. The oocyts stain red taking the color of the primary stain because of mycolic acid and the background stain green because of malachite green.

### 3.9.5. McMaster Concentration Technique

It is based on the principle of differential density. Parasite eggs sinks in water, but they will float in various chemical solutions that are more dense than water (i.e. solutions that have higher specific gravity) because the eggs are lighter than the fluid used as floatation solution. For this research project we used zinc sulphate, 7, hydrate salt (UNI CHEM, Mumbai, India).

## 3.10. Data Quality Assurance

### 3.10.1. Pre-Analytical Data Quality Assurance

1. Participants screening for eligibility criteria were conducted by trained nurses. The screening procedure was monitored by the principal investigator.
2. Venous blood and stool specimens collected from participants were properly labeled
3. Socio-demographic characteristics and clinical data of the participants were collected by trained data collectors
4. Appropriate protocols or SOPs were written and/or used according to the manufacturer's instructions for all the laboratory tests
5. All reagents were checked for their expiry date and prepared and handled according to manufacturer's instructions

### 3.10.2. Analytical Quality Assurance

1. Protocols for each laboratory tests were strictly followed
2. Quality control materials were used to check for accuracy and precision of hematological (three level controls) and CD4 (low, medium and high controls) analyzers
3. Appropriate reagents were used for each analysis and before their expiry date.
4. All tests were run by experienced laboratory technologists

### 3.10.3. Post Analytical Data Quality Assurance

1. The data were rechecked on daily basis.
2. All clinical and laboratory result of each test were recorded and documented properly
3. Randomly selected samples were reanalyzed and checked by the supervisor or the principal investigator

Moreover, Zewditu Memorial Hospital Laboratory participates in an External Quality Assurance (EQA) program which all in all earned it 3 stars, on an annual onsite assessment performed in 2005 E.C. and 2006 E.C. as per WHO AFRO Checklist. This indicated that the Laboratory had a good performance.

### 3.11. Statistical Analysis

Data were double entered into Microsoft excel and IBM SPSS version 20(SPSS INC, Chicago, IL, USA) for analysis. We checked the consistency of data by tabulating variables and making simple frequencies using both Microsoft Excel and SPSS. Descriptive statistics (mean and standard deviation (SD) were used to summarize continuous variables and simple frequencies were done to show the distribution of the socio-demographic and clinical characteristics of the patients. Chi square tests were used to show association between categorical variables. Unpaired T tests were computed to compare the means of all continuous variables between HAART Naïve and HAART experienced participants. Bivariate analysis was performed to calculate unadjusted odds ratio with 95% confidence interval to quantify the association between anemia and its possible predictors. Then, multiple logistic regressions were performed by entering variables that have shown significance during bivariate analysis to obtain adjusted effects. P- Value $< 0.05$ was considered as statistically significant. Correlation analysis was done to quantify the direction and strength of the linear association between hemoglobin values and risk factors.

### 3.12. Ethical consideration

Ethical clearance was obtained from departmental research and ethics review committee (DRERC) of the department of medical laboratory science. The proposal was also reviewed by ethical review board of Addis Ababa city administration Health Bureau. Permission was obtained from hospital authorities to conduct the study. Written informed consent (signed or thumb print) was gained from each participant. Confidential identifiers were used to code participant's identities. Results and any information regarding patients are kept confidential during and after the completion of the research project by password protected electronic programs and locking hard copy files. Hematological and parasitological analysis results were available to the treating physicians.

# 4. Result

## 4.1.Socio-demographic and Clinical Characteristics of the Participants

A total of 340 (170 HAART Naïve and 170 HAART Experienced)HIV patients were recruited intothis study. Table 1 describes the proportion of socio-demographic and clinical characteristics of the study population. The majority of the patients were females both in HAART naïve and HAART experienced groups with the proportion of 62.4% (106/170) and 59.4% (101/170) respectively, though the difference did not reach to a statistically significant level. The mean age of the patients was 39.92± 11.24 years. HAART experienced patients were older (42.62 ± 11.09) when they were compared to their HAART Naïve counter parts (37.22 ± 10.76). This difference was statistically significant (p =0.000).

All of the study participants were urban residents. Most of the patients (89.71%) (305/340) had working functional status. The majority (67.65 %) (230/340) of patients had CD4 + T-lymphocyte count greater than two hundred and 83.82 % were classified as WHO clinical stage I/II. However, half of HAART Naïve patients have CD4 + T cell count less than 200 per a micro liter of whole blood.Of the total 340 study patients, 22.65% were co-infected with opportunistic infections. HAART-Naïve participants (42.4%) had higher proportion of opportunistic infection than those on HAART (2.9%). Tuberculosis infection (pulmonary or extra-pulmonary TB) was present in 17 (5%) of the study participants and 16 were detected from HAART Naïve patients. The body mass indicesof 326 patients were calculated to assess for nutritional status of the participants and 17.79%, 55.21% and 26.99 % were underweight, normal and overweight respectively. The majority of the patients (55.29 %) (188/340) were taking other therapeutic drug(s) at the time of enrollment. Of which, 122 (64.89%) were from HAART- Naïve participants (Table 1).

Thick blood films was prepared and examined to assess malaria infection but no patient was found to have the hemoparasite. Stool samples of 157 HAART Naïve and 163 HAART experienced patients were screened. Intestinal parasites were detected in 10 (5.88%) of HAART Naïve and 13 (7.7 %) of HAART experienced participants (Table 1). Except for gender, the difference between the two groups for all the listed variables in the table did reach to a statistically significant level.

**Table1:- Socio-demographic and Clinical Parameters of HAART Naïve and HAART Experienced adult HIV Patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia, 2015.**

| Variable | | ART Status | | P- Value |
|---|---|---|---|---|
| | | HAART Naïve n= 170 (50%) | HAART Experienced n=170 (50 %) | |
| **Sex** | Male | 64 (37.6%) | 69(40.6%) | |
| | Female | 106 (62.4%) | 101(59.4%) | 0.657 |
| **Age** | 18-35 | 106 (62.4%) | 72(42.41%) | |
| | 36-59 | 59(34.7%) | 90 (52.91%) | |
| | >60 | 5 (2.9%) | 8 (4.7%) | |
| **Mean± SD** | | 37.22 ± 10.76 | 42.62 ± 11.09 | 0.000 |
| **Employment status** | Employed | 140 (82.4%) | 154 (90.6%) | |
| | Unemployed | 30 (17.6%) | 16 (9.4%) | **0.039** |
| **Residence** | Urban | 170 (100%) | 170 (100%) | |
| | Rural | 0 (%) | 0 (0%) | |
| **Functional status** | Working | 140 (82.4%) | 165 (95.1%) | **0.000** |
| | Ambulatory | 27 (15.9%) | 3 (1.8%) | |
| | Bedridden | 3 (1.8%) | 2 (1.2%) | |
| **TB-infection** | Yes | 16 (9.4%) | 1 (0.6%) | **0.000** |
| | No | 154 (90.6%) | 169 (99.4%) | |
| **CD4 Count** | <200 | 85 (50%) | 25 (14.7%) | |
| | 200-500 | 44 (25.9%) | 81 (47.6%) | |
| | >500 | 41 (24.1%) | 64 (37.6%) | |
| **Intestinal parasite** | Yes | 10 (5.88) | 13(7.7%) | |
| | No | 147 (86.4%) | 150 (88.2%) | **0.004** |
| | Missing | 13 (7.64%) | 7 (4.1%) | |
| **BMI (kg/ $m^{2)}$** | <18.5 | 31 (18.2%) | 27(15.9%) | |
| | 18.5-24.99 | 88 (51.8%) | 92(54.1%) | |
| | ≥25 | 40 (23.5%) | 48(28.2%) | |
| | Missing | 11 (6.5%) | 3(1.8%) | |
| **Opportunistic infection** | Yes | 72 (42.4%) | 5(2.9%) | **0.000** |
| | No | 98 (57.6%) | 165(97.1%) | |
| **Malaria infection** | Yes | 0 (0%) | 0(0%) | |
| | No | 170 (100%) | 170(100%) | |
| **WHO clinical Stage** | I | 100 (58.8%) | 166 (97.6%) | **0.000** |
| | II | 18 (10.6%) | 1(0.6 %) | |
| | III | 29 (17.1%) | 2 (1.2%) | |
| | Iv | 21 (12.4%) | 1 (0.6%) | |
| | Missing | 2 (1.2%) | _ | |
| **Other therapeutic drugs** | Yes | 122 (71.8%) | 66 (38.2%) | **0.000** |
| | No | 48 (28.2%) | 104 (61.2%) | |

Both groups combined, the most prevalent intestinal parasites were cryptosporidium species (47.83%) followed by *Entamoeba histolytica/ dispar* (21.74%) and *Giardia lamblia* (17.39%) (Figure 2)

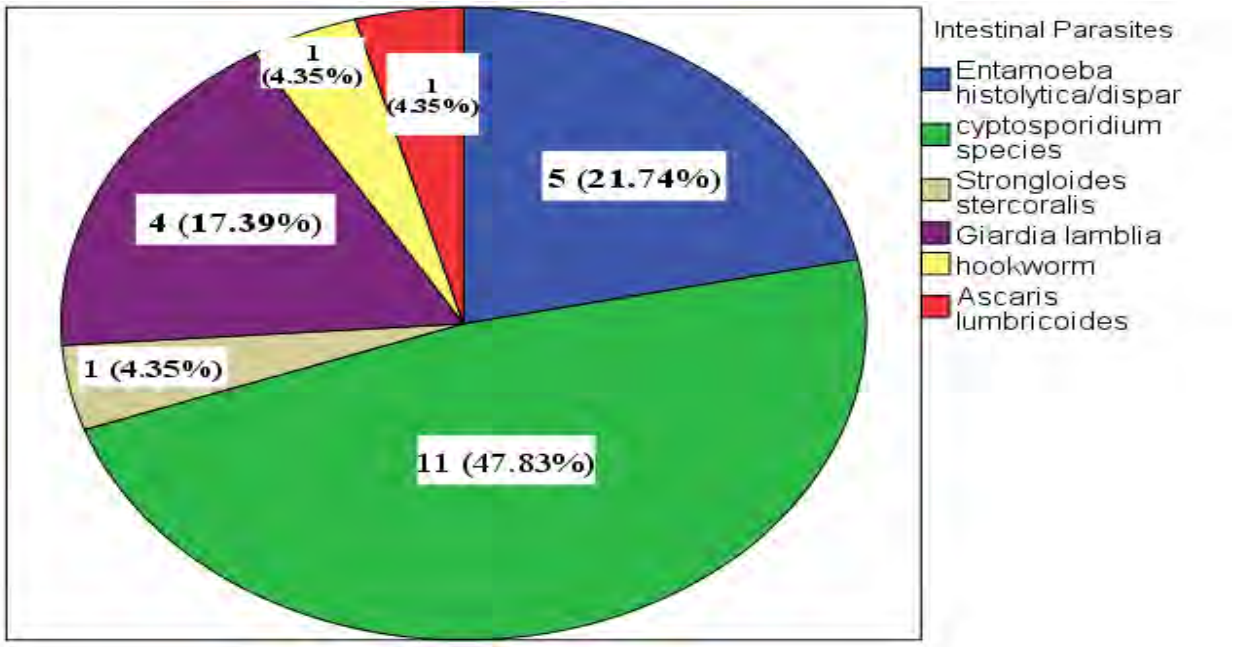


**Fig 2:- Frequency of Intestinal Parasites among Study participants at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015**

