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CHANGES IN HEMATOLOGICAL PARAMETERS IN HIV-1 INFECTED PATIENTS BEFORE AND AFTER ANTIRETROVIRAL THERAPY IN TIKUR ANBESSA SPECIALIZED TEACHING HOSPITAL ART CLINIC, ADDIS ABABA, ETHIOPIA

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY

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ADDIS ABABA UNIVERSITY

November, 2011

Addis Ababa, Ethiopia

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Title of the thesis:

Changes in hematological parameters in HIV-1 infected patients before and after antiretroviral therapy in Tikur Anbessa Specialized Teaching Hospital ART clinic.

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Declaration

I, the undersigned, assure that this M.Sc research project proposal is my original work and has not been presented for a degree in any other university. False statements could be cause for invalidating this research thesis and may lead to other administrative or legal action.

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ABBREVIATIONS

AIDS- acquired immunodeficiency disease

ADRs- adverse drug reactions

ART- antiretroviral therapy

AZT- azidothymidine

CBC- complete blood count

CDC- center for disease control

FACS- fluorescent activated cell sorter

HAART- highly active antiretroviral therapy

HIV- human immunodeficiency virus

MCH- mean cell hemoglobin

MCHC- mean cell hemoglobin concentration

MCV- mean cell volume

NIH- national institution of health

NNRTI- non-nucleoside reverse transcriptase inhibitors

NRTI- nucleoside reverse transcriptase inhibitors

PCV- packed cell volume

PI- protease inhibitors

RDW- red cell distribution width

TTP- thrombotic thrombocytopenic purpura

WHO- world health organization

ZDV- zidovudine

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ACKNOWLDGEMENTS

First of all, I would like to thank God for being with me throughout my work.

I wish to thank Department of Biochemistry, Faculty of Medicine, Addis Ababa University for financing and school of graduate studies of the university for allowing me to carry out my research and Dessie University for sponsoring of my MSc. study.

My deepest and heartfelt gratitude forwarded to my advisor, Dr. Daniel Seifu for his unreserved encouragement, hospitability, and support while I was seeking his assistance from the beginning till the accomplishment of this research.

My cordial appreciation also forwarded to my co-advisor, Dr. Aster Tesgaye for her unreserved assistance and support in conducting the research.

I am very grateful to thank my co-advisor Dr. Wondwossen Amogne for his constructive assistance and guide.

I want to thank the management office of Tikur Anbessa specialized Teaching Hospital and head of the ART clinic for allowing me to carry out a research in the hospital.

Finally, my gratitude extends to the ART clinic staff members and Medical Laboratory workers for their day to day cooperation while conducting the research.

ABSTRACT

HIV/AIDS is characterized by progressive damage to the body's immune system that results in a number of opportunistic infections, immunological and hematological complications. Hematological complications have been documented to be the second most common cause of morbidity and mortality and are estimated to affect from 11% to 75% of patients with HIV disease. They are generally marked with cytopenias such as anemia, neutropenia, lymphopenia and thrombocytopenia. These abnormalities may be of greater concern in populations in sub-Sahara Africa, where comorbidities such as malnutrition, malaria, and parasitic infections are common, and capacity to monitor adverse events related to ART is often limited. Although treatment of HIV infected patients using ART is accepted as the gold standard, few studies tried to assess hematologic responses to ART.

A comparative cross sectional study was conducted to determine changes in hematologic parameters in HIV infected patients before and after ART from July to August 2011 in TASTH ART clinic. Socio-demographic, baseline hematologic parameters and ART related information of study participants were collected using a structured questionnaire. CBC and CD4+ T cell count was done for 340 HIV infected patients by hematological analyzer and FACS count, respectively. The data was entered and analyzed in simple descriptive statistical methods using SPSS window version 16.

From this study, WBC, neutrophils, lymphocyte, hemoglobin and platelet count showed increment from the baseline by mean 0.36 x 10^3/ μL, 0.12 x 10^3/ μL, 0.25 x 10^3/ μL, 0.98 g/dL and 20 x 10^3/ μL, respectively after ART. Proportion of leucopenia, neutropenia, lymphocytopenia, anemia and thrombocytopenia was dropped from baseline 25.9% to 11.5% (p-value= 0.000), 23.2% to 17.4% (p-value= 0.057), 24.1% to 9.1% (p-value= 0.000) 45% to 20.88% (p-value= 0.000) and 28.5% to 11.2% (p-value= 0.000), respectively. Nevertheless, reduction in mean hemoglobin (p-value= 0.070) and high proportion of anemia (p-value= 0.000) was observed in AZT than non-AZT containing ART treatment.

In conclusion, ART was effective in improving blood cell count and decreasing hematologic abnormalities in HIV infected patients.

Key words: HIV, ART, Anemia, Neutropenia, Thrombocytopenia, CD4+ T cells

1. INTRODUCTION

Human immunodeficiency virus is an RNA retrovirus. It is the infectious agent that causes AIDS, a disease that leaves a person vulnerable to life threatening infections. Two types of this virus have been identified. HIV-1 is the primary cause of AIDS worldwide. HIV-2 is found mostly in West Africa. AIDS is now a pandemic and sub-Saharan Africa has been described as the worst hit region by AIDS. UNAIDS estimates in 2009 suggested that 33.3 million people worldwide had HIV infection. It caused death in 1.8 million people and 1.3 million (76%) of those deaths occurred in sub-Saharan Africa and 1.8 million people became infected with HIV in this region **[**UNAIDS Report on the Global HIV/AIDS Epidemic, 2010**]**. National projections estimate approximately 1.1 million (2.1%) Ethiopians were living with HIV in 2007 and prevalence increased slightly to 2.3 percent by 2009 **[**UNAIDS Report on HIV/AIDS Prevalence in sub-Saharan Africa, 2010**].**

HIV/AIDS is characterized by progressive damage to the body's immune system which results in a number of opportunistic infections, immunological and hematological complications. Hematological complications have been documented to be the second most common cause of morbidity and mortality in HIV patients and are generally marked with cytopenias such as anemia, neutropenia, and thrombocytopenia **[**Moyle, 2002**]**. These abnormalities are estimated to affect from 11% to 75% of patients with HIV disease. Of these abnormalities, anemia is the most common hematologic manifestation. The incidence and severity of the cytopenia generally correlate to the stage of the disease with anemia being the most commonly encountered hematologic abnormality and a significant predictor of progression to AIDS or death. In addition, hematologic toxicities comprise a significant proportion of the adverse events secondary to antiretroviral therapy **[**Odunukwe and Idigbe, 2005**]**.

The introduction of highly active antiretroviral therapy (HAART), a cocktail of nucleoside and non-nucleoside analogues capable of inhibiting reverse transcriptases and proteases, in industrialized countries during the mid 1990s led to well documented reductions in the risk of AIDS defining illness, AIDS related mortality and hence, improve the quality of life of people living with HIV/AIDS in developed and developing countries **[**Palella and Delaney, 1998**]**.

Treatment with HAART generally improves hematologic abnormalities associated with advanced HIV disease even though antiretroviral drugs such as zidovudine can cause anemia and leucopenia **[**Richman and Fischl, 1987**]**.

Hematologic abnormalities may be of greater concern in populations in sub-Saharan Africa, where comorbidities such as malnutrition, malaria, and parasitic infections are common and capacity to monitor adverse events related to antiretroviral therapy (ART) is often limited. Hematological adverse drug reactions are the most common cause of ART interruption **[**Fellay and Boubaker, 2001**]**. A combination of genetic and environmental factors may make hematologic abnormalities more common in African populations as compared to Western populations.

Estimates of anemia in sub-Saharan African range from 58% and 75% with infants and pregnant women bearing the highest burden [Sullivan and Hanson, 1998]. Indeed, many ART programs in Africa have used stavudine (d4T) instead of ZDV as part of the first-line ART regimen, partly because of concerns about anemia in this population. Even in ART programs that do not use ZDV as part of the first-line ART regimen, a significant proportion of patients receiving d4T will likely be switched to ZDV because of toxicity [Fatu and David, 2007].

With AIDS becoming a global emergency, treatment using ART became the most effective health care intervention and accepted as the gold standard in the management of HIV patients **[**Amballi and Ajibola, 2007**]**. However, it has been documented up to 25% of patients discontinue their initial HAART regimen because of treatment failure (inability to suppress HIV viral replication to below the current limit of detection, 50 copies/mL), toxic effects or noncompliance within the first 8 months of therapy. One of such HAART is ZDV and Lamivudine/3TC.They belong to a class of antiretrovirals known as nucleoside analogue reverse transcriptase inhibitors (NRTIs) which act by inhibiting reverse transcription by incorporating into the newly synthesized viral DNA and preventing its further elongation. The NRTIs are the backbone of HAART. Combining Zidovudine and Lamivudine has been shown to have synergic antiretroviral effect **[**URL:http//www.who.int/medicine/publications/ARV survey, accessed on 3 May 2011**]**.

