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Hematological profile of HIV Infected Adult Individuals After Receiving Highly Active Antiretroviral Therapy (HAART) at Black Lion Specialized Hospital, Addis Ababa Ethiopia

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ABBREVIATIONS

AIDS--- Acquired Immune Deficiency Syndrome
ART--- Anti Retroviral Therapy
AZT/ZDV --- Zidovudine
BLH --- Black Lion Hospital
CBV ---Combivir
CDC---Centre for Disease Control and Prevention
CD--- Cluster of Differentiation
CI--- Confidence interval
d4T --- Stavudine
DTH--- Delayed Type Hypersensitivity
EFV ---Efavirenz
HAART--- Highly Active Antiretroviral Therapy
Hgb--- Hemoglobin
HIV--- Human Immunodeficiency Virus
IQR ---Inter Quartile Range
LPV ---Lopinavir
MOH--- Ministry of Health
NVP ---Nevirapine
PI--- Protease Inhibitors
RTV--- Rotanavir
SPSS--- Statistical Package for Social Science
SQV ---Saquinavir
STP--- Severe Thrombocytopenia
TDF- Tenofovir
3TC --- Lamivudin
UNAIDS--- United Nations joint programme on HIV/AIDS
WBC---White blood cells
WHO--- World Health Organization

OPERATIONAL DEFINITIONS

Adult people--Individuals greater than 18 years of old.

Anemia--Hemoglobin values less than 13gm/dl for males, less than 12gm/dl for non pregnant women and less than 11 gm/dl for pregnant women.

ART Unit--A unit in hospitals responsible for counseling, investigating, and treating HIV infected persons with antiretroviral drugs.

Hematological Improvement—statistically significant improvement of hematological parameters of an individual after starting the treatment.

Hematological profile-- Hemoglobin, White blood cells, CD4 + T cells and platelets value of individual.

Highly Active Antiretroviral Therapy (HAART)--Two or more antiretroviral drugs for at least 6 months.

Immunodeficiency(Immunosuppression)--CD4+T lymphocyte counts < 200cell/ μ l

Leucopenia--White blood cell count < 3000/ul.

Mild anemia--Hemoglobin values of 10-12gm/dl for women and 10-13 gm/dl for men

Moderate anemia—Hemoglobin values of 8-9.9 gm/dl.

Severe anemia—Hemoglobin values less than 8 gm