HAART experienced patients had significantly higher mean hemoglobin, hematocrit, MCV, MCH, and MCHC values than HAART Naïve participants **(p < 0.001).** With regard to CD4 + T cells count, HAART Naïve patients had high Mean ± SD value when compared to HAART experienced patients (450.38 ± 229.48 versus 315.76 ± 292.73) **(P= 0.000).** There were no statistically significant difference in the mean values of RBC count (**P= 0.281**) and BMI (**P= 0.243**) between HAART Naïve and HAART experienced participants (Table 2)

**Table 2:- Comparison of mean values Among HAART Naïve And HAART Experienced Adult HIV Patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November,2015**

| Variables | HIV Adult Patients | | P -value | T value (95% CI) |
|---|---|---|---|---|
| | HAART Naïve (mean± SD) | HAART Experienced (mean ±SD) | | |
| **CD4 count/ µ1** | 450.38 ± 229.48 | 315.76 ± 292.73 | .000 | 4.719 (78.5,190.732) |
| **Hb (g/dl)** | 13.74 ± 2.44 | 15.04 ± 2.05 | .000 | 5.320 (0.821,1.7839) |
| **HCT (%)** | 41.16 ± 7.18 | 44.1 ± 5.70 | .000 | 4.185 (1.56, 4.3252) |
| **RBC(x10$^6$/µ1)** | 5.41 ± 10.64 | 4.53 ± 0.68 | **0.281** | -1.080 (-2.4916,0.72) |
| **MCV (fl)** | 88.65 ± 11.11 | 97.98 ± 10.87 | .000 | 7.83 (6.98, 11.67) |
| **MCH (Pg)** | 29.95 ± 3.11 | 33.5 ± 4.19 | .000 | 8.86 (2.76, 4.34) |
| **MCHC(g/dl)** | 33.31 ± 1.602 | 34.15 ± 1.602 | .000 | 4.493 (0.471,1.204) |
| **BMI** | 22.21 ± 4.77 | 22.82 ± 4.60 | **0.243** | 1.171 (-0.413, 1.63) |

## 4.2.Prevalence of anemia in HIV patients

The prevalence of anemia was determined using hemoglobin values of study participants. The overall prevalence of anemia in our study was 54 (15.88%). 12(7.1%) of HAART- experienced and 42(24.71%) HAART Naïve patients were anemic from their respective groups (Table 3). Though not statistically significant (P= 0.909), anemia was more prevalent among females (15.4%) than their male counter parts (16.5%). The difference in proportions of anemia among different categories of CD4+T cells counts, Functional status, TB- infection, BMI, opportunistic infection, WHO clinical stages, Cotrimaxazole usage, and HAART-Status of participants was statistically significant (Table 3). Of the anemic individuals, 2 (0.58%), 25 (7.35%), 27 (7.94%) had sever, moderate and mild anemia respectively. There was statistically significant difference in the degree of anemia between HAART Naïve (24.8%) and HAART experienced patients (7.06%) ($p<0.001$) (Table 4).

**Table 3:- Frequency of Anemia among Adult HIV Patients attending ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015**

| Variables | | Anemia | | P- Value |
|---|---|---|---|---|
| | | Present N (%) | Absent N (%) | |
| **Sex** | Male | 22 (16.5) | 111 (83.5) | 0.909 |
| | Female | 32 (15.4) | 175 (84.5) | |
| **Age** | 18-35 | 32 (18.0) | 146 (82.0) | |
| | 36-59 | 18 (12.1) | 131 (87.9) | 0.130 |
| | ≥60 | 4 (30.8) | 9 (69.2) | |
| **Employment status** | Employed | 45 (15.3) | 249 (84.7) | 0.604 |
| | Unemployed | 9 (19.6) | 37(80.4) | |
| **Functional status** | Working | 42 (13.8) | 263 (86.2) | |
| | Ambulatory | 11 (36.7) | 19 (63.3) | **0.013** |
| | Bedridden | 1 (20) | 4 (80) | |
| **CD4 count** | <200 | 36 (32.7) | 74 (67.3) | |
| | 200-500 | 13 (10.4) | 112 (89.6) | **0.000** |
| | >500 | 5 (4.8) | 100 (95.2) | |
| **TB infection** | Yes | 6 (35.3) | 11 (64.7) | **0.044** |
| | No | 48 (14.9) | 275 (85.1) | |
| **Intestinal parasite (n= 320)** | Yes | 7 (30.4) | 16 (69.6) | 0.150 |
| | No | 43 (14.5) | 254 (85.5) | |
| **BMI (kg/ $m^{2)}$ (n= 326)** | <18.5 | 21(36.2) | 37(63.8) | **0.000** |
| | 18.5-24.99 | 21(11.7) | 159(88.3) | |
| | > 25 | 7(8.0) | 81 (92.0) | |
| **Opportunistic infection** | Yes | 29 (37.7) | 48 (62.3) | **0.000** |
| | No | 25 (9.5) | 238 (90.5) | |
| **WHO clinical Stage** | I | 26 (9.8) | 240 (90.2) | **0.000** |
| | II | 5 (26.3) | 14 (73.7) | |
| | III | 15 (48.4) | 16 (51.6) | |
| | IV | 7 (31.8) | 15 (68.2) | |
| **HAART-Status** | Naïve | 42 (24.7) | 128(75.3) | **0.000** |
| | Experienced | 12 (7.1) | 158 (92.9) | |
| **Anti-TB drugs** | Yes | 10 (22.7) | 34 (77.3) | 0.267 |
| | No | 44 (14.9) | 252 (85.1) | |
| **Cotrimaxazole use** | Yes | 35 (21.34) | 129 (78.65) | **0.012** |
| | No | 19 (10.8) | 157 (89.2) | |

Table 4:- Severity of Anemia among HAART Naïve and HAART Experienced Adult HIV Patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015

| Severity of Anemia | HAART-Status | | Total N (%) | P- value |
|---|---|---|---|---|
| | HAART Experienced N (%) | HAART Naïve N (%) | | |
| **None** | 158 (92.94) | 128(75.30) | 286(84.12) | |
| **Sever** | 0(0.0) | 2(1.18) | 2(0.58) | |
| **Moderate** | 7 (4.12) | 18 (10.68) | 25(7.35) | **0.000** |
| **Mild** | 5(2.94) | 22 (12.94) | 27(7.94) | |
| **Total** | 170 (50) | 170 (50) | 340 (100) | |

MCV < 100 fl and MCHC <31 g/dl were used to morphologically characterize anemia among study participants identified as having anemia. Out of the total anemic individuals, Microcytic Hypochromic (24.07%) anemia and normocytic normochromic (NCNC) (51.85%) anemia were more commonly found in HAART Naïve anemic study participants. On the other hand, macrocytosis was more common in HAART experienced anemic patients (5.56%) than in HAART Naïve patients (1.85%) (Figure 3).

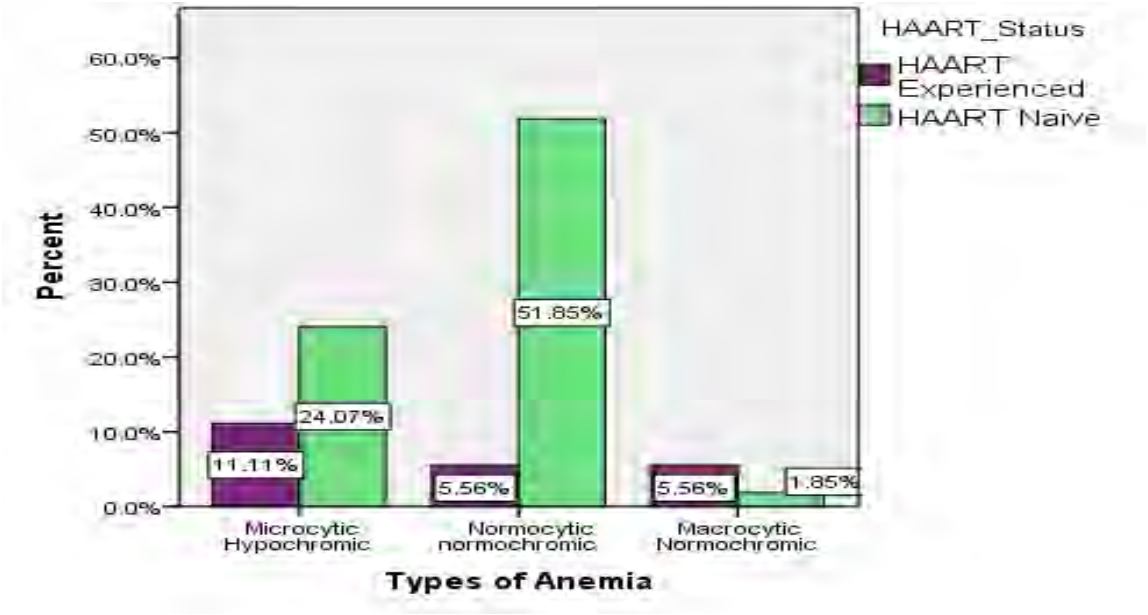


**Fig 3: Types of Anemia among HAART Naïve and HAART Experienced Adult anemic HIV Patients at Zewditu Memorial Hospital, Addis Ababa Ethiopia from April-November, 2015.**

## 4.3. Associated Factors of anemia in HAART Naïve adult HIV patients

Different factors were observed to be associated with being anemic in HAART Naïve Adult HIV patients (Table 5). In bivariate analysis, presence of opportunistic infection (COR= 3.32; 95 % CI: 1.60-6.68), WHO clinical stage III (COR= 6.07; 95% CI: 2.44-15.11), BMI <18.5 (COR=5.32; 95 % CI: 2.12-12.73), CD4 + T cells Count < 200 (COR= 12.38; 95% CI: 2.79-12.73), intestinal parasites (COR= 4.99; 95 % CI: 1.33-18.62) and being in ambulatory functional state(COR= 2.52; 95 % CI: 1.06-6.01) were statistically associated with increased odds of anemia. Multivariate analysis depicted that WHO clinical stage III (AOR= 9.63; 95 % CI: 1.06-86.95), BMI <18.5 (AOR= 7.56; 95 % CI: 2.11-27.01), and CD4+ cells count <200 (AOR= 7.57; 95% CI: 1.27-27.01) were independent risk factors for anemia in HAART naïve patients. There was lack of significant association between anemia and opportunistic infection, age category, presence of intestinal parasites, use of cotrimaxazole, anti-TB drugs and functional status of HAART naïve HIV adult patients (Table 5).