ZDV should be used with caution in patients who have bone marrow compromise evidenced by granulocyte count <1,000 cells/mm^3 or hemoglobin <9.5 g/dL. Hematologic toxicities appear to be related to pretreatment bone marrow reserve and to dose and duration of therapy. In patients with advanced symptomatic HIV-1 disease, anemia and neutropenia were the most significant adverse events observed. In patients who experience hematologic toxicity, a reduction in hemoglobin may occur as early as 2 to 4 weeks and neutropenia usually occurs after 6 to 8 weeks. There have been reports of pancytopenia associated with the use of ZDV, which was reversible in most instances after discontinuance of the drug. However, significant anemia, in many cases requiring dose adjustment, discontinuation of ZDV and/or blood transfusions, has occurred during treatment with the drug alone or in combination with other antiretrovirals **[**Molina and Bentata, 2005**]**.

Frequent blood counts are strongly recommended to detect severe anemia or neutropenia in patients with poor bone marrow reserve, particularly in patients with advanced HIV-1 disease who are treated with ZDV. If anemia or neutropenia develops, dosage interruption may be needed **[**Richman and Fischl, 1987**]**.

1.1. Literature Review

Clinically significant hematologic abnormalities are common in persons with HIV infection. Impaired hematopoiesis, immune mediated cytopenia, peripheral destruction of blood cells and altered coagulation mechanisms have all been described in HIV infected individuals. These abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and associated conditions **[**Zon and Groopman, 1988**]**.

Both ineffective hematopoiesis and peripheral destruction of blood cells may lead to cytopenia, which are common in AIDS patients. Ineffective hematopoiesis in patients with AIDS has several potential causes, which may include, for example: (1) Suppression of the bone marrow by the HIV infection through abnormal cytokine expression and alteration of the bone marrow environment; (2) the formation of circulating inhibitors (viral envelope glycoprotein gp120 and tumor growth factor beta-1); and (3) the direct infection of hematopoietic stem cells by HIV **[**Folks and Kessler, 1988**]**.

Anemia is a very common finding in patients with HIV infection, particularly in individuals with more advanced HIV disease **[**Spivak and Barners, 1989**]**. It occurs in 10 – 20% at initial presentation but could increase to involve approximately 70– 80% of patients during the course of the disease. In both antiretroviral treated and untreated individuals, anemia is independently associated with an increased risk of disease progression and death. This may be due to decreased in production of RBC due to infiltration of the bone marrow by neoplasm or infection, use of myelosuppressive medications like AZT, HIV infection itself, a decreased production of endogenous erythropoietin, a blunted response to erythropoietin, or hypogonadism.

Another cause of anemia is due to increased or premature RBC destruction in the spleen or the circulatory system, which may occur in patients with HIV infection. Hemolytic anemia may result from RBC autoantibodies, hemophagocytic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, or glucose-6-phosphate dehydrogenase deficiency. Hemolysis may also develop because of the use of various medications. ZDV (AZT) therapy is probably the most common cause of anemia **[**Richman and Fischl, 1987**]**.

Ineffective RBC production because of deficiencies in iron, folic acid, or vitamin B_{12} can also lead to anemia **[**Zon and Groopman1987**]**.

Neutropenia (granulocytopenia) is another commonly encountered hematologic abnormality in patients with HIV infection. Although low granulocyte counts usually reflect the toxicity of therapies for HIV infection or associated conditions, studies of untreated patients have also shown a high incidence of granulocytopenia, particularly in patients with more profound immunodeficiency **[**Kalsow and Phair, 1987**]**.

The pathogenesis of granulocytopenia in patients with HIV infection is multifactorial. An autoimmune mechanism involving antigranulocyte antibodies [Van der Lelie and Lange, 1988**]** and impaired granulopoiesis **[**Folks and Kesslers, 1988] has been postulated, but not yet proved, to account for granulocytopenia in some patients. In clinical practice, however, drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV infection. Neutropenia, as in the other peripheral blood cytopenias in the setting of HIV infection has multiple etiologies, which may be present either singly or in combination. Thus, decreased colony growth of the progenitor cells leads to decreased production of both granulocyte and monocytes produced by the infected cells known to suppress neutrophils production **[**Moses and Nelson, 1998**]**.

A number of medications (ART) commonly used in the setting of HIV infection can cause granulocytopenia, but AZT therapy is probably the most common cause of low granulocyte counts **[**Shaunak and Bartlett, 1989**]**.

Association between thrombocytopenia and HIV infection is well known for over 20 years. Possible etiologies of thrombocytopenia in patients with HIV infection include immune mediated destruction, thrombotic thrombocytopenic purpura, impaired hematopoiesis, and toxic effects of medications. The most common cause is immune thrombocytopenic purpura (ITP) **[**Bettaied and Fromont, 1992**]**.

In many instances, however, thrombocytopenia is a relatively isolated hematologic abnormality associated with a normal or increased number of megakaryocytes in the bone marrow and elevated levels of platelet-associated immunoglobulin. These patients have the clinical syndrome commonly referred to as ITP **[**Karpatkin and Nardi, 1988**]**.

A patient with thrombocytopenia has true HIV-ITP if there is no other condition or treatment that could cause thrombocytopenia. Most such patients are otherwise well. In fact, HIV-ITP is most often an early manifestation of HIV infection, occurring before the development of any CDC AIDS-defining condition **[**Jost and Tauber, 1988**]**. CD4+ lymphocyte counts in reported series of patients with HIV-ITP have averaged between 300 and 600 cells/mm^3. HIV-ITP is therefore commonly included among those conditions characterizing the middle-stage HIV disease**.** ITP typically improves as HIV disease progresses **[**Abrams, 1988**]**.

Several hypotheses have been advanced to explain the pathogenesis of HIV-ITP. One theory holds that circulating immune complexes are nonspecifically deposited on platelet membranes, resulting in reticuloendothelial clearance. Studies have shown that these immune complexes contain anti-HIV gp120 and complementary anti-idiotypic antibody. The hypothesis that a specific antiplatelet antibody binds to the platelet membrane, resulting in platelet destruction, has fallen out of favor **[**Karpatkin and Nardi, 1989**]**.

ZDV therapy has improved platelet count without changing platelet survival and this suggests improved production. Megakaryocytes are infected by HIV virus and HIV viral particles have been documented in the megakaryocytes by electron microscopy. HIV p24 antigen has also been documented in the megakaryocytes by immunohistochemical techniques while HIV RNA was detected using *in situ* hybridization studies. Treatment is unnecessary unless patient is symptomatic or platelet count drops below 30,000/μL. Almost 20% of patients with HIV associated thrombocytopenia undergo spontaneous remission. The most effective treatment is the use of HAART therapy, however, historical treatment with AZT alone was also effective **[**Gruszeeki and Wehrli, 2002**]**.

Thrombotic thrombocytopenic purpura (TTP) is another well documented complication of HIV infection. It was recorded in 1.5% of affected patients prior to introduction of HAART therapy. It is a clinical syndrome characterized by the classic pentad of fever, neurologic dysfunction, renal dysfunction, microangiopathic hemolytic anemia, and thrombocytopenia. The finding of hyaline microvascular thrombi in tissue biopsy specimens supports the diagnosis. Abnormal interaction between platelets and endothelium (HIV related endothelial cell perturbation) are thought to be responsible for the clinical and pathologic findings, but the mechanism accounting for this

observation remains undefined. Plasmapheresis is generally accepted as standard therapy for TTP, although plasma infusions, exchange transfusions, antiplatelet drug therapy, corticosteroids, and splenectomy have all been used with varying degrees of success **[**Shepard and Bukowski, 1987**]**. Therapy consists of plasma exchange. It is unclear whether HAART therapy is helpful in prevention or treatment of TTP **[**Gruszeeki and Wehrli, 2002**]**.

In general, ART is known to profoundly suppress viral replication, increases CD4+ T cell count and delays disease progression and death. Nevertheless, the direct role of HIV-1 replication in hematologic disorders and the effectiveness of antiretroviral therapy to correct them are not fully understood. HAART has been shown to improve HIV related cytopenia, presumably by inhibiting virus replication. Clinical observations supporting this assumption have, however, rarely been reported in the medical literature **[**Mildvan and Creagh, 2007**]**.

Despite the positive therapeutic effects, ART may cause undesirable adverse effects, which largely challenge the management of HIV infected patients. Adverse drug reactions are the most common reasons for ART interruption among HIV infected patients and over 25% of these patients discontinue ART within the first year of treatment due to side effects. Immune deregulation, altered drug metabolism and polypharmacy in these patients result in the presence of some degree of ADRs in about 80% of HIV-infected patients. From these ADRs, some are clinically presented and the others are laboratory documented. The etiologies of these effects are thought to be complex. Each antiretroviral drug is associated with specific adverse effects. Among the antiretroviral drugs, ZDV (AZT) remains to be the most widely used drug resulting in myelosuppression. The hematological toxicity observed is proposed to be caused by inhibition of hematological progenitor cells **[**De Jesus and Herrera, 2004**]**.

A cross sectional study was conducted in Ghana (2008-2009) to assess the efficacy and ability of HAART to resolve immunological and hematological abnormalities in HIV infected patients. Using packed cell volume less than 30% as an indicator for anemia, 37.6% (88/234) of the HAART naive patients were 3 times at risk of having reduced PCV compared to 15.2% (28/151) of the patients on HAART. The odds of PCV being reduced in both male and female patients on HAART compared to those of HAART naive was not, however, significant and the calculated mean PCV (35.68 ± 0.55%) in patients on HAART was significantly higher than that of patients who were HAART-naive (32.64 ±0.48%) [Owiredu and Quaye, 2011].