We also investigated the strength of association between those independent risk factors and hemoglobin levels. There was a weak, positive correlation between BMI and hemoglobin (r= 0.383, p<0.001), and between CD4+ cells count and hemoglobin (r=0.294, P<0.001) (Figure 4).

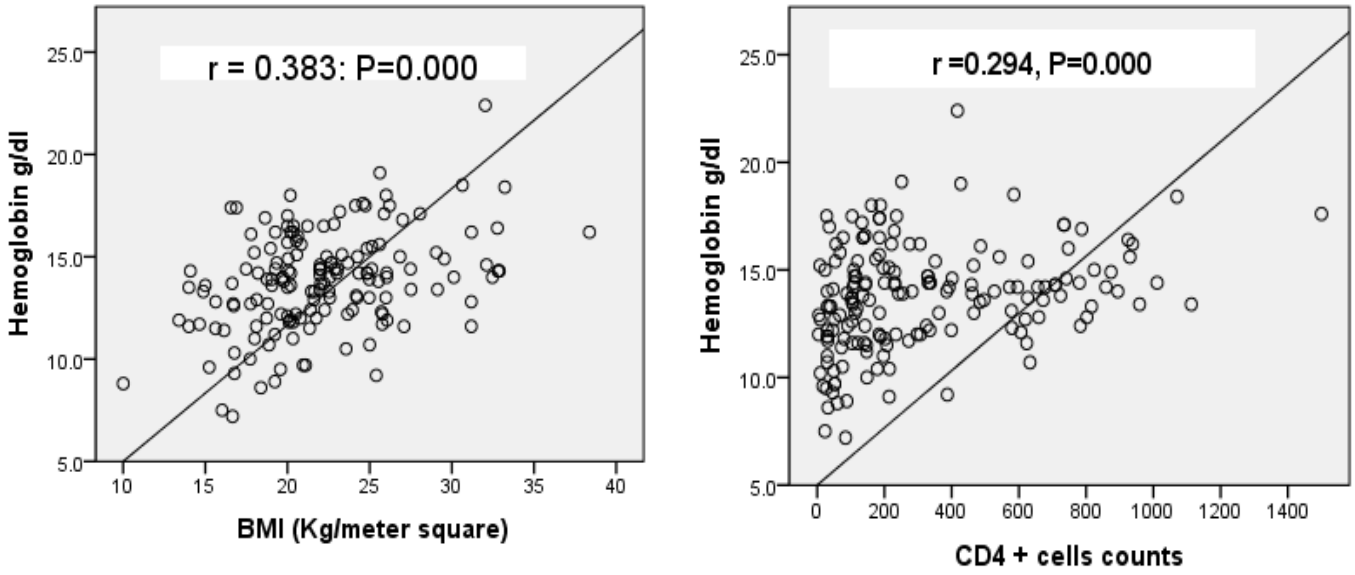


**Fig 4:- Relationship of hemoglobin with risk factors of anemia in HAART Naïve Adult HIV patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015.**

**Table 5:- Possible Risk factors of anemia in HAART Naïve adult HIV patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015.**

| Variables | | COR (95% CI) | P-Value | AOR (95% CI) | P-Value |
|---|---|---|---|---|---|
| **Opportunistic infection** | Yes | 3.32( 1.60-6.88) | 0.001 | 0.81(0.10-6.35) | 0.837 |
| | No | **1(referent)** | | **1(referent)** | |
| **WHO clinical stage** | **I** | **1 (referent)** | | **1 (referent)** | |
| | II | 1.62 (0.47-5.59) | 0.446 | 3.69(0.31-42.77) | 0.296 |
| | III | 6.07(2.44-15.11) | 0.000 | 9.63(1.07-86.95) | **0.044** |
| | IV | 2.83(0.98-8.18) | 0.054 | 1.06(0.08-13.48) | 0.966 |
| **Age category** | **18-35** | **1(referent)** | | **1(referent)** | |
| | 36-59 | 0.87(0.41-1.86) | 0.718 | 0.59(0.19-1.83) | 0.360 |
| | ≥60 | 4.62(0.73-29.15) | 0.104 | 22.92(0.93-564.19) | 0.055 |
| **BMI** | <18.5 | 5.319(2.12-12.73) | 0.000 | 7.56(2.12-27.01) | |
| | **18.5-24.99** | **1 (referent)** | | **1 (referent)** | |
| | >25 | 0.859(0.31-2.407) | 0.772 | 2.84(0.69-11.67) | 0.148 |
| **CD4 Count** | <200 | 12.38(2.79-54.71) | 0.001 | 7.57(1.27-27.01) | **0.027** |
| | 200-500 | 3.67(0.72-18.92) | 0.118 | 0.736(0.08-7.23) | 0.793 |
| | **>500** | **1 (referent)** | | **1 (referent)** | |
| **Intestinal parasite(s)** | Yes | 4.99(1.33-18.67) | 0.017 | 6.01(0.62-58.27) | 0.122 |
| | **No** | **1 (referent)** | | **1 (referent)** | |
| **Functional status** | Ambulatory | 2.52(1.06-6.01) | 0.037 | 0.889(0.21-3.69) | 0.872 |
| | Bedridden | 1.83 (0.16-20.91) | 0.626 | 0.583(0.02-20.51) | 0.776 |
| | Working | **1 (referent)** | | **1 (referent)** | |
| **Cotrimaxazole use** | Yes | 1.79(0.81-3.98) | 0.150 | 0.901(0.21-3.79) | 0.887 |
| | **No** | **1 (referent)** | | **1 (referent)** | |
| **Anti-TB Drugs** | Yes | 2.22(0.88-5.59) | 0.090 | 1.32(0.32-5.46) | 0.699 |
| | **No** | 1 (referent) | | **1 (referent)** | |

## 4.4. Associated Factors of anemia in HAART Experienced adult HIV patients

Both bivariate and multivariate analysis were done to identify the risk factors of anemia in HAART experienced HIV positive persons. In bivariate analysis, having opportunistic infection (COR= 10.33; 95% CI: 1.55-69.09) were significantly associated with increased odds of being anemic. However, after adjusting for these variables using a multiple logistic regression model, no variable was shown to be associated with odds of anemia in HAART experienced patients (Table 6). There was very weak, positive correlation between BMI and hemoglobin levels of HAART experienced HIV positive adults individuals (r = 0.015, P >0.05) (Figure 5).

**Table 6:- Possible Risk factors of anemia in HAART Experienced adult HIV patients at ART Clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015**

| Variables | | COR (95% CI) | P-Value | AOR (95% CI) | P-Value |
|---|---|---|---|---|---|
| **Opportunistic infection** | Yes | 10.33(1.55-69.09) | **0.016** | 5.57(0.76-41.08) | 0.092 |
| | No | **1(referent)** | | **1(referent)** | |
| **BMI** | < 18.5 | 3.26 (0.909-11.67) | | 2.67 (0.701-10.13) | |
| | **18.5-24.99** | **1 (referent)** | | **1 (referent)** | |
| | ≥25 | 0.305(0.036-2.609) | 0.278 | 0.329(0.038-2.833) | 0.311 |

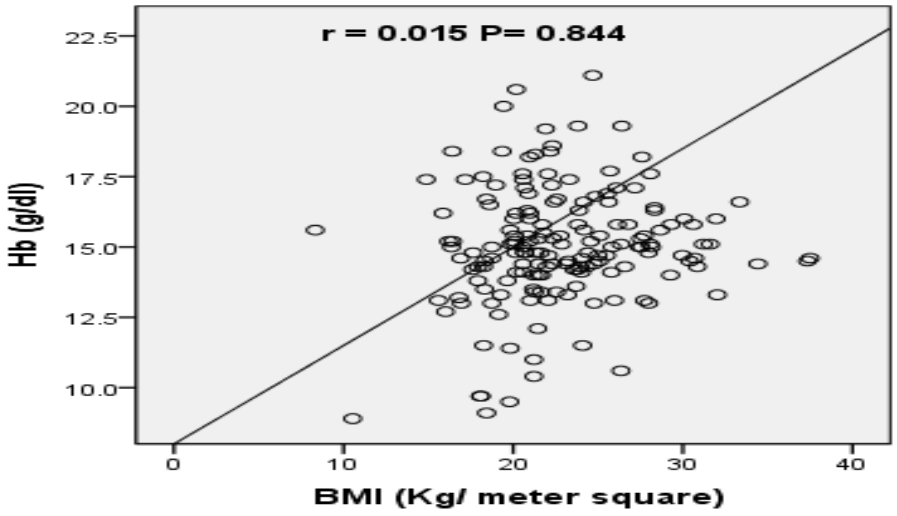


**Fig 5:- Relationship of hemoglobin with risk factors of anemia in HAART Experienced Adult HIV patients at Zewditu Memorial Hospital, Addis Ababa, Ethiopia from April-November, 2015.**

# 5. Discussion

Anemia is a common and serious complication of both HIV infection and its treatment. It can compromise a patient's functional status, quality of life and survival [45]. The aims of this study were to determine the prevalence of anemia in HAART naïve and HAART experienced HIV positive adult patients and to compare the burden anemia between these groups of patients as well as to identify possible predictors of anemia in adult HIV patients attending ART clinic of Zewditu memorial hospital, Addis Ababa, Ethiopia.

The prevalence of anemia in HAART naïve HIV positive participants was higher than in HAART experienced patients. CD4+ T cells count <200/µl, BMI <18.5 and WHO clinical stage III in HAART naïve participants were identified as risk factors for anemia. In contrary, no variable was found to be significantly associated with anemia in HAART experienced participants.

The overall prevalence of anemia in this study was 15.88% which was in line with finding from another study by Muqisha *et al* which reported 18.9% anemia prevalence [46]. However, the finding of our study was higher than a report from a study done in Iran (10.3%) (2009) [33]. In contrast, it was much lower compared to the prevalence reported by Alem *et al* [25], Dikshit*et al* [47], Mildivan *et al* [48], and Gedefaw *et al* [2] who reported an overall prevalence of 70.1%, 65.5%, 36 % and 23.1% respectively. The difference might be due to differences in socio-economic characteristics, geographic location of the study settings, and nutritional status of study participants. For example, the study by Mildivan *et a*l [48] was conducted in USA which is different geographic setting from ours. In addition, Nutritional status of their study participants could have contributed less to the prevalence of anemia when it is compared to our findings.

The prevalence of anemia in HAART experienced HIV positive adult patients was lower and their mean hemoglobin value was higher than those HAART naïve patients. This finding is supported by Gedefaw *et al* [2], Tesfaye *et al* [28], Asgeir *et a*l [49] and Moore *et al* [50]. This is due to the fact that HAART suppresses viral replication and increases immunity of HIV patients. Therefore, there will be reduction in opportunistic infections such as TB, Herpes Zoster, cryptosporidiosis and others, as well as reduction in the occurrence of neoplasms. This may account for the low prevalence of anemia in HAART experienced patients.