According to WHO/ACTG grading system, anemia toxicity grades gave a 63% and 46% calculated incidence of anemia (Hb < 10.5 g/dL) in HAART naive patients and those on HAART respectively. The calculated mean hemoglobin of (11.54 ± 0.33 g/dL) and (10.33 ± 0.16 g/dL) in males and females on HAART, respectively, were significantly higher when compared to their naïve counterparts (10.59 ± 0.29 g/dL and 9.45 ± 0.13g/dL). However, there was no statistical significance between the mean hemoglobin values of patients on HAART and those who are HAART-naive (10.59 ± 0.15 and 9.80 ± 0.12) [Owiredu and Quaye, 2011].

Using neutrophil count (<60%) and platelet count (<150 x 10^3/μL) as indicators of neutropenia and thrombocytopenia, respectively, there was no significant difference in the relative risk of developing neutropenia in HAART naïve and those with HAART (96.2% and 99.3%), respectively. Similarly, there was no significant difference in the odds of developing thrombocytopenia in the study populations (50.4% in HAART naive and 51.7% in patients on HAART with mean platelet count 174.40 ± 7.20 and 148.80 ± 6.07 x 10^3/μL), respectively [Owiredu and Quaye, 2011].

A study conducted in Uganda (2003-2007) showed 279 (25.6%) patients who initiated d4T containing ART had anemia, 94 (8.6%) had leukopenia, and 147 (13.5%) had thrombocytopenia. At enrollment, 69 (23.8%) of the patients who later had ZDV substitution had anemia, 24 (8.3%) had leukopenia, and 39 (13.4%) had thrombocytopenia. Hematologic parameters generally improved among patients taking d4T containing ART and cotrimoxazole in this rural Ugandan population Uganda [Fatu and David, 2009].

A higher incidence of anemia and leukopenia was observed after single-drug substitution from d4T to ZDV. However, median hemoglobin levels for patients who switched to ZDV remained higher than levels before starting HAART, suggesting that initial treatment with HAART improved hematologic abnormalities associated with advanced HIV disease, thus allowing patients to better tolerate ZDV. Overall, hematologic toxicity was not a major complication after single drug substitution from d4T to ZDV in Uganda [Fatu and David, 2009].

Similar result has been found in United Kingdom meta analysis studies. Data were available for 679 AZT treated patients and 984 d4T treated patients at treatment week 24 and 649 AZT treated patients and 946 d4T treated patients at treatment week 48. Mean (SE) hemoglobin levels decreased with AZT treatment by a mean 0.4 (0.05) g/dL and 0.2 (0.06) g/dL at weeks 24 and 48, respectively, but increased with d4T treatment by 0.45 (0.03) g/dL and 0.58 (0.04) g/dL, respectively. These differences (0.87 and 0.79 g/dL at weeks 24 and 48), respectively, were statistically significant (p-value < 0.05). Anemia was consistently more common in the AZT treated groups relative to the d4T treated groups. Neuropenia events of all grades also were consistently more common with AZT based regimens (26%-43%) relative to d4T based regimens (15%-31%) **[**Graeme and Will, 2004**]**.

On other hand, a longitudinal study carried out in Belgium (2001) showed presence of hematologic reconstitution during PI based HAART from month 6 onward. The study included 116 patients of them 27(23.3%) had thrombocytopenia (<150,000 platelets/μL), 24(20.7%) had anemia (<12 g/dL of hemoglobin), 36(31%) had neutropenia (<2,000 neutrophils/μL) at the time of PI initiation. Compared with baseline values, the progression of hematologic parameters was statistically significant from month 6 on ward for all patient groups**[**Servias and Nkoghe, 2001**]**.

Platelet counts increased from 110 to 180 × 10^{3}/μL at month 24, resulting in 53% of patients with thrombocytes within normal ranges at that time point. Hemoglobin levels increased from 10.7 to 12.3 g/dL at month 9, but slightly declined to 11.4 g/dL at month 21, in parallel with the CD4 cell counts. Even so, 35% of patients remained non-anemic at that time. There appears to be no influence of ZDV containing treatment on hemoglobin levels at any time point. Neutrophil counts rose from 1260 to 2,000 cells/μL by month 6, finally reaching a level of 2240 cells/μL at

month 24 and 53% prevalence rate of neutropenia after 2 years of receiving HAART **[**Servias and Nkoghe, 2001**]**.

Another study was conducted in Nigeria (2008-2009) to evaluate hematologic changes in HIV patients placed on ART. The research showed that PCV in percentage, which is 36.67±0.45 for HIV negative control subjects significantly reduced to 31.56±0.65 in HIV positive subjects without ART treatments. But, upon administration of ART there was no significant increase over HIV positive subjects compared to those without the treatment. The increase observed did not returned to the obtained value in control subjects **[**Amegor and Oyesola, 2009**]**.

In the same study, the hemoglobin concentration showed a significant reduction from 12.24±0.15 g/dl to 10.30±0.22 g/dL for HIV positive subjects without ART treatments while to 10.44±0.17 g/dL when the subjects received the treatment. This observed increase did not return the value back to the recorded value for HIV negative subjects. The pattern observed in the WBC count (measured in cells/mm^3) showed that there was a significant reduction from 6.33±0.33 in HIV negative subjects to 4.07±0.25 in HIV positive subjects without ART treatments. However, the observed increase when subjects receive ART was significant (value was 4.7 ± 0.15) when compared to subjects without the treatment. It was also noted that although the increase was significant, the observed value of 4.7±0.15 was not up to the recorded value for the HIV negative control subjects (6.22 ± 0.33) **[**Amegor and Oyesola, 2009**]**.

A study was carried out in the Netherlands (2003-2007) to evaluate changes in hematological parameters after switching treatment of HIV infected patients from ZDV to ABC or TDF. Mean hemoglobin and leukocyte count showed significant increases from 8.2 g/dL to 8.8 g/dL and from 5.8 × 109 cells/L to 6.5 × 10^9 cells/L, respectively. Mean MCV and platelet count showed significant decreases from 106 fL to 93 fL and from 256 × 10^9 cells/L to 241 × 109 cells/L, respectively. Overall anemia and leucopenia prevalence dropped from 40.5% to 15.2% and from 19.5% to 11.7%. This study showed that switching antiretroviral medication from AZT to either ABC or TDF in a multidrug regimen results in a significant increase in hemoglobin and leukocyte count **[**Rodrek and Viergever, 2009**]**.

1.2.Significance of the study

Although treatment of HIV infected patients using ART has dramatically improved the length and quality of life, few studies have assessed hematologic reposes to ART in sub-Saharan Africa. In addition, the impact of ART on the hematological profile of Ethiopian HIV/AIDS patients is not well known.

Therefore, the significance of this study is to quantify changes in hematologic parameters in HIV infected patients before and after treatment of ART. This will serve as supporting information for health workers to emphasize on impacts of ART on hematological parameters, for proper management and close monitoring of hematologic toxicities. The information will also be useful for health professionals to encourage of HIV infected patients to make themselves available to be treated at the early stage with the appropriate drug regimens.

1.3. Research hypothesis

a. There is significant association between AZT-based ART treatment and anemia.

b. ART treatment improves neutropenia in HIV patients.

c. ART treatment improves HIV related thrombocytopenia.

2. OBJECTIVES

2.1. General objectives

- To determine changes in hematologic parameters in HIV infected patients before and after ART treatment.

2.2. Specific objectives

- To determine the prevalence of AZT induced anemia in HIV infected Ethiopian patients taking HAART.
- To determine the prevalence of Neutropenia in Ethiopian patients taking HAART.
- To evaluate prevalence of Thrombocytopenia in HIV infected Ethiopian patients taking HAART.
- To evaluate the frequency of common hematological disorders at the various CD4+ T cell categories of HIV infected patients.
- To forward specific recommendations based on the research findings.

3. MATERIALS AND METHODS

3.1. Study design

A cross sectional study was conducted to determine changes in hematologic parameters in HIV infected patients before and after ART.

3.2. Study area and period

This study was carried out at TASTH ART clinic starting from July to August 2011. TASTH is the largest of all the hospitals in Ethiopia and provides a tertiary level referral treatment and is also open 24 hours for emergency services.

The hospital is administered by Addis Ababa University and is the largest and oldest teaching hospital among all in Ethiopia providing teaching for about 300 medical students and 350 residents every year. The hospital offers diagnosis and treatment for approximately 300,000 patients a year and is situated at the heart of the capital city on Churchill avenue. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees around 80,000 patients a year and they just started a new trauma unit in the recent months. TASTH also deals with palliative care, HIV counseling and testing, STI services, and post exposure prophylaxis services. Currently the ART clinic offers treatment for 2445 HIV infected patients.

3.3. Study population

HIV infected patients who have been taking ART for a period of one month or more in TASTH ART clinic were included in the study.

3.4. Sampling technique

All study subjects available during the study period were included for the study.