The commonest type of anemia in the current study was of mild to moderate anemia as has been reported by other studies. Severe anemia was detected in two of HAART naïve patients while in none of the HAART experienced ones. Though the overall prevalence of anemia is much higher than reported in the current study, Meiden and colleagues [5] published a study describing the prevalence of mild to moderate anemia and severe anemia in 67% and 4 % of anemic patients, respectively. Frede *et al* [27] from Ethiopia also indicated prevalence of mild to moderate anemia in 32 % and severe anemia in 3% of HAART naive study participants.

In the current study, Microcytic Hypochromic (24.07 %) and Normocytic Normochromic (51.85%) anemia were more common in HAART Naïve anemic patients than HAART Experienced anemic patients. However, Macrocytosis was more common in HAART Experienced anemic patients (5.56%) than HAART Naïve (1.85%) anemic individuals. The lower MCV in patients may attribute to iron deficiency secondary to poor nutritional status or chronic blood loss. Anemic HIV patients with normal MCV values may have anemia of chronic disease (ACD) due to underlying chronic inflammation. Anemia of inflammation is characterized by up regulation of pro-inflammatory cytokines like TNF which may directly suppress bone marrow activity or play a major role in hepcidin mediated and iron–restricted erythropoiesis [51, 52]. Normal MCV may also be due to use of myelosuppressive drugs, or bone marrow infiltration by tumor or infections [50]. Macrocytosis in HAART experienced patients may be owing to the effect of Zidovudine in the regimen.

The prevalence of anemia was significantly higher in patients with CD4 +T cells <200 cells/ µl , advanced WHO clinical stage, BMI < 18.5, co-infected with opportunistic infections and those who were taking cotrimaxazole prophylaxis for sometimes during the study period. The higher prevalence of anemia in patients with low CD4 + T cells count could be explained by the direct infection of CD4 + T cells by HIV virus which renders decrease in the number of these cells. This in return decreases the immunity of the patients leaving the individuals to be more susceptible to opportunistic infections. Concequently, opportunistic infections such as tuberculosis, pneumocytosis, and non-hodgkin's lymphoma can infiltrate the bone marrow, generally causing pancytopenia. [4, 53].

Lower BMI reflects malnutrition as a result of low calorie intake, coexistent opportunistic infections, and hyper-catabolic action of the body in an attempt to control viral replication and reconstitute the immune system. The resulting nutritional deficiencies contribute to the occurrence of anemia in adult HIV patients [7, 54- 55]. The high prevalence of anemia in patients using cotrimaxazole could be as a result of haematotoxic nature of the drug itself or the synergistic effects \which exist between co-trimaxazole and zidovudine combined therapy in patients on ART [56, 57].

There was no significant difference between female and male study participants with regard to the prevalence of anemia. (P= 909), which is in agreement with findings of Tesfaye *et al* [28], and Addis *et al* [41]. However, it is in contrast to many studies [1,23,25,27,28.58] that reported the presence of significantly higher prevalence among female HIV patients and other studies done by Omoregie *et al* in Nigeria [59] and Assefa *et al* in Ethiopia [30] which showed significantly higher prevalence of anemia in male patients than in their female counter parts. Although the majority of anemic cases were observed in the age group of 18-35, there was no significant association between anemia and age category (p=0. 130). This report contradicts the findings of other studies [23, 34].

Advanced WHO clinical stage particularly clinical stage III, low BMI, and CD4 +T cells count <200 cells /µl were found to be risk factors of anemia in HAART naïve patients. Individuals at WHO clinical stage of III had 9.64 times more risk of developing anemia than patients at other clinical stages (p=0.044 95% CI= 1.07-86.95). The risk of developing anemia in patients with CD4 +T cells count <200 cells was 7.57 times more than those having CD4 count > 200 cells /µl. This may indicate the likelihood of anemia occurrence with deterioration of immunity and disease progression. This finding is consistent with Levine *et al* [60], curkendall *et al* [61], Voldebering *etal* [62], Denue *et al*[16] and Ferede *et al* from Ethiopia [27] who reported high risk of anemia in patients having low CD4 + T cells count and having clinically diagnosed AIDS. In addition, we found weak, positive correlation between hemoglobin and CD4 + T cells count of HAART naïve patients. This finding is in consistent with a finding reported by Mata-Marin and collaborators [38] which showed a strong, positive correlation between these two parameters in HAART naïve patients.

However, the relationship of anemia and CD4 count is at variance. In contrast to our finding which indicated CD4+ T cells count less than 200 to be predictor of anemia, Studies conducted by Moyle *et al* [63] and Nadler *et al* [64] reported that CD4 count <50 cells/µl is a significant factorfor the development of anemia in HIV patients. However, Assefa and collegues [30] showed that CD4 count less than 350 cells/µl was independently associated with odds of being anemic. The effect of CD4 count on anemia can be clearly portrayed by further studies. We also identified a 5.3 times increase for the risk of developing anemia in patients with low body mass index. A study conducted by Takuva *et al* [7] in South Africa supported our result. A study conducted in Ethiopia by Assefa *et al* [30] also reported that overweight and obese BMI are associated with decreased odds of anemia in HIV patients.

Significant and independent association was absent between anemia and opportunistic infection, age category, intestinal parasitosis, and use of cotrimaxazole and anti-TB drugs in HAART naïve patients despite their major contribution to the development of anemia. In contrast, the presence of opportunistic infection was shown to be associated with increased odds of anemia in some previous studies [2, 30, 32]. In Nigeria, a study was conducted by Akinbo *et al* [65] which reported that A. lumbricoides, hookworm and Tenia species in HIV infected individuals were the parasitic agents associated with anemia which contradicted the finding of our study. The study also stated that parasites are potent factors to change blood both qualitatively and quantitatively. Unlike a report by Sara Jam and colleagues [33], no significant and independent relationship was observed between anemia and use of Cotirimaxazole or anti-TB drugs.Nevertheless, this finding is in line with a report by Assefa *et al* [30]

The observed prevalence of anemia in HAART experienced patients participated in this study (7.1%) is comparable with a report by Tadele *et al* from northern Ethiopia [36] but is very low compared to a study done by Oweridu *et al* in Ghana [66], Denue et al in Nigeria [16], Adane et al [19] in Addis Ababa, Ethiopia Gedefaw*et al* [2] and associates in southwest, Ethiopia which reported 37.6%, 24.3%, 37.4% and 16.2%, respectively. No variable was identified as significantly independent predictor of anemia in HAART experienced patients unlike most studies [2, 31-35, 66]. This difference might be due to sample size difference, all being urban residents in our study and difference in study design. For instance, in Gedefaw and colleagues' study [2], participants were both from urban and rural areas. Therefore, the high prevalence of anemia in the rural residents was

explained to be due to the lack of information about nutrition and other factors contributing to the occurrence of anemia.

All in all, the current study tried to investigate the prevalence and risk factors of anemia comparatively in HAART naïve and HAART experienced patients. We believe that our study will contribute to the improvement of quality life of HIV patients by providing information about individuals who need better attention and close monitoring. However, further longutidinal study with long-term follow up is necessary to assess every possible risk factor for anemia development in HIV adult patients.

# 6. Strength and Limitations of the Study

## 6.1.Strength

- The study tried to compare the burden of anemia and possibly related factors between participants stratified based on their HAART status
- Three different techniques were used to detect intestinal parasites among participants which increased the quality of our data
- The study focused on adult HIV patients who are part of the working force of the society

## 6.2.Limitations of the Study

- Serum erythropoietin level was not measured
- The presence of other underlying chronic diseases and pro-inflammatory cytokines were not assessed
- HIV RNA viral load was not determined which could have indicated the contribution of the virus itself to the occurrence of anemia
- Thin blood films were not made to determine the morphology of RBCs under light microscope

# 7. Conclusion and Recommendations

## 7.1.Conclusion

- Anemia is the common manifestation both in HAART naïve and HAART experienced patients. However, Prevalence of anemia is higherin HAART naïve HIVindividuals than those on HAART.
- Severity of anemia is significantly high in HAART naïve patients compared to HAART experienced individuals
- Macrocytosis is more prevalent in HAART experienced patients than in HAART naïve HIV patients
- Poor nutritional status, immunosupression and advanced disease progression contribute independently for the occurrence of anemia in HAART naïve patients.
- There is weak positive correlation between CD4+ T cells count, BMI and Hemoglobin value of HAART naïve individuals
- HAART intiation has resulted in remarkable increment of mean hemoglobin value and reduction in opportunistic infection thereby decreases the prevalence of anemia in HIV adult patients.

## 7.2.Recommendation

- Clinical service providers should give due attention to mitigate adverse effect of anemia in HIV/ AIDS patients with CD4 + Lymphocyte count less than 200 cells/ µl, those who are malnourished (BMI <18.5) , co-infected by opportunistic infections, who are at advanced WHO clinical stages and having one or more intestinal parasites.
- It is important to routinely screen for abovementioned parameters which permits early and adequate clinical management of HIV patients.
- Differential diagnosis of HIV-related anemia to cease the etiology of the disorder. Besides, therapeutic modalities for anemia in HIV/AIDS patients should address the underlying cause(s).
- Hematological side effects of antiretroviral therapies should be monitored regularly.
- Further longitudinal study should be conducted to map every possible correlates of anemia and compare its burden in HAART Naïve and HAART experienced adult HIV patients.

## References


1. Coyle TE. Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Med Clin North Am.1997; 81 (2): 449-470.
2. Gedefaw L, Yemane T, Sahlemariam Z, Yilma D. Anemia And Risk Factors In HAART Naïve And HAART Experienced HIV Positive Persons In South West Ethiopia: A comparative study. *PLoS ONE*. 2013; 8 (8): e72202.
3. WHO. Haemoglobin Concentrations for the Diagnosis of Anemia and Assessment of Severity, Vitamin and Mineral Nutrition Information System. Geneva, Switzerland. WHO, 2011.
4. Kreuzer KA, Rockstroch JK. Pathogenesis and Pathophysiology of anemia in HIV infection. Ann Hematol.1997; 179-187.
5. Meidani M, Rezaei F, Maracy MR, Avijgan M, Tayeri K. Prevalence, severity, and related factors of anemia in HIV/AIDS patients. *J Res Med Sci*. 2012; 17(2): 138-42.
6. Volberding PA, Levine AM, Dietrich D, Mildivan D, Mitsuyasu R and Saag M. Anemia in HIV Infection: Impact and Evidence based Management Strategies. *CID*. 2004; 38: 1454-63.
7. Takuva S, Maskew M, Brennan AT, Sanne I, Macphail PA, Fox MP. Anemia among HIV-infected patients initiating antiretroviral therapy in South Africa: Improvement in Hemoglobin regardless of degree of immunosuppression and initiating ART regimen. *Journal of tropical Medicine*. 2013; 10 (12): 124-129.
8. Balkrishnan A, Valsalan R, Sheshadri S, Pandit VR, Madep V, Argawal RK. Zidovudine-induced reversible pure red cell Aplasia. *Indian J pharmacol*. 2010; 42(3): 189-191.
9. Allen LH. Causes of Vitamin B12 and Folate Deficiency. *Food and Nutrition Bulletin*. 2008; 29(2): 20-34.
10. James A, Sudakar R, Yasawini B, Manimalika K, Kumar TRA, Sivakumar T. A case report on zidovudine induced anemia and its management in HIV-1 infected patient. *Int. J. Pharm. sci.Rev.Res*.2014; 27(10): 67-68.
11. Sharma SK. zidovudine – Induced Anemia in HIV/AIDS. Indian J Med Res. 2010; 132: 359-361.
12. Abebe M, Alemseged F. Hematologic Abnormalities among Children on HAART, in JimmaUniversity Specialized Hospital, Southwest Ethiopia. *Ethiop J Health Sci*.2009; 19 (2): 83-89.
13. UNAIDS. Fact Sheet 2015:Global Statistics. Accessed from www.who.int/gho/hic/epidemic-respose/ART/en/ Accessed on December 16, 2015.