3.5. Sample size determination

The sample size was determined by the following formula:

$$N = 2 \cdot [z_{crit} \sqrt{2\bar{p}(1-\bar{p})} + z_{pwr} \sqrt{p_1(1-p_1) + p_2(1-p_2)}]^2 / D^2$$

Where p1 and p2 are pre-study estimates of the proportions of anemia before (0.63) and after (0.46) ART in HIV infected patients, respectively.

- *D= /p1-p2/* (*0.17)*
- Z_{crit} and Z_{pwr} are Z-values at specified significance criterion and statistical power. Assumptions are 95% confidence interval and at 80% power.
- Therefore N = 280

* Proportion of anemia was used to determine the appropriate sample size because it is the well characterized hematologic abnormalities in HIV patients.

3.6. Eligibility criteria

3.6.1. Inclusion criteria

After informed consent, all volunteer HIV infected patients who were on ART treatment in the study hospital for a period of one month or more with a complete baseline hematologic test results and CD4 T cell count available were recruited for this study.

3.6.2. Exclusion criteria

HIV patients who are pregnant, children under 18 years of age, and patients who are on medications (antibiotics, vitamin supplements, and tuberculosis treatment) other than ART at the time of sampling were excluded from the study.

3.7. Variables

3.7.1. Dependent variables

- Anemia
- Neutropenia
- Thrombocytopenia

3.7.2. Independent variables

- HIV
- ART regimen
- CD4+ T cell count
- Age
- Gender
- Educational status

3.8. Sample Collection

The principal investigator and one recruited laboratory technologist with prior experience were engaged in the laboratory procedures. Appropriate information about the study was provided for the study participants.

A volume of 5 ml of venous blood was collected from each patient from the antecubital vein using a disposable plastic syringe and dispensed into vacutainer EDTA tubes. Each sample was mixed gently and thoroughly to ensure anticoagulation and prevent cell lyses. The 3 ml sample was used for hematological analysis and the 2 ml sample used for CD4+ T cell estimation using Cell Dyn 1800 and FACS count, respectively. All of the information collected from the study participants was identified by a code number.

3.9. Complete blood count (CBC) determination by Hematology analyzer (Cell Dyn 1800)

3.9.1. Measurement principle of WBCs, RBCs, and Platelets

WBC, RBC, and platelets are measured by the electrical impedance method (resistance to current flow). The blood sample is suspended in a highly conductive diluent solution, and then made to pass through an aperture between two electrodes. As the sample passes between the electrodes, it causes a change in the impedance between the electrodes according to the volume and number of blood cells. The number of blood cells can be determined from the number of pulses as the impedance changes, and the volume (type) of the cell can be determined from the height of the pulse.

The shape of blood cells is not necessarily fixed according to the type of blood cell, and the shape of a cell may even change during the course of measurement. To correct for these effects and obtain an accurate measurement, the obtained pulse heights are divided into multiple channels according to each blood cell and measured in the optimum pulse height range.

A small sample of the blood is aspirated into a chamber (the WBC counting bath) and diluted with a balanced isotonic saline solution that is free of particles. The diluted blood sample is split into two parts, one for counting RBCs and platelets and the other for counting WBCs. The RBC portion is transferred to the RBC/platelet counting bath where it is diluted further. The other portion remains in the WBC bath and lysing agent (Cyanide Free differential lyse reagent 1600/1800) is added to destroy (hemolyze) the red blood cells.

A small portion of the diluted fluid in each bath is allowed to flow past a small aperture. An electrical current is produced in each aperture by two electrodes, one on the inside and the other on the outside of the aperture. The saline solution is responsible for conducting current between the electrodes. The cells move through the aperture one at a time. When a cell enters the aperture, it displaces a volume of electrolyte equal to its size. The cell acts as an electrical resistor, and impedes the flow of current. This produces a voltage pulse, the magnitude of which is proportional to the size of the cell. Instrument electronics are adjusted to discriminate voltage pulses produced by different cells. These adjustments are called thresholds. For example, the threshold for counting a RBC is equivalent to a cell volume of 36 femtoliters or higher.

Voltage pulses that are equivalent to volumes of 2–20 femtoliters are counted as platelets. This process is repeated two more times so that the RBC, WBC, and platelet counts are performed in triplicate. Each time frame for counting is several seconds and many thousands of cells are counted. The computer processes the counting data first by determining the agreement between the three counts. If acceptable criteria are met, the counts are accepted and used to calculate the result.

The voltage pulses produced by the white blood cells depend upon the size of the cell and its nuclear density. Therefore, the pulses may be analyzed to differentiate between the types of WBCs based on forward and angular light scattering. For example, lymphocytes are the smallest WBCs and comprise the lower end of the size scale. Monocytes, prolymphocytes, and immature granulocytes comprise the central area of the WBC histogram, and mature granulocytes comprise the upper end. In addition to cell sizing, automated instruments may use any of three other methods to distinguish between subpopulations. These are radio frequency conductance and fluorescent staining.

3.9.2. Measurement principle of hemoglobin Concentration

The hemoglobin concentration is determined optically by colorimetric (cyanmethemoglobin) method measured optically using the solution in the WBC bath, which has been established as an international standard by the International Council for Standardization in Hematology (ICSH). When the hemolyzing agent is added to the blood sample, the red blood cells are lysed and hemoglobin is released. The cyan in the reagent joins the hemoglobin to form cyanmethemoglobin. A light emitting diode (LED) is mounted on one side of the bath and emits a beam of monochromatic light, whose central wavelength is 540 nm. The light passes though the sample and is then measured by an optical sensor that is mounted on the opposite side. The cyanmethemoglobin absorbs light at 540 nm and thus the hemoglobin concentration can be determined from the degree of light absorption.

3.9.3. Measurement principle of hematocrit and cell indices

The hematocrit is a test that measures the volume of blood in percent that is comprised of the red blood cells. Automated cell counters calculate the hematocrit by multiplying the RBC count by the mean red cell volume. A decrease in the number or size of red cells also decreases the amount of space they occupy, resulting in a lower hematocrit. Conversely, an increase in the number or size of red cells increases the amount of space they occupy, resulting in a higher hematocrit. The main RBC indices, MCV, MCH, MCHC and RDW, are used to determine the average size and hemoglobin content of the RBCs and they help determine the cause and type of anemia.

3.10. Procedure for CBC determination

- Collect 3mL of venous blood from patients via the antecubital vein with minimum stasis using a sterile disposable plastic syringe into EDTA anticoagulation tube.
- Mix the sample gently and thoroughly to ensure anticoagulation and prevent cell lyses.
- Run the sample on hematology analyzer.

3.11. Principle and procedure of CD4+ T lymphocyte determination by FACSCount system

3.11.1. Principle

The FACSCount system is an automated instrument designed specifically for enumerating the absolute and percentage of $CD4^+$ T lymphocyte in unlysed whole blood. When whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigen (CD4 antigen) and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. During sample acquisition, the cell come in contact with the laser light, which causes the fluorochrome labeled cells and fluorescently dyed cells to fluoresce. The light scattered by the individual particle and the fluorescence emitted by the cells is used for analysis and sorting of the cells based on the fluorescent antibody directed against a specific surface. This combination of scattered and fluorescence light is picked up by the detectors in the flow cytometer. These detectors then produce electronic signals that are proportional to the optical signals received.

The visible light undergoes deflection based on the size and internal structures of the cell. FSC (Forward Scatter) correlates with the cell volume. SSC (Side Scatter) depends on the inner complexity of the particle (i.e. shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). The fluorescence emitted by the cell depends upon the florescence tagged specific monoclonal antibodies against the cell surface markers. The data collected on each cell or event are stored in the computer. This data is then processed and analyzed to provide information about cell populations within the sample.

In addition, the reagent tubes also contain a known number of fluorescent reference beads. A precise volume of whole blood is stained directly in the reagent tube. The software automatically identifies lymphocyte populations and calculates CD_4 count (cell/μL) by comparing cellular events to internally calibrated bead events. Result includes CD_4 count and percentage, which are printed immediately after sample is run.

3.11.2. Procedure

- Venous blood is collected into a vacutainer EDTA anticoagulation tube.
- Label the blood sample with the patient serial number.
- Vortex reagent tubes inverted and upright for 5 seconds.
- Open the reagent tubes with coring station.
- Mix and pipette 50 μL blood into the reagent tube.
- Vortex for 5 seconds and incubate the tubes for 30 minutes at room temperature.
- Pipette 50 μL of fixative solution into the reagent tube.
- Vortex upright and run the sample on the FACSCount instrument.

3.12. Data collection

Data from the study participant was collected using structured pretested questionnaire. The questionnaire had three parts, the first part for collecting data about socio-demographic characteristics of the study subjects, the second part for collecting ART related information, and the third part for collecting data concerning hematologic test results (at baseline and after ART). Data related to socio-demography and ART was collected by two nurses who had experience in care and treatment of patients with HIV whereas laboratory test results were collected by the principal investigator.

3.13. Quality control

Data quality was ensured through:

- ✓ Careful selection and training of data collectors
- ✓ Supervision in every step of data collection
- ✓ Collected data was checked for completeness and internal consistency
- ✓ Performing daily instruments check
- ✓ Running periodic controls
- ✓ Data was double entered using SPSS window version 16.0.