14. Khan K, Khan AH, Sulaiman SA, Soo CT and Aftab RA. Adverse Effect of highly active anti-retroviral therapy (HAART) in HIV/AIDS patients. IJOPP. 2014; 7(3): 29-35.
15. Manzardo C, Zaccarelli M, Agüero F, Antinori A, Miró JM. Optimal timing and best antiretroviral regimen in treatment-naive HIV-infected individuals with advanced disease. JAIDS.2007; 46: 9–18.
16. Denue BA, Kida IM, Hammagabdo A, Dayar A, Sahabi MA. Prevalence of Anemia and Immunological Markers in HIV-Infected Patients on Highly Active Antiretroviral Therapy in Northeastern Nigeria. *Infectious Diseases: Research and Treatment*.2013:6 25–33.
17. Subbaraman R, Chaguturu SK, Mayer KH, Flanigan TP, Kumarasamy SW. Adverse Effects of Highly Active Antiretroviral Therapy in Developing Countries. *Clic infect Dis*. 2007; 45 (8): 1093-110.
18. Semba RD, Martin BK, Kempen JH, Thorne JE, Wu AW: The impact of anemia on energy and physical functioning in individuals with AIDS.Arch Intern Med. 2005, 165:2229–2236.
19. 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.
20. WHO/UNICEF. Focusing on Anemia: Towards an integrated approach for effective anemia control. Available at www.who.int/topics/anaemia/en/who_unicef-anaemiastatement.pdf. Accessed on September 23, 2015.
21. WHO. Worldwide prevalence of anemia 1993-2005: WHO Global Database on anemia. Geneva,Available at www.whqlibdoc.who.int/publications/2008/9789241596657_eng.pd. Accessed on September 23, 2015
22. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf Sk, Johns N, Lozano R. et al .A systematic analysis of global anemia burden from 1990 to 2010. *Blood*.2014; 123(5):615-24.
23. Rodríguez EJS, Mayor AM, Santos DMF, Candelaria YZ, Mallado RFH. Anemia in a cohort of HIV-infected Hispanics: prevalence, associated factors and impact on one-year mortality. *BMC Research Notes*. 2014; 7:439-446.
24. Agarwal D, Chakrauarty J, Chaube L, Rai M, Agarwal NR, Sundar S. High incidence of zidovudine induced anemia in HIV infected patients in eastern India. *Indian J Med Res*.2010; 132: 386-389.
25. Alem M, Kena T, Baye N, Ahmed R, Tilahun S. Prevalence of Anemia and Associated Risk Factors among Adult HIV Patients at the Anti-Retroviral Therapy Clinic at the University of Gondar Hospital, Gondar, Northwest Ethiopia. *Scientific Reports*.2013; 2:662-667.

26. Redig AJ and Berliner N. Pathogenesis and clinical implications of HIV- related anemia in 2013. *Hematology*.2013; 377-381.
27. Ferede G and wondimeneh Y. Prevalence and related factors of anemia in HAART-naïve HIV patients at Gondar University Hospital, northwest Ethiopia. *BMC Hematology*. 2013; 13: 8-12.
28. Tesfaye Z and 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; 7:745-749.
29. Federal HIV/AIDS Prevention and Control Office (FHAPCO). Country Progress Report On The HIV Response ,2014.
30. Assefa M, Abegaz WE, Shewamare A, Medhin G and Belay M. Prevalence and correlates of anemia among HIV infected patients on highly active antiretroviral therapy at Zewditu Memorial Hospital, Ethiopia. *BMC Hematology*.2015; 15:6-13.
31. Reddy Ak, Lihite RJ, Lahkar M, Choudhury U, Baruah Sk. A Study on Adverse Drug Reactions in HIV Infected Patients at ART Centre of Tertiary Care Hospital in Guwahati, India. *Asian J Pharm Clin Res*.2013; 6 (2): 102-104.
32. Mijiti P, Yuexin Z, Min L, Wubuli M, Kejun P, Upur H. Prevalence and predictors of anemia in patients with HIV infection at intiation of combined antiretroviral thrapy in Xinjiang. China Int J STD AIDS. 2014; Accessed from www.mcbi.nlm.nih.gov/pubmed/24810220. Accessed at January 18, 2015.
33. Jam S, Sabzvari D, Badie BM, Allinghahi SAS, Jabbari H, Fattahi F, Mohraz M. A Cross-Sectional Study of Anemia in Human ImmunodeficiencyVirus-Infected Patients in Iran. *Arch Iranian Med.* 2009; 12 (2): 145 – 150.
34. Mildivan D, Creagh T, Leitez G. Prevalence of anemia and correlation with biomarkers and specific antiretroviral regimens in 9690 human immunodeficiency virus infected patients: findings of the anemia prevalence study. *Curr Med Res opin*. 2007; 23(3): 343-355.
35. Okinbo FO and Omeregie R. Plasmodium falciparum Infection in HIV-Infected Patients on Highly Active Antiretroviral Therapy (HAART) In Benin City, Nigeria. *JRHS*. 2012; 12(1): 15-18.
36. Tadele A, Kedir A, Enawgaw B, Melku M, Terefe B. Prevalence of Zidovudine induced megaloblastic anemia among Human Immunodeficciency virus positive patients attending university of Gondar hospital antiretroviral therapy clinic, northwest Ethiopia. *HIV Adv. Res. Dev. Open access.* 2014; 1 (2): 106-112.

37. Wiasakana R, Sumantri R, Indrati AR, Zwister A, Jusuf H, Mast QD, et al. Anemia and iron homeostasis in a cohort of HIV infected patients in Indonesia. *BMC Infectious Diseases*.2011; 11: 213-222.
38. Mata-Marin JA, Martinez JEG, Martinez REM, Anduiza CA, Allen JLF, Ramirez MC. Risk factors and correlates for anemia in HIV- treatment naïve infected patients : a cross sectional analytical study. *BMC Research Notes*. 2010; 3: 230-234.
39. Shen Y, Wang Z, Lu H, Wang J, Chen J, Liu L, et al. Prevalence of anemia among adult with newly diagnosed HIV/AIDS in China.*PLos One*. 2013; 8(9):e73807.
40. Munyazesa E, Emile I, Mutimura E, Hoover DR, Shi Q, McGinn AP et al. Assessment of Hematological Parameters In HIV-Infected And Uninfected Rwandan Women: A Cross-Sectional Study. *BMJ Open*. 2012; 2:e001600.
41. Addis Z, Yitayew G, and Tachebele B. Prevalence of Some Hematological Abnormalities among HIV Positive Patients on Their First Visit to Tertiary Health Institution in Ethiopia: A cross sectional study. *IBRR*. 2014; 2 (6): 270-278.
42. Teklemariam Z, Abate D, Mitiku H, Dessie Y. Prevalence Of Intestinal Parasitic Infection Among HIV Positive Persons Who Are Naïve and on Antiretroviral Treatment in Hiwot Fana Specialized University Hospital, Eastern Ethiopia. *ISRN AIDS*. 2013; 10: 324-329.
43. Zewditu Hospital to get a new outpatient Departement. Available at www.ethiopia.usembassy.gov. Accessed on March 20, 2015.
44. Chada V. Sampling: Sample size determination in Health studies. *NTI Bulletin*. 2006; 42 (3): 55-62.
45. Wills TS, Nadler JP, Somboonwit C, Vincent A, Leitz G, Marino K, et al. Anemia prevalence and associated risk factors in a single center ambulatory HIV clinical cohort. *AIDS Read*.2004; 14(6):310-315.
46. Muqisha J, Shafer L, Van der Paal L, Mayanja BN, Eotu H, Hughes P, et al. Anemia in a rural Ugandan HIV cohort: prevalence at enrolment, incidence, diagnosis and associated factors. *Trop Med Int Health*.2008; 13(6): 788–94.
47. Dikshit B, WanchuA, SachdvaRK, SharmaA, DasR. Profile of hematological abnormalities of Indian HIV infected individuals. *BMC Blood Disorders*. 2009; 9(5). Doi: 10.1186/1471-2326-9-5.
48. Mildvan D, Terri C, Gerhard L. Anemia prevalence and correlation with biomarkers and ART in HIV/AIDS. *Curr Med Res Opin*. 2007; 23(2): 343–355.
49. Asgeir J, Ezra N, Svein G, and Johan B. Antiretroviral treatment reverses HIV-associated anemia in rural Tanzania.*BMC Infect Dis*.2011; 11(190). Doi: 10.1186/1471-2334-11-190

50. Moore RD, Forney D. Anemia in HIV-infected patients receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndtr*. 2002; 29(1): 54-57.
51. Claster S. Biology of anemia, Differential Diagnosis and treatment options in Human Immunodeficiency virus infection. *J infect Dis.*2002; 185(2); 105-109
52. Ganz T and Nemeth E. Iron sequestration and anemia of Inflammation. *Semin Hematol*.2009; 46 (4): 387-393.
53. Enawgaw B, Alem M, Addis Z, and Melku M. 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.doi: 10.1186/2052-1839-14-8.
54. Salomon J, Truchis P, Melchior JC. Nutrition and HIV infection. *Br J Nutr*.2002; 87 (1): 111-119.
55. Santos ACO and Alemieda AMR. Nutritional status and CD4 cell counts in Patients with HIV/AIDS reeiving antiretroviral therapy. Rev. Soc. Bras. Med. Trop. 2013; 46 (6): 698-703.
56. Moh R, Daniel C, Sorho S, Sauvageot D, Anzian A, Minga A, et al. Haematological changes in adults receiving a zidovudine containing HAART regimen in combination with cotrimaxazole in cote divoire. Anti. Ther. 2005; 10(5): 615-624.
57. Venugopalan V, Thoronton AC, Steinke DT, Rapp RP, Romanelli F and Feola DJ. Trimethoprim- sulfamethoxazole exposure alters *ex vivo* function of B lymphocyte isolated from human immunodeficiency virus infected patients receiving Zidovudine. Pharmacotherapy. 2009, 29 (4): 373-382.
58. Wolde HM, Lerebo WT, Melaku YA, Kiday and Girmay KH. Incidence and Risk Factors of Anemia among HIV/AIDS Patients Taking Anti-Retroviral Therapy at Tertiary Hospitals in Addis Ababa, Ethiopia: A Retrospective Cohort Study. *J HIV AIDS Infect Dis*. 2014; 2(303): 1-6.
59. Omoregie R, Omokaro EU, Palmer O, Ogefere HO, Egbeobauwaye A, Adeghe JE, et al. Prevalence of anaemia among HIV-infected patients in Benin City, Nigeria. Tanzan J Health Res. 2009; 11:1.1-4.
60. Levine AM, Berhane K, Masri-Lavine L,Sanchez M, Young M, Augenbraun M et al: Prevalence and correlates of anemia in a large cohort of HIV-infected women: Women's Interagency HIV Study. J acquir Immune defic syndr. 2001; 26:28–35.

61. Curkendall SM, Richardson JT, Emons, MF, Fisher AE, and Everhard F. incidence of anemia among HIV infected patients treated with HAART. HIV Medicine.8: 483-490.
62. Volberding PA, Dieterich D, Mildvan D, Mitsuyasu R, Saag M: Anemia in HIV infection: clinical impact and evidence-based management strategies. Clin Infect Dis. 2004; 39:1088–9.
63. Moyle G. anemia in person with HIV infection: Prognostic marker and contributor to morbidity. AIDS Rev.2002; 4: 13-20.
64. Nadler JP, willis TS, Sonboonwit C, Vincent A, Leitz G, Marino K, et al. Anemia Prevalence among HIV Patients: ART and other risk factors. Anti. Ther. 2003; 8 (1)
65. Akinbo FO, Okaka CE, Omoregie R. Prevalence of intestinal parasites in relation to CD4 counts and anemia among HIV infected patients in Benin city, Edo state, Nigeria. *Tanzan J Health Res*.2011; 13 (1): 8-13.
66. Owridu WKBA, Quaye L, Amidu N, Addai-Mensah O. Prevalence of anemia and immunological markers among Ghanaian HAART- naïve HIV- patients and those on HAART. *African Health Science*.2011; 11 (1): 2-15.

# Annexes

## Annex 1:- Laboratory Methods

1. SOP for CD3/CD4 positive cell count determination

Purpose to: - To enumerate the absolute and percentage of lymphocytes that are CD4 + T lymphocyte by lysing whole blood using FACScalibur machine

Principle: -when whole blood is added to the reagent, the flourochrome labeled antibodies in the reagent bind specifically to leucocytes surface antigens. During acquisition, the cells travel past the laser beam (488 nm Argon and 635 nm red diode and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals are detected by the instrument provide information about cell's size, internal complexity, and relative fluorescence intensity. TriTEST reagents employ fluorescence triggering allowing direct fluorescence gating of the lymphocyte population to reduce interference of unlysed or nucleated red blood cells in the gate

**Sample**

| **Sample Type** | **Amount Required** | **Transport and Storage** | **Stability** |
|---|---|---|---|
| Whole blood with anticoagulant of $k_2/k_3$EDTA | 4-5 ml<br><br>NB:- Note less than 1/3ml of the standard collection tube | Room Temperature ($20^0$c -$25^0$c) | 72 hours before preparing (staining) & 24 hours after preparing (staining) |

**Materials**

| **Reagents and Supplies** |
|---|
| **Name** |
| 1. TriTest CD3/ CD45/CD4+ TRU COUNT |
| 2. BD CaliBRITE$^{TM}$3 |
| 3. FACSlysing Solution |
| 4. FACS flow |
| 5. FACS Clean |
| 6. FACS rinse |
| 7. TruCOUNT$^{TM}$ Tubes |

| **Equipment** |
|---|
| 1. FACSCalibur flow cytometer |
| 2. Apple Computer with monitor |
| 3. HP laserjet printer |
| 4. Racks |
| 5. Sample mixer |
| 6. Vortex |
| 7. Precision adjustable micropipette |
| 8. Supply tank |
| 9. Waste Reservoir |
| 10. refrigerator |

Reagent preparation

- if FACS clean is not available, make 1:3 dilution of bleach (%) with distilled water
- Diluted FACS lysing solution make 1:10 dilution with distilled water

Reagent Stability and storage

- Diluted bleach stable for two days and store at room tempreture
- Reagent stable until expiry date and store at 2-8$^0$c
- Tru count tube store at room temperature
- BD TriTest CD3FITC/ CD4PE/ CD45perCP stable until expiry date and store at 2-8$^0$C
- FACS lysing solution stable until expiry date and store at room temperature
- Diluted FACS lysing solution stable for one days and store at room temperature
- FACS Clean stable until expiry date and store at room temperature

---

FACS caliber Calibration procedure

To calibrate use

- BD calibrate 3 beds
- BD calibrate APC beads (only for 4 color set up)
- Sheath fluid

1. Label tube one A and tube two B
2. Vortex the stock vials of the BD calibrates beads to thoroughly suspend them
3. Add 1ml of sheath fluid to tube A and 3ml to tube B
4. Use the following table to choose set up that you are to run in BD FACScomp software

| Set Up | Unlabled tube A | Mixed tube B |
|---|---|---|
| Three color lyse/ No wash | Unlabled beads | Unlabled, FITC, PE, Percp |
| Four color lyse/No wash | Unlabled and APC beads | Unlabled, FITC, PE, PerCP and APC Beads |

5. Add one free falling drop from each of the BD calibrate beads based on the information in each column
6. Cap the tube and mix by gently inversion
7. Select BD FACScomp from the display. And click ‘accept’.
8. Click lyse/ No wash under assay selection if not selected.

9. Enter each BD calibrate beads lot ID as it appears if you use calibrator with different lot from previously set lot.
10. Gently mix tube A and install into the cytometer sample injection port
11. Set the flow rate to HI and press the RUN button on the cytometer
12. In the software MENU click the RUN and start to begin acquiring event
13. For 4 colour lyse/ No wash APC beads are detected
14. Gently mix tube B install into the cytometer sample injection port and click START
15. Remove the tube from the cytometer
16. Install a tube of distilled water into the sip and select STANDBY on the cytometer

**Procedure**

1. First calibrate the machine before run the sample
2. Label each BD TruCount tube with the sample identification number that is corrected with laboratory unique ID (barcode) of the sample
3. Pipette 20µl of BD Tritest CD3/CD4/CD45 in the bottom of each trucount tubes
4. Pipette 50µl of respective well-mixed $k_3$EDTA blood into the bottom of each trucounttube. Cap the tube and vortex gently to mix for at least 15 seconds
5. Incubate for 15 min. in the dark at room temperature (20-25$^0$c)
6. Add 450µl 1: 10 diluted BD FACS lysing solution to each tube
7. Incubate for 15 minutes in the dark at room temperature (20-25$^0$c).The sample is now ready to analyze on the FACSCalibur. Then, enter the barcode of each sample sequentially on the multiset software which is attached to the FACSCalibur machine at sample ID column and save
8. Run the samples within 24 hours
9. After finishing the test copy the export file from DATA-2 and paste on polytech to export the results to LIS
10. Check that whether all results are exported to LIS or not by using sample ID or barcode and review the result. If there is a result <200 ceells/µl or >1000 repeat the test by new preparation to confirm the result. Accept the result if it is not discordant with the first one and file both results print out.
11. Lastly print and attach result summary

Absolute Count from the machine is determined by ratio:

Calculation: $\frac{\text{observed counts from the population of interest}}{\text{Observed reference bead counts}} \times \frac{\text{Reference bead count}}{\mu\text{l whole blood}}$ = absolute count

Daily CD4 QC in house run

1. Select three samples

   High ---------- result greater than 1000

   Normal----------result between 500-600

   Low ----------result 100-200
2. Store at appropriate temperature (20-25$^0$c)
3. Run each sample using sample testing procedure in the next day
4. Compare the result with the previous day result
5. The allowable difference should be within $\pm$ 7.02%

   **When do we use in house control?**

   Note: use in house control when TruCount controls and MultiCHEK controls are not available. But it is possible to use either TruCount control or MultiCHEK control. In some cases it may be difficult to get all the three levels of in house controls. In this circumstance it is possible to use the available ones.

Limitation and Interference

1. Blood and control bead delivery must be performed by reverse pipetting
2. Sample must be collected in $K_3$EDTA vacutainer brand collection tubes
3. Don't store whole blood longer than 48 hours before preparing
4. Do not refrigerate whole blood
5. Do not dilute whole or use any volume other than 50µl
6. Store prepared samples at RT (20-25$^0$c) in the dark and run within 24 hrs
7. Store the Trites Antibody at 2-8$^0$ C. do not use the reagent after the expiration date
8. Do not freeze the reagent
9. Do not expose the reagent to direct light.
10. Do not expose the reagent to direct light during incubation with cell.
11. Open the pouch only after it has reached room temperature

12. Carefully reseal the pouch immediately
13. Examine the desiccant each time you open the pouch. If the dessicant has turned from blue to lavender, discard the remaining tube
14. Use tubes within one hour after removal from the foil pouch.
15. Do noy use the Tru- count tubes beyond the expiration date indicating on the packing
16. Do not use the reagent if you observe any change in appearance
17. Take care to avoid the reagent from microbial contamination, which can cause erroneous results.
18. Never pipette by mouth
19. Wera suitale protective clothes and gloves
20. Pipette must be calibrated to deliver 50 µl of smaple
21. Do not mix multiple lots of tubes in the same assay
22. It is critical to use the bead count shown on the current lot of TruCount tubes
23. Do not use previously fixed and stored patient specimen
24. Whole blood sample refrigerated before staining ca give aberrant result
25. Sample obtained from patient taking immunosuppressive drug can yield poor result
26. Blast cell can interfere with test result

**Result interpretation: -** the sample printout reports percent and absolute counts for CD3+, CD4+ and CD45+ lymphocytes.