3.14. Data entry and analysis

Data from the questionnaire and the laboratory test results was double entered using SPSS version 16 for statistical analysis. Descriptive analysis was done and paired student t-test was used to determine association between mean hematologic values. The strength of association between proportion of hematologic disorders and ART status was measured by Pearson's chi-square (χ^2) test and OR (odds ratio) using 95% CI (confidence interval). All p-values were two-sided and p-value of <0.05 was considered to be statistically significant.

3.15. Definition of terms/ standard or working terms

- Anemia: a reduction below normal levels in the quantity of hemoglobin.
- Highly active antiretroviral therapy (HAART): a combination of protease inhibitors taken with reverse transcriptase inhibitors; used in treating AIDS and HIV.
- Leukopenia: any situation in which the total number of leukocytes in the circulating blood is less than normal, the lower limit of which is generally regarded as 4000–5000/mm^3 (WHO/NIH grading of hematologic toxicity).
- Macrocytosis: elevated in mean cell volume of RBC.
- MCV: the average size of the red blood cells expressed in femtoliters.
- MCH: the average amount of hemoglobin inside an RBC expressed in picograms.
- MCHC: the average concentration of hemoglobin in the RBCs expressed in percent.
- Neutropenia: a hematological disorder characterized by an abnormally low number of neutrophils (WHO/NIH grading of hematologic toxicity).

- RDW: coefficient of variation of the red blood cell volume distribution (size).
- Myelosuppression: a decrease in the ability of the bone marrow to produce blood cells.
- Thrombocytopenia: is any disorder in which there is an abnormally low amount of platelets i.e., when the platelet count is below140 x 10^9/L (WHO/NIH grading of hematologic toxicity).

3.16. Ethical considerations

Ethical approval was obtained from research and ethical committee of the department of biochemistry before the study. Permission to conduct the research was obtained from the management and head of the ART unit of TASTH after explaining the purpose and the procedure of the study.

Informed written consent from study subjects was obtained before sample collection. Inclusion of the study participants was voluntary and confidential and private information was protected. Information about the study was given to the participants, including purposes and procedures, potential risk and benefits. The right of the participant to withdraw from the study or not to participate was respected. Patients were treated according to the Helsinki declaration. To ensure confidentiality of data, study subjects were identified using codes and unauthorized persons had no access to the collected data. Blood from the study participant was drawn by the principal investigator.

4. RESULTS

Three hundred forty HIV infected adults with a mean age of 38±10 years were evaluated during the study period; with female (mean age 36 ± 9) to male (mean age 42 ± 11) ratio of 2:1. Majority of patients, 140(41.2%), were married and concerning their educational status and occupation, patients who had completed elementary school and unemployed accounted the highest percentage, 131(38.5%) and 213(62.6%), respectively (Table-4.1).

According to WHO clinical staging 24(7.1%) of the study participants were in stage 1, 83(24.4%) in stage 2, 133(39.1%) in stage 3 and 100(29.4%) in stage 4 at the time of ART initiation. With regard to the type of ART regimen, 149(43.5%) and 191(56.5%) were taking AZT-based and non-AZT based ART drug regimens, respectively. Among the non-AZT based regimens, TDF- 3TC- EFV was the most commonly prescribed ART drug regimen, used in 121(63.35%) of patients. The mean duration of patients on ART was 30 ± 26 months.

Table 4.1. Socio-demographic characteristics of HIV infected subjects on ART at TASTH ART clinic, 2011.

Demography	N	%
Sex		
M	115	33.8
F	225	66.2
Age		
18-28	54	15.9
29-39	149	43.8
40-49	84	24.7
50-59	38	11.2
60-69	14	4.1
$\geq$ 70	1	0.3
Marital status		
Single	87	25.6
Married	140	41.2
Divorced	55	16.2
Widowed	58	17
Education status		
Illiterate	6	19.1
Elementary school	131	38.5
Secondary school	89	26.2
High school	25	7.4
College	22	6.5
Graduate	8	2.4
Occupation		
Government employed	26	7.6
Private employed	92	27.1
Unemployed	213	62.6
Others	9	2.6

Table 4.2 shows the mean results of hematological values of the study participant before and after initiation of ART. On comparison of the results between baseline and after ART initiation, all parameters except RBC were increased. Statistically significant differences were observed in mean WBC (p-value= 0.014), lymphocyte (p-value= 0.000), hemoglobin (p-value= 0.000) and platelet count (p-value= 0.003). However, hemoglobin concentration was lowerd in AZT-based treatment by a mean 0.11 g/dl (p-value= 0.070) but significantly increased in non-AZT based treatment by 1.83 g/dl (p-value= 0.010). No significant differences were observed in the mean hematocrit (p-value= 0.488) and RDW (p-value= 0.894) whereas RBC count was significant lower (p-value = 0.000) after ART treatment.

Table 4.2. Mean hematological values of HIV infected subjects before and after initiation of ART at TASTH ART clinic, 2011.

Variables	**Hematological Values**			
	Before ART Mean(n=340)	After ART Mean(n=340)	Mean change (95% CI)	P-value
WBC(cellx10^3/ μL)	5.56	5.93	0.36(0.66, 0.74)	0.014
Neutrophil(cellx10^3/ μL)	3.22	3.34	0.12(0.33, -0.09)	0.266
Lymphocyte(cellx10^3/ μL)	1.64	1.89	0.25(0.37, 0.12)	< 0.001
RBC (cellx10^6/ μL)	4.14	3.76	-0.38(-0.27,-0.49)	< 0.001
Hemoglobin (g/dL)	12.48	13.46	0.98(1.29, 0.66)	< 0.001
AZT based	12.93	12.82	-0.11(-0.08,-0.21)	0.070
non-AZT based	12.14	13.97	1.83(1.98, 1.2)	0.001
Hematocrit (%)	37.01	37.35	0.34(1.30, -0.62)	0.488
MCV(fL)	89.82	100.30	1.04(11.8, 9.1)	< 0.001
MCH(pg)	30.15	35.91	7.75(4.86, 12.76)	< 0.001
MCHC(%)	33.04	35.91	2.87(3.57, 2.17)	< 0.001
RDW(%)	14.19	14.22	0.03(0.46, -0.40)	0.894
Platelet (cellx10^3/ μL)	208.33	228.31	20 (7.05, 32.89)	0.003
CD_4 (cell/ μL)	145.29	299.67	1.54(172.54, 136.20)	< 0.001

Hematological abnormalities were present both before and after treatment with ART. However, overall prevalence of the major hematological abnormalities showed significant reductions after ART treatment in the study participants (Table 4.3). Leucopenia was significantly dropped from 25.9% before to 11.5% after ART treatment (x^2 test= 23.25, df= 1, p-value= 0.000). The odds of developing leucopenia was two times more before than after ART treatment at 95% CI. Proportions of neutropenia was dropped from 23.2% to 17.4% (x^2 test =1.44, p-value= 0.057), lymphocytopenia from 24% to 9.1% (x^2 test= 2.03, p-value= 0.000), thrombocytopenia from 28.5% to 11.2% (x^2 test= 32.17, p-value= 0.000) before after ART, respectively. There was no significant difference in odds of developing neutropenia whereas odds of developing lymphcytopenia and thrombocytopenia was three times more before than after initiation of the treatment at 95% CI.

Anemia was found in 45% of subjects before and in 21% of the subjects after initiation of ART and the difference was statistically significant (x^2 test=28.6, p-value= 0.000). The odd of developing anemia was three times more before than after initiation of the treatment at 95% CI.

Using the MCV range of 80 –100 fL and MCH range of 26 – 32 pg as pointers in distinguishing between the different types of anemia where low MCV (<80 fL) is indicative of microcytosis, high MCV (>100 fL) indicates macrocytosis and low MCH (<26 pg) indicates hypochromia, microcytic hypochrommic anemia decreased from 6.3% to 0.8% (p-value= 0.002), but normochromic macrocytic anemia showed increment from 0.8% to 3.96% (p-value= 0.000). The odd of developing microcytic hypochromic and normochromic macrocytic anemia was three times more and three times less before than after ART treatment at 95% CI, respectively. High prevalence of macrocytosis was found after, 48%, than before, 10%, initiation of ART (p-value= 0.000).

Among the anemic cases after initiation of ART, 46 (65%) proportion of anemia was found in AZT-based drug regimen. In addition, out of 149 patients who received AZT containing ART, 15(10.06%) of them subsequently changed to other nucleoside analogous because of AZT induced anemia of them 7(5%) patients discontinue the drug because of severe anemia (<6.5 g/dL).

Table 4.3. Prevalence of hematological abnormalities in HIV infected subjects before and after initiation of ART at TASTH ART clinic, 2011.

Variables	Before ART (n=340) N(%)	After ART (n=340) N(%)	OR(95% CI)	P-value
Leucopenia	88(25.9)	39(11.5)	2.70(1.78-4.07)	< 0.001
Neutropenia	79(23.2)	59(17.4)	1.44(0.99-2.10)	0.057
Lymphopenia	82(24.1)	31(9.1)	3.16(2.03-4.94)	< 0.001
Anemia	153(45)	71(20.88)	2.24(1.86-3.94)	< 0.001
Micro. Hypo.	16(6.3)	2(0.8)	3.05(1.45-6.42)	0.002
Normoc. Macro.	2(0.8)	10(3.96)	3.2(1.2-5.9)	< 0.001
Macrocytosis	26(10.3)	122(48)	0.13(0.08-0.21)	< 0.001
Thrombocytopenia	97(28.5)	38(11.2)	3.17(2.10-4.78)	< 0.001

As shown in Figure 4.1, there was high prevalence rate of anemia in AZT containing ART regimens (31%) as compared to non-AZT based treatments(13%) and the difference was statistically significant (p-value < 0.001).

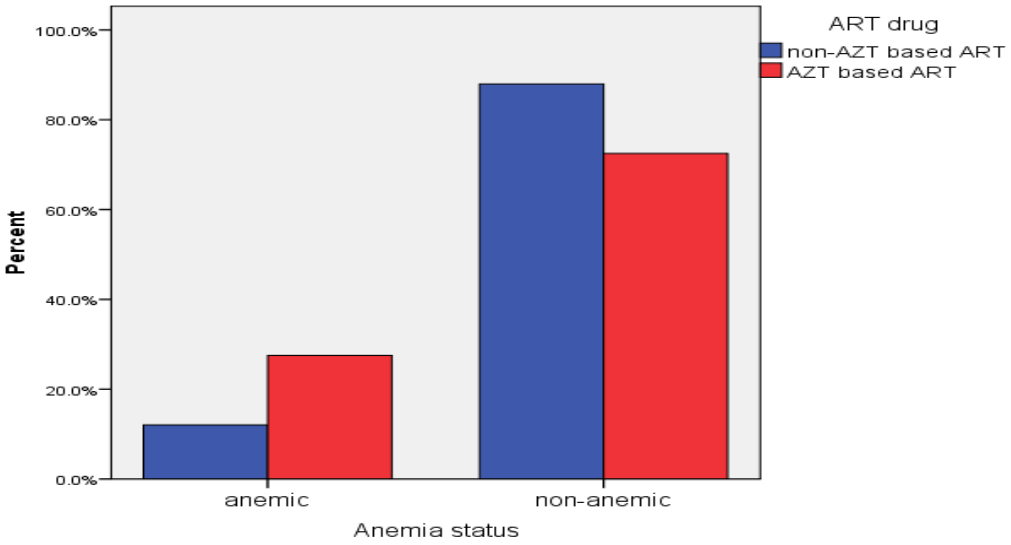

Figure 4.1. Prevalence of anemia in AZT-based and non-AZT based ART treatments in the study patients.

In Table 4.4, the CDC criteria was used to classify the study population into three CD4 count categories to analyze the proportions of hematologic disorders in each category. A gradual increase in proportion of most of hematologic disorders was observed as the CD4 count categories increased and the difference in proportions was significant (p-value <0.05).

Table 4.4. Proportions of hematologic abnormalities of the study population stratified by CD4 T cell counts after initiation of ART at BLH ART clinic, 2011.

Cytopenia	**CD4 counts (cell/µL)**			P-value
	0-199	200-499	≥ 500	
Leucopenia	24(7%)	23(6.8%)	2(0.6%)	0.004
Neutropenia	26(7.6%)	32(9.4%)	1(0.3%)	0.002
Lymphocytopenia	20(5.9%)	10(2.9%)	1(0.3%)	< 0.001
Anemia	30(8.8%)	36(10.6%)	5(1.5%)	0.011
Thrombocytopenia	21(6.2%)	17(5%)	0(0%)	< 0.001

Table 4.5. Correlations among HIV clinical stage, baseline CD4 count and hematological parameters in the study participants.

	Neutrophils	Hemoglobin	Platelets
Stage	0.079	-0.113*	0.007
CD4 count	0.183*	0.236*	0.062

Note: Pearson correlation (r) was used to calculate strength of association between parameters. *Correlation is significant at the 0.05 significance level.

Table 4.5 shows correlations of the selected hematological parameters with HIV clinical stage and baseline CD4 counts. There was no significant correlation between HIV clinical stage with neutrophil count (r= 0-079, p-value= 0.147) and platelet count (r= 0.007, p-value= 0.902). However, hemoglobin concentration showed statistically significant negative correlation (r= -0.113, p-value= 0.037). On the other hand, neutrophil count(r= 0.183, p-value= 0.001) and hemoglobin concentration(r= 0.236, p-value= < 0.001) showed statistically significant positive correlation with CD4 count whereas platelet count showed no significant association (r= 0.062, p-value= 0.256).

5. DISCUSSION

Hematological complications have been documented to be the second most common cause of morbidity and mortality in patients with HIV disease. Persons with HIV infection continue to live longer primarily because of advances in antiretroviral therapy. A variety of hematologic abnormalities associated with HIV infection has been described in different studies. However, due to the general acceptability of ART as the gold standard in the treatment of HIV patients, it is essential to evaluate the hematological response to ART. This will give a clear pointer to the overall impact of the treatment regimen in HIV patients.

From this study, mean hemoglobin concentration was increased significantly from the baseline by 0.98 g/dl and proportion of anemia showed significant reduction from a baseline 45% to 21% after initiation of ART. This finding confirms data from study carried out in Ghana by Owiredu et al (2008-2009), which report 0.8 g/dL mean increment in hemoglobin concentration in HIV patients with HAART as compared to their naïve counter parts. A Significant difference in proportion of anemia was also reported with 64% and 46% prevalence rate of anemia in patients before and after the treatment, respectively, even if the proportion is higher in both study groups as compared to the present study.

In another study, Egger et al (2002-2004) reported a 0.2 g/dL increment in mean hemoglobin concentration from the baseline than after initiation of ART. The increment in hemoglobin level observed was not significant and lower than the present study. This may be due difference in study design which was cohort study with repeated hemoglobin measurements were done for each patient where as in the present study cross sectional study was conducted. However, comparable result was obtained in prevalence rate of anemia, which showed a significant reduction from baseline 35% to 26% after ART initiation.

Nevertheless, in the present study, a 0.11g/dL reduction in mean hemoglobin concentration in patients after treatment of AZT containing drug regimens was observed. This finding is consistent with a result obtained by Graeme et al (2004) and Fatu et al (2009) that found statistically significant reduction in hemoglobin level from the baseline by mean 0.4 g/dl and 0.3 g/dl than after the treatment. However, the reduction in mean hemoglobin showed in the present study was lower and was not statistically significant as compared to the above reports.

This may be due to difference in duration of treatment, sex, and baseline hemoglobin level of the study populations. For example the reduction in hgb conc. observed in the above reports was after six months of therapy with AZT based ART whereas in the present study was after a mean of 27 months.

However, Christopher et al (2008) reported contrasting result in South Africa in which mean hemoglobin increased by 0.28 g/dl with AZT containing treatment. This difference could be due to difference in study design and degree of immunodeficiency of the study populations.

This study found statistically significant difference in proportion of anemia in patients taking AZT and non-AZT based ART treatment, which was 31% and 13%, respectively. This suggests the myelosuppressive effects AZT usage. Similar finding was reported by Raoul et al (2004) in Cote d'Ivoire that found 31% and 17% prevalence rate of anemia with AZT and non-AZT based treatments, respectively. In addition, comparable result was obtained in a study conducted by Curkendall et al (2007) in USA. The result showed statistically significant difference in anemia prevalence (24% and 8.4%) in patients under AZT and non-AZT containing HAART treatments after six months of treatment.

Regarding to the type of anemia, significantly high proportion of microcytic hypochromic anemia was found at the baseline (6.3%) as compared to after ART (0.8%). This may reflect the overall nutritional deficiencies (malnutrition and malabsorption) associated with HIV patients. Similar findings have been reported by Owiredu et al in which microcytic hypochromic anemia was 14.5% in HAART naive and 3.3% in patients on HAART.

However, significantly high prevalence of macrocytosis observed after the treatment (48%) as compared to the baseline (10%) and it can be used as a marker of adherence to ZDV therapy. Moyle (2004) reported that macrocytosis (elevated MCV) is typically associated with vitamin B12 or folate deficiency and in the setting of HIV treatment reflects the use of zidovudine (AZT). Conversely, Hepburn et al (2004) reported that HAART may increase serum vitamin B12 levels and patients did not display characteristic findings of vitamin B12 deficiency, namely macrocytic anemia.

In this study, reduction in proportion of neutropenia after ART treatment was observed as compared to the baseline value, which was 23% and 17%, respectively. The reduction in proportion of neutopenia was not statistically significant.

However, contrasting results were obtained by a research carried out by Raoul et al (2004) in Cote d'Ivoire and Christopher et al (2008) in South Africa. Raoul et al (2004) found statistically significant increment in prevalence of neutropenia from a baseline 40% to 49% after ART initiation. Similarly, Christopher et al (2008) reported 24% and 51% proportion of neutropenia before and after the treatment, respectively.