**Biological reference interval**

| Analyte | Reference range | unit |
|---|---|---|
| **CD3+ cell** | **55-84** | **Cells/µl** |
| **CD4+cell** | **410-1590** | **Cells/µl** |
| **CD3 average** | **690-2540** | **Cells/µl** |

Clinical utility: - to monitor disease progression, establish decision points for initiating therapy and to monitor the effects of therapy in HIV infected individuals

Maintenance:-

| Step | Action |
|---|---|
| **1** | **Daily Cleaning** |
| **2** | **Weakly Cleaning** |
| **3** | **Monthly Cleaning** |
| **4** | **Periodic Cleaning** |

Safety precaution: 1. when laser radiations open do not stare into the beam

2. Follow national health and safety guideline

Sample disposal procedure:

1. Refer the sample disposal log and separate samples that is retention time is completed
2. Prepare 0.5% bleach solution
3. Add 500µl of 0.5% bleach solution to each blood specimen and stained sample and cap.
4. Wait for 30 minutes
5. Put into biohazard bag
6. Pack and send to incinerator

**Reference**


1. BDFacscalibur- instruction for use
2. BD FACSCalibur Manual (safety and limitation)

## 2. Procedure and reagents of Cell-Dyn 1800 Hematology Analyzer


Picture Cell Dyn 1800 hematology analyzer

**Procedure for cell Dyn 1800 hematology analyzer**

1. Whole blood collected in an EDTA tube with a Minimum sample volume is 0.5 ml using the Open Sample Mode. The instrument aspirates 30 µl of patient sample.
2. Run three levels of QC at the beginning of each day of patient testing. Do not perform patient testing until QC tests are performed and within acceptable limits. Rerun at least one of the three levels of QC again after eight hours of patient testing to assure the instrument is still functioning properly.
3. Press MAIN to return to the MAIN MENU. At the MAIN MENU, enter in the operator ID and press RUN, next press SPECIMEN TYPE. If the instrument has been idle for fifteen minutes or more, press normal background. Press the Touch Plate to run an Open Mode Background test. Verify that the Open Mode Background count results are acceptable.
4. Press MAIN to return to the MAIN MENU screen. Enter in the Operator ID and press RUN. Press SPECIMEN TYPE then press PATIENT SPECIMEN. Verify that RUN Ready is displayed in the Status Box. Scan patient specimen number and patient name using the keyboard. Expected ranges for blood counts differ based on gender and age.
5. The Cell-Dyn is programmed to display the correct reference range. The operator, however, must first manually type in the correct gender prior to running the patient sample. Once RUN Ready is displayed in the Status Box, use the ↓key to scroll to the Limit prompt. Enter either “1” for Male or “2” for Female. Mix the patient sample well and remove the cap. Place the sample

probe in the tube so that the end is immersed in the sample but not resting on the bottom of the tube.

6. Press the Touch Plate to start the run. The Status Box on the RUN menu indicates the stage of the run. When Remove Specimen is displayed in the Status Box and the probe has moved up through the wash block remove the sample tube and replace the tube cap.
7. A beep will indicate that the probe cleaning cycle has begun. After the probe cleaning cycle is complete, the probe will move down into position for the next sample and the results will be displayed on the screen.
8. If needed, press PRINT REPORT for a hardcopy of the report. After sampling is complete, press MAIN to return to the MAIN MENU. Change the Operator ID to "000"for the next user.

**Reagents for Cell-Dyn 1800**

1. **Cell-Dyn Diluents:**

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.
- Do not use if reagent has been frozen.

2. **Cell-Dyn Lytic Agent:**

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.
- Do not use if reagent has been frozen.

3. **Cell-Dyn Detergent:**

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.
- Do not use if reagent has been frozen.

4. **Enzymatic Cleaner:**

- Stable at 2-8°C until the expiration date on the container.
- Do not use if reagent has been frozen.

5. **Cell-Dyn Whole Blood QC**:

- Unopened QC vials are stable at 2-8°C until the expiration date on the vial. Opened QCvials are Stable at 2-8°C for 7 days after opening. Do not use expired QC.
- Allow QC to sit at room temperature for fifteen minutes before testing.
- Mix QC vial by rolling the vial between palms for 20 seconds.
- Invert the vial and roll it back and forth for another 20 seconds.
- Gently invert the vial 10 times.
- Do not shake.
- Continue to mix in this manner until cells are completely suspended (3-5 times).
- Gently invert the pre-mixed vial 5 times immediately before testing.
- Return vial to refrigerator when testing is complete.

6. **Whole Blood Calibrator:**

- Unopened calibrator vials are stable at 2-8°C until the expiration date on the vial. Opened calibrator vials are stable at 2-8°C for 7 days after opening. Do not use expired calibrators.
- Allow the calibrator to sit at room temperature for fifteen minutes before testing.
- Mix the calibrator vial by rolling the vial between the palms for 20 seconds.
- Invert the vial and roll it back and forth for another 20 seconds.
- Gently invert the vial 10 times.
- Do not shake.
- Continue to mix in this manner until cells are completely suspended (3-5 times).
- Gently invert the pre-mixed vial 5 times immediately before testing.
- Return vial to refrigerator when calibration is complete.

3**. Preparation of thick blood film and staining with Geimsa stain**

**Principle**: - during staining of the drop of dried blood, the emoglobin in the red cells dissolves and is washed out by the water in the staining solution. All that remains are the parasites and the white cells which can be seen under the microscope.

**Materials required:**

- Microscope slides
- Staining through
- Glass rod
- Wash bottle
- Slide forceps
- Slide rack
- Timer
- Giemsa stain
- Buffered water
- Lead pencil
- Applicator stick
- Funnel
- Filter paper

**Preparing Thick Film**

1. Make a thick smear in the center of the slide. Spread the blood with the corner of a clean slide or applicator stick to an even thickness. Smears that are too thick or too thin will not stain well.

   NB: a correct thickness enables the hands, but not the figures, of a watch to be seen through the smear.
2. Label the end of the slide with patient's number using a lead pencil.
3. Leave the thick film o dry in the air. The smear should be protected from flies and dust.

**Staining of thick film with Giemsa**

1. Make a 1 in 10 dilution of Giemsa stain

   Example: use 10ml of Giemsa stock solution and 90ml buffered water
2. Mix gently with a glass rod
3. Filter the diluted, working Giemsa stain using filter paper
4. Place the slides over the staining rack
5. Cover the dried smears with diluted Giemsa stain.
6. Leave for 30 minutes.

7. Wash off the stain with buffered water. Do not tip off the stain and then wash, as this will leave a deposit of stain over the smear.
8. Drain off the water. Place the slides in a rack to dry. Place them in a sloping position, the slides with the stained films facing downwards to protect them from dust in the air.

---

Methods for large numbers of smears

1. Using forceps, pick up the slides one by one and slot them into the rack of the staining trough in a Z pattern
2. Make up sufficient stain to fill the staining through. Slowly fill the staining trough containing the slides. Cover and leave them for 30 minutes out of the sunlight.
3. Remove the lid. Slowly pour clean water from a beaker into the trough, to remove the deposit on the surface of the staining.
4. Gently, pour off all the staining solution from the trough.
5. Fill the staining trough with buffered water
6. Take out the slides one by one, using forceps
7. Drain the slides. Place them in a rack to dry (the side with blood film facing downwards)

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Result interpretation:

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Wet mount stool smear preparation

Principle:- The direct wet mount is used primarily to detect motile protozoan trophozoites. These organisms are very pale and transparent, two characteristics that require the use of low light intensity. The direct wet smear is prepared by mixing a small amount of stool (about 2gm) with a drop of 0.85% NaCl. This mixture provides a uniform suspension under a 22 by 22 cover slip .The entire 22 by 22 mm cover slip should be systematically examined with the low power objective (10X) and low intensity; any suspicious objects may then be examined with the high dry objective (40X).

---

Materials

- Microscope slide
- Coverslip,22mm x22mm
- Wooden applicator
- Lead pencil

- Sodium chloride solution
- Microscope

Procedure

1. Take a slide and put 1 drop of sodium chloride solution in the middle of the microscope slide
2. Using an applicator stick, take a small portion of the stool. If the stool is formed, take the portion from well inside the sample and from the surface. If the stool contains mucus or liquid, take the portion from the blood stained mucus or from the surface of the liquid.
3. Mix the sample with the drop of sodium chloride solution on the slide
4. Place a cover slip over the mixture in a way possible to avoid the formation of air bubbles
5. Examine the preparation under the microscope. Use 10 x and 40x objectives. As the eggs and cysts are colorless, reduce the amount of light using the condenser aperture or lower the condenser to increase the contrast.

Result interpretation: - Report the presence of the ova, cyst, trophozoite or adult stage of the intestinal parasite.

Procedure for modified Ziehl- Neelsen technique

**Principle**: The lipid capsule of acid fast oocyts of protozoa takes up carbol fuschin and resists decolorarization with a dilute acid rinse. The lipoid capsule of the organisms is of such high molecular weight that is waxy at room temperature and successful penetration by the aqueous based staining solutions is prevented. The oocyts stain red taking the color of the primary stain because of mycolic acid and the background stain green because of malachite green.

**Materials required**

- Carbol fuschin
- Methanol
- Methylene blue
- 1% acid alcohol
- Applicator stick
- Microscope slide
- Staining rack
- Wash bottle
- Microscope

- timer

---

Method:

a. Fecal smears are made either directly from the stool sample or from the concentration deposit
b. Allow to air dry
c. Fix in methanol for 3 minutes
d. Stain with strong carbol fuschin for 15- 20 minutes
e. Rinse thoroughly in tap water
f. Decolorize in acid alcohol (1% HCl in methanol) for 15-20 minutes.
g. Rinse thoroughly in tap water.
h. Counter stain with 0.4% malachite green (or methylene blue) for 30-60 seconds.
i. Rinse thoroughly and air dry
j. Examine using x40 and x100 objectives.

---

Result interpretation: Report the presence of oocysts based on the distinguishing characteristics under 100 X objective

## 2. Procedure to take weight measurement from the study participants

1. Zero the scales before the clients steps onto them
2. Ask the client to remove any heavy items from their pockets (keys, wallets, etc) and remove any heavy items of clothing or apparel (big jackets, shoes, woolen jersey etc)
3. Ensure you note the clients state and time of day for testing to ensure any subsequent tests can be taken under identical conditions ( check state of hydration, food consumed recently)
4. When measuring weight, ask clients to look straight ahead, stay still on the scale. Wait for the needle digital screen to settle for recording the measurement.

## 3. Procedure to Take Height Measurement

Ideally height measurement will be taken using 'drop down' tape measure fixed at about 2 meters on a wall or a specific piece of measuring equipment. A reliable measurement can be taken without this equipment by making a point (top of clients' head, against a wall and measuring up to it.

1. Ask the clients to remove their shoes prior to the measurement
2. Ask the clients to stand their back to the wall and look directly forward. The back of their feet, calves, bottom, upper back and the back of their head should all be in contact with the wall. They should be positioned directly under knees the 'drop down' measuring device
3. Lower the measuring device until it rests gently on the top of the clients head and record the measurement.

## 4. Calculating Body Mass Index (BMI)

The calculation is based on comparing a person's weight against height. It applies equally to men's and women.

The equation for BMI is $BMI = \frac{\text{weight (KG)}}{\text{Height } (m^2)}$

The following table categorized people according to their BMI result

- ✓ Underweight <18.5
- ✓ Normal 18.5-24.9
- ✓ Overweight 25-29.9
- ✓ Obesity >30

Annex II**:** Information sheet **(for participants, English version)**

**Title of the Research Project:** Prevalence and Associated Factors Of Anemia In HAART Naïve And HAART Experienced Adult HIV Patients At A Selected Hospital, Addis Ababa, Ethiopia, 2015.