The difference between the observation in the present study and reports from Cote d'Ivoire and South Africa may be attributed due to difference in study settings like study design, dose and duration ART treatment in the study populations. Furthermore, AZT inhibits the proliferation of blood cell progenitor cells in a dose-dependent manner and can cause neutropenia. This may contribute for the significantly higher prevalence rates of neutropenia in patients on ART in the above studies even if lower prevalence rate of neutropenia was observed after treatment initiation in the present study.

In addition, data by Owiredu et al (2008-2009) from Ghana found a very high proportion of neutropenia in patients with and without HAART (96.2% and 99.3%) even if the difference was not significant among the study subjects. The result was in contrast with this finding and it may be due to difference in the study design and grading system of neutopenia of the study patients. The study defines neutropenia by percent of neutrophil count (< 60%) whereas this study used absolute neutrophil count (< 2 x 10^3/ μL).

Platelet count of the study patients, in this study, was significantly increased from the baseline by mean 20 x 10^3/μL after ART treatment. Huang et al (2000) in California obtained similar result, but the increment was higher as compared to this study, which was 38 x 10^3/μL after the treatment. This may be due difference in platelet count before starting ART in the study patients.

On the other hand, this result was in sharp contrast to that of Owiredu et al (2008-2009) where significant reduction in platelet count was observed by mean 26 x 10^3/μL in HIV patients on HAART as compared to their naive counter parts. This may be due to difference in the study design.

Regarding to proportion of thrombocytopenia, this study found a significant reduction from baseline 28.5% to 11% after ART treatment. This is contrast with a study by Fatu et al (2009) in Uganda that reported similar proportions of thrombocytopenia, 13.5% and 13.4%, in patients with and without ART treatment, respectively. This could be due to difference in dose, type and duration treatments with ARV drugs.

In a study carried out by Servias (2001) in Belgium found high proportion of thrombocytopenia in patient after initiation of ART, 47%, as compared to the baseline value, 23% and the difference was statistically significant. The difference in the result obtained between the above reports and in this study could be due to difference in the study design.

In this study, most of the hematologic abnormalities showed decrement as the CD_4 count increased. Depletion of lymphocytes, primarily of the CD4 T-cell subset subsequent to cellular immunodeficiency has been noted as the hallmark of HIV infection with cytopenia being documented in different proportions in HIV patients. Highest proportion of hematologic abnormalities was observed with CD_4 count between 0-199 cell/ μL as compared to other categories. This suggests that ART treatment can reduce hematologic disorders by improving CD4 T cell numbers and restoring immunological functions.

Similar finding was reported by Odunukwe et al (2005). As related in this study, the ability of HAART to improve upon CD4 counts and hematologic abnormalities can be attributed to the effectiveness of HAART in reducing viral replication and viral load and this proves the ability of HAART to reduce morbidity and mortality in HIV infected patients.

In general, in this study, the overall improvement in blood cell count and proportion of hematologic abnormalities after ART initiation indicates that HIV associated cytopenia can be reversed by the treatment. This may be due inhibition of viral replication and prevention of HIV related complications in the bone marrow by the treatment which accelerate recovery of the hematopoietic system resulting in improvement in blood cell counts.

However, in this study, anemia remained the commonest cause of AZT discontinuation with high proportion of macrocytosis. The mechanism of induction of anemia by AZT is possibly related to the reduction of globin mRNA synthesis due to inhibition of β-globin gene expression by the drug. It is claimed also AZT induce a metabolic defect (impairs DNA synthesis) in developing RBC precursors. In addition, the drug has bone marrow cytotoxicity particularly the erythroid progenitor cells, leading to the generation of fewer but larger RBCs.

This study has limitations. Firstly, blood cell count measurements were done for each study participants at one time in their attendance at the ART clinic and patients did not had tests at study determined time intervals. Secondly, blood test was done for patients with a wide range of duration of ART treatment and the data obtained was analyzed together. These limits to conclude about the relationship between duration of ART therapy and hematologic abnormalities.

6. CONCLUSION

The study has showed that ART treatment is effective in improving hemoglobin concentration, neutrophil and platelet counts. Furthermore, overall prevalence rate of HIV associated anemia, neutropenia and thrombocytopenia also showed reduction after initiation of the treatment. This positive impact can leads to increased survival and improvement in quality of life of people living with HIV/AIDS.

However, a reduction in hemoglobin concentration and significantly higher proportion of anemia was observed in patients with ZDV containing HAART as compared to non-ZDV based treatments. In addition, anemia was the first cause of AZT discontinuation. The study further revealed that ART usage and higher CD4 cell counts are associated with reduction in prevalence rates of hematologic abnormalities.

7. RECOMMENDATIONS

Based on these findings, it is recommended detailed hematologic work up to be done to investigate and treat any hematologic abnormalities in HIV infected patients before and after ART treatment. Additionally, the study encourages health professionals to carry out close clinical monitoring of blood cell count profiles and acute interventions to prevent hematologic complications.

Furthermore, administration of ZDV should be done with caution, as the hemoglobin level has declined in infected patients after the treatment. Hence, it is suggested that documentation of baseline hemoglobin level, repeated testing and serial monitoring to be strengthened after the treatment. The studies also recommend physicians to avoid ZDV prescription in patients with high background levels of anemia.

Finally, large scale and longitudinal studies are recommended to estimate the magnitude of hematological abnormalities in depth in HIV patients before and after ART treatment.

8. ANNEXES

8.1. Questionnaire

Data collection questionnaire to determine changes in hematologic parameters in HIV infected patients before and after ART treatment in TASTH ART clinic, Addis Ababa, Ethiopia, 2011.

Date of patient Visit_____ Patient code number_____

Part-I. Socio-demographic characteristics

1. Age _____
2. Sex : Male____ Female____
3. Marital status
   - Single
   - Divorced
   - Married
   - Widowed
4. Occupation
   - Government employed
   - Private employed
   - Unemployed
   - Others
5. Level of education
   - Illiterate
   - Elementary school
   - High school
   - Secondary school
   - College
   - Graduate

Part-II. ART related information

WHO HIV clinical stage	ARV drugs dispensed	Months on ART	Drugs changed to:	Reason for change

Part-III. Hematologic test results

Variables	Baseline	After ART
WBC (cells x 10^3/ μL)		
GRAN(cells x 10^3/ μL)		
LYMPH(cells x 10^3/ μL)		
MID(cells x 10^3/ μL)		
RBC(cells x 10^6/ μL)		
HGB(g/dl)		
HCT(%)		
MCV(fL)		
MCH(pg)		
MCHC(g/dL)		
RDW (fL)		
PLT(cell x10^3/ μL)		
CD4 (cell/ μL)		

8.2. WHO/ NIH grading system hematologic toxicity

Hematologic Toxicity	Cell count
Leucopenia	WBC count < 3.9 x 10^3/ μL
Neutropenia	ANC < 2.0 x 10^3/ μL
Lymphocytopenia	Lymphocyte count < 1.0 x 10^3/ μL
Anemia Male Female	 Hemoglobin conc. < 13.0 g/dL Hemoglobin conc. < 12.0 g/dL
Thrombocytopenia	Platelet count < 140 x 10^3/ μL

8.3. WHO clinical classification system of HIV disease

- Stage I: Asymptomatic, persistent generalized lymphadenopathy
- Stage II: Weight loss <10 percent, prurigo, fungal nail infection, herpes zoster, recurrent URTIs
- Stage III: Weight loss > 10 percent, chronic diarrhea or fever, oral candidiasis, pulmonary TB, severe bacterial infections
- Stage IV: AIDS defining illnesses: for example, HIV wasting syndrome, PCP, brain toxoplasmosis, candida esophagitis, extrapulmonary TB, CMV retinitis, Kaposi's sarcoma, non-Hodgkins lymphoma, and/or performance score 4: bedridden >50 percent of the day during the last month

8.4. Patient information and consent form

Research title: Changes in *hematologic parameters in HIV infected patients before and after antiretroviral therapy in TASTH, Addis Ababa, Ethiopia 2011.*

Principal investigator: Chalachew Abiyu

- Address: mobile- 0913526578

E-mail- *chalachewabiyu@yahoo.com*

Addis Ababa University Medical Faculty Institutional Review Board (IRB)

- Address : Tel- 0115 538734

E-mail- ***aaumfirb@yahoo.com***

PART I: patient information

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

Ask the principal investigator (Mr. Chalachew Abiyu) if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Purpose of the study

The aim of this study is to determine effects of ART on anemia, neutropenia and thrombocytopenia in HIV infected patients. This will serve as supporting information for health workers to emphasize on adverse effects of ART on hematological parameters, for proper management and understand better your problem as you are living with HIV. It is also helpful to treat you with appropriate ART drug regimens with lowest hematological toxicity.

Procedure

If you are volunteer to participate in this study, we will ask you the following

1. We will use the blood you gave to determine hematological parameters.
2. The researcher may ask you different questions related to this study.

Potential risks

You will not be required to take anything or undergo any procedure except that your blood will be used for other purpose. There will be no risk to your health or ability to receive appropriate therapy.

Benefits from the study

This study will allow your physician to better manage your medical condition. Medical treatment would be provided depending on your test result from the study. We will arrange for medical treatment, if not you will cover your treatment by yourself, knowing your medical condition or test result. However, it is possible you may not directly benefit from your participation in this study, provided that your test result of the study will not indicate any abnormality.

Confidentiality

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. We will not use your name in any of the information we get from this study or in any of the research reports.

Sharing the result

At the end of this research, the study findings will be published and it will be disseminated to all responsible bodies for taking actions and other purposes.

Right to refuse

Please know that your participation in this study is entirely voluntary and you are free to withdraw at any point. Allowing your blood sample to be used for this research is completely voluntary. Your decision will not affect your right to take medication or any other health service facility now or in the future.You may choose not to have your sample tested for the purpose of the study or may withdraw at any time before your sample is being tested without providing any reason and without affecting your service in the ART clinic or in the Hospital.

PART-II: Consent form

By my signature below, I confirm that I have read and understood this informed consent. I understood this is a research study and my participation is voluntary. I understood that I may change my mind about participating at any time, without my medical care or legal rights being affected. I have had the opportunity to ask questions and my questions have been answered. I have been given adequate explanation and understood the purpose, procedures, risks and benefits of this research study. By signing this form, I gave permission for these individuals to have access to my blood sample for the purpose of the study.

Patient code number	**date**	**signature**
Name of principal investigator	**date**	**signature**

Patient information and consent form (Amharic version)

አዲስ አበባ ዩኒቨርሲቲ

የሕክምና ፋኩልቲ

ባ□ኬሚስትሪ ት/ክፍል

<u>የጥናት ተሳታ□ች የመረጃና የስምምነት ቅፅ</u>

<u>የጥናቱ ርዕስ</u>፡ በጥቁር አንበሳ ስፔሻላ□□□ ሆስ□ታል □□ረ-ኤች አይ ቪ መድሃኒት እየወሰዱ ባሉ የኤች አይ ቪ ህመምን ያለውን የደም ማነስ እና የደም ሴሎች ብዛት (የነጭ የደም ሴልና ፕላትሌት) ለውጥ ለማነፃፀር ፣አዲስ አበባ፣ኢትዮጵያ::

<u>የጥናቱ ባለቤት</u> :- ቻላቸው አብዩ□

- **አድራሻ :-** ሞባ□ል- *0913526578*

  ኢ-ሜ□ል- chalachewabiyu@yahoo.com

አዲስ አበባ ዩኒቨርሲቲ የሕክምና ፋኩልቲ ኢንስቲቱትሽናል ሪቪው ቦርድ(IRB) ቢሮ

- **አድራሻ :-** ስ.ቁ - *0115 538734*

  ኢ-ሜ□ል- <u>aaumfirb@yahoo.com</u>

□□ል አንድ:- <u>የጥናቱ ተሳታ□ች የመረጃ ቅፅ</u>

<u>መ□ቢ□</u>፡- በዚህ ጥናተዊ ፅሁፍ ላይ □እንዲሳተፉ □እየተጋበዙ ነው:: በጥናቱ ላይ ለመሳተፍ ከመወሰን በፊት ግን ጥናቱ ለምን እንደሚካሄድና ምን ምን ዓይነት ነገሮች እንደሚያስፈልጉት ማወቅ በጣም ጠቃሚ ነው:: ስለዚህ እባ□□ ጥቂት ግዜ ይውሰዱና የሚከተለውን ስለ ጥናቱ በተመለከተ መረጃ ይመልከቱ:: አስፈላጊ ከሆነም ከሌሎች ሰ□ች ጋር ይወያዩበት:: ማንኛ□ም ግልፅ ያልሆነ ነገር ካለ ወይም ተጨማሪ መረጃ ከፈለጉ የጥናቱ ባለቤት (ቻላቸው አብዩ) መ□□ቅ ይችላሉ::

<u>የጥናቱ ዓላማ</u>:- የዚህ ጥናት ዋና ዓላማ □ረ-ኤች አይ ቪ መድሃኒት በኤች አይ ቪ ህመማን የሚያመጣውን የደም ማነስና የደም ሴሎች ብዛት ለውጥ ለማወቅ ነው።

ይህ መድሃኒት ለኤች አይ ቪ ህሙማን በጣም አስፈላጊ እና ቫ□ረሰ□□ማ□መ□□□ □□ት ለማስታ□ስ የሚሰጥ ነው። ስለዚህ መድሃኒቱ የሚያመጣውን የደም ማነስና የደም ሴሎች ብዛት ለውጥ ማወቅ ለጤና ባለመ□□□ች መድሃኒቱ በሴሎች የሚያመጣውን ጉዳት በጥልቅ ለመገንዘብ፤ በኤች አይ ቪ ህሙማን ቀላል ጉዳት የሚያመጡ የመድሃኒቱን አይነቶች ተመርጠው እንዲሰጣቸው እና በአግባቡ እንዲታከሙ፤የተለመዱ ጉዳቶችን አውቀው መድሃኒቱን እንዲወስዱ ለማድረግ የሚረዳ ነው።

<u>የጥናቱ ሂደት</u>፡- በዚህ ጥናት ለመሳተ□ □ቃ□ኛ ከሆኑ ከርስ□ የሚጠበቁ የሚከተሉት ናቸው፡፡

1. ለሃ□ሎበ□ና ደም ሴሌች ብዛት ምርመራ ብለው የሰጡት ደም ለጥናቱ ይውላል፡፡ ለዚህ ጥናት ሲባል ግን ተጨማሪ ደም እንዲሰጡ አይጠየቁም፡፡
2. የጥናቱ ባለቤት ጥናቱን በተመለከተ አንዳንድ ጥያቄች ሊጠይቅት ይችላል፡፡

<u>ጉዳት</u>፡- ከዚህ ጥናት ጋር በተያያዘ በጤናም ሆነ በሚያገኙት ተገቢ ህክምና ምንም አይነት ጉዳት ስለማያስከትል አይስጉ፡፡

<u>ጥቅም</u>፡- በጥናቱ ላይ በመካፈል የሚከተሉትን ጥቅሞች ያገኛሉ፡-

ሀ. ስለ መድሃኒት በቂ መረ□ □ሰ□□ታል።

ለ. □ሃ□ሎበ□ና የደም ሴሌች ምርመራ ይደረግልዎታል። ውጤቱንም ወዲያውኑ □□ቃለ።

ሐ. □ምርመራ ውጤት መሰረት በማድረግ ተገቢዉን ሕክምና □እንዲያገኙ ይመቻችል□ታል፡፡ የመድሃኒት □□ ራስ ይችላሉ፣ የላቦራቶሪ ምርመራው ግን በጥናቱ የሚሸፈን ይሆናል፡፡

መ. በተ□ ማሪም □ህ □ናት ለህኪሞች ስለ ፀረ-ኤች አይ ቪ የበለጠ መረጃን ይሰጣል።

<u>ሚስጥራዊነት</u> ፡- ማንኛውም ከዚህ ጥናት ጋር የተያያዘ የግል መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ ስለዚህ የጥናቱ መረጃ ይፋ የሚሆነው ለርስ ብቻ ነው፡፡ ለሚወሰደው ማንኛ□ም መረጃም ሆነ የጥናቱ ውጤት ለማሰራጨት በስም ሳይሆን በሚስጥር ቁጥር(ኮድ) የሚመዘገብ ይሆናል፡፡

<u>የተሳትፎ መብት</u> ፡- በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በዕርስ□ ፍቃድ የተመሰረተ መሆኑን ልናሳስብ እንወዳለን፡፡ በመሆኑም በማንኛውም ሰዓት ምንም ዓይነት ምክንያት ሳይሰጡ ከጥናቱ ራስን የማግለል መብት የተጠበቀ ነው፡፡ የሰጡት ደም ለዚህ ጥናት እንዲውል ማድረግ በእርስ□ ሙሉ ፈቃድ ብቻ ሲሆን በጥናቱ ላይ ለመሳተፍ በመ□ሰን ወይም ባለመወሰን ግን መድሃኒት ወይም ሌላ የጤና አገልግሎተ የማግኘት መብት□ አሁንም ሆነ ለወደፊቱ ምንም አይነት ተፅዕኖ አያሳድርብ□ትም፡፡

ክፍል ሁለት፡- <u>የስምምነት ቅፅ</u>

የዚህን ጥናት መሰረታዊ □ላማ እና ሌሎችመረጃ□ችን በሚገባ ተገንዝቤያለሁ፡፡ ተሳትፎየ በፍቃደኝነት ላይ ብቻ የተመረኮዘ እንደሆነም ተረድቻለሁ፡፡ ማንኛውም ሰብዓዊም ሆነ ህጋዊ መብቴ ሳይነካ ከጥናቱ ራሴን ማግለል እንደምችልም እንድሁ፡፡ ስለ ጥናቱ ዝርዝር ጉዳይ በግልፅ ከተረዳሁት ባሻገር ተጨማሪ ማብራሪያ ብፈልግ መጠየቅ እንደምችልም አውቂያለሁ፡፡ በመሆኑም በፈቃዴ የዚህ ጥናት አካል እንድሆን ስፈልግ የሚጠበቅብኝን ሁሉ ለማድረግ በመወሰን መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

□ሚስጓ ር ቁጓ ር	**ቀን**	**□ርማ**
የጥናቱ ባለቤት ስም	**ቀን**	**□ርማ**

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