**Name of Investigator:** Rahel Alemu (BSc, Msc candidate)

**Name of the Organization:** Addis Ababa University, College of Health Science, school of allied sciences, Department of medical Laboratory Science.

**Introduction**

You are invited to participate in a study to be conducted by MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It is aimed at determining the prevalence of HIV– induced anemia and to assess the associated and aggravating factors of the anemia. After the result of the study is disseminated, strategies will be designed to prevent and control the predisposing factors. Moreover, it will also be a useful reference for drug choice. Please read the following statements and ask any unclear points before you agree to participate.

Participation in the study is exclusively voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign the consent form and you can get a copy of this information sheet.

Participation in this study is exclusively voluntarily. If you are not interested to participate to you or if you once decide to participate and want to draw from participation at any time, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign the consent form and may obtain a copy of this information sheet.

**What is expected from you as a participant of the study?**

As a participant of this study you are expected to give 3-4 ml blood and stool specimen. In addition you are expected to give answers for some questions about yours health and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals out of this hospital. But your name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

**How long participation will take you?**

You will spend 20-35 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

**What are the risks of participating in this study?**

There are no anticipated risks to your participation except minor discomfort during venipuncture because well experienced professionals will collect blood samples.

**How the information is to be kept confidential?**

All information that you give and the results from your specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

**What are the benefits from participation?**

Since this study is MSc student research, there will not be payment for participants. But your participation is important for studying the prevalence and associated factors of anemia which will be useful in the improvement of management of HIV positive patients.

**What are your rights as a participant of this study?**

You can ask any question questions for further explanation. The principal investigator and the data collectors are responsible to clear any doubt you may have during participation. You have the right to get the results of the analysis.

**What can I do if I have a problem or a question?**

Please forward any question or problems you may encounter during this study to

Rahel Alemu

Department of medical laboratory science

School of Allied health sciences

College of health sciences

Addis Ababa University

Mob: +251-929- 04 54 54/0923-48-59-43

Email:Rahelalemu2014@gmail.com

**Agree to participate?**

- Yes
- No

**Annex III- Subject information sheet (for participants, Amharic version)**

አዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ ,የአላይድ ጤና ሣይንስ ት/ቤት ,የሕክምና ላቦራቶሪ ሣይንስ ክፍል እድሜያቸው ከአስራስምንት አመት በላይ ከሆኑ አዋቂዎች ላይ የደምና የሠገራ ናሙና ተወስዶ ለሚሰራው የደምማነስ፤ለችግሩተዛማጅ ና አባባሽ ይሆናሉ ተብለው የሚገመቱትን ሁኔታዎች ለማጥናት ታስቦ ለተሳታፊዎች የተዘጋጀ መረጃ ሲሆን እርሶም በአዲስ አበባ ዩኒቨርሲቲ ፡ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል ፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን /ኑትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

***መግቢያ***

**የጥናቱ ርዕስ፡** በዓዋቂነት የዕድሜ ክልል በሚገኙ የኤድስ ቫይረስ ተጠቂ ግለሰቦች ላይ የሚታይ የደም ማነስ ግዝፈት፤ ለችግሩ አጋላጭና ተዛማጅ የሆኑ ሁኔታዎች›› እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም ፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅብዎታል፡፡ ፡፡ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

**የጥናቱ ተሳታፊ በመሆኖ የሚጠበቅቦት ምንድን ነው?**

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደምና የሠገራ ናሙና ለመስጠት መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉልና ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

**በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?**

የተዘጋጀዉን መጠይቅ ለመሙላት፤ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-35 ደቂቃ ያስፈልጋል፡፡

**በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?**

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምምትምምክንያቱምናሙናውየሚወሰደውልም ድ ባላቸው የጤና ባለሙያዎች በመሆኑ ነው።

**የእኔየህክምናመረጃበሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?**

የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው። ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው። ከዚህም በላይ ስለእርሶ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባላው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረገል።

**በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?**

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ ፅሁፍ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም። ሆኖም ከጥናቱ የሚገኘው መረጃ የኤች አይ ቪ ህሙማንን ህክምና ለማሻሻል አስተዋፅዎ ያደርጋል።

**የዚህጥናትተሳታፊመብቱ ምንድን ነው ?**

ከዚህምበተጨማሪጥናቱንበተመለከተ
ማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአለዎት።የላብራቶሪምርመራውጤቱንምበነጻማግ ኘትይችላሉ።

**ጥያቄካለኝወይምችግርቢያጋጥመኝምንማድረግይገባል?**

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይምጥያ ቄካለዎትበሚመለከተውአድራሻይጠቀሙ።

ራሄል ዓለሙ

የህክምናላባራቶሪሳይንስት/ክፍል

የአላይድጤናሳይንስት/ቤት

የጤናሳይንስ ኮሌጅ

አዲስአበባዩኒቨርሲቲ

**ሞባይል** +251-929-04-54-51/0923-48-59-43

**ኢሜይል**Rahelalemu2014@gmail.com

**ለመሳተፍይስማማሉ?**

**እስማማለሁ□አልስማማም□**

## Annex-IV- Consent Form (for participants, English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed at determining the prevalence of anemia and assessing the associated factors. For this study blood and stool samples are required from a participant. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my blood and stool sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my sample measured for free via the counselor nurse.

Participant's signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

**Please direct any questions or problems you may encounter during this study to:**

Rahel Alemu

Department of medical laboratory science

School of Allied health science

College of health sciences

Addis Ababa University

Mob: +251-929-04-54-51/0910-27-05-36

Email: Rahelalemu2014@gmail.com

For additional information, please contact Addis Ababa University, College Of Health Science, and Departmental Research and Ethics Review Committee (DRERC) office at:

Tell. +251-11-2-75-51-70

Fax +251-11-2-75-46-69

P.O.Box 1176, Addis Ababa, Ethiopia

Email: SMLT@ethionet.et

**Annex-V** - Consent form (for participants, Amharic Version)

**<u>የተሳታፊዎችስምምነትማረጋገጫቅጽ</u>**

**የሚስጥርቁጥር ----------**

**የተሳታፊዉስም ----------**

**እኔስሜከላይየተጠቀሰውተሳታፊበዓዋቂነትየዕድሜክልልበሚገኙየኤድስቫይረስተጠቂግለሰቦችላይየሚታ ይየደምማነስግዝፈት፤ለችግሩአጋላጭናተዛማጅየሆኑሁኔታዎችላይስለሚደረገውጥናትበቂገለጻተደርጎ ልኛል፡፡ለጥናቱምከእኔየተወሰደየደምናየሰገራ ናሙናእንደሚያስፈልተገልጾልኛል፡፡የጥናቱንምአላማዎችበሚገባ ተረድቻለሁ፡፡**

**በመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል፡፡በጥናቱላይያለ መሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብቱየተጠ በቀእንደሆነተገልጾልኛል፡፡**

**ስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነ ትነው፡፡የምሰጠውም ናሙና ለምርምርብቻእንደሚውልምተረድቻለው፡፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትን ያህልማብራሪያአግኝቻለሁ፡፡የዚህጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻ ማግኘትእንደሆነተረድቻለሁ፡፡**

**የተሳታፊዉ ፊርማ /የጣት አሻራ ----------**

**የምስክር ሙሉ ስምናፊርማ**

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

**(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)**

**የመረጃ ሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------**

**---**

**ጥናቱን የሚያካሂደዉ ሰዉማረጋገጫ**

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

ራሄል አለሙ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251-929-04-54-51/0910-27-05-36

ኢሜይል: Rahelalemu2014@gmial.com

ለተጨማሪ መረጃዎች የአዲስ አበባ ዩኒቨርሲቲ ፤ጤና ሳይንስ ኮሌጅ ፤ሜዲካል ላቦራቶሪ ሳይንስ ት/ቤት የምርመር ስነ-ምግባር ተቆጣጣሪ ኮሚቴ ቢሮ ይደውሉ፡፡

ስ.ቁ+251-11-2-75-51-70

ፋክስ +251-11-2-75-46-69

ኢሜይል: SMLT@ethionet.et

## ANNEX VI: Data Extraction Form

**Introduction**

I_____ am working as a data collector in this study to assess the prevalence and related factors of anemia in HAART naïve and HAART experienced adult HIV infected individuals in Zewditu memorial hospital, Addis Ababa, Ethiopia ART clinic. The name of the participant is not going to be written but the demographic and clinical data. All the extracted data will be kept entirely confidential.

Identification code_____

Date of data collection _____

Name of the data collector_____

Supervisor_____

**Instruction**: fill the information, either thick in the appropriate boxes by using "X" or a word or phrases where required.

Section I: socio-demographic and clinical characteristic of the study participants

1. Sex Female □ male □
2. Age (in complete years) _____
3. Weight (in kilogram)_____
4. Height (in meter):_____
5. Occupation of the participant
   □ Employee
   □ unemployed
6. Functional Status
   □ Working
   □Ambulatory
   □Bedridden
7. Opportunistic infection
   Yes □ No □

8. WHO clinical stage of HIV/AIDS

   Stage I □ Stage II □ stage III □ stage IV none □

9. Intestinal parasite

   Positive □

   Negative □

10. If positive to question No. 12, name of the parasite _____

11. Hemoparasite

    Positive □

    Negative □

12. If Positive to question No.14, Name of the parasite_____

13. Status of the patient

    HAART- naïve □ HAART experienced □

14. HAART regimen (For HAART experienced participants only)

    1. D4T/3TC/NVP
    2. ZDV/ 3TC/NVP
    3. TDF/ 3TC/NVP
    4. TDF/ 3TC/EFV
    5. D4T/3TC/EFV
    6. D4T/3TC/EFV

15. Duration of HAART in months_____

16. Other therapeutic drugs

    Yes □ No □

17. If yes, type/s of drugs:_____

**Section II hematological and immunological variables of the study participants**

WBC_____ x $10^3$cells/µL

RBC_____ x $10^6$ cells/µL

Hgb_____ g/dl

Hct_____ %

MCV_____fL

MCH_____pg

MCHC_____%

Platelet_____x $10^3$cells/μL

CD4 count_____ cells/μL

## Declaration

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

Principal investigator: Rahel Alemul (Bsc, MSc. Student, CLS, AAU)

E-mail: Rahelalemu2014@gmail.com Phone: +251-929-04-54-51

Signature----------

Approval of advisors

1. Dr. Aster Tsegaye (PhD,AAU)

E-mail:tsegayeaster@yahoo.com Phone: +251-911-69-60-85

Signature----------Date----------

2. Jemal Alemu (Msc, AAU)

E-mail-Jemalalemu@gmail.com Phone: +251-911-42-99-89

Signature----------Date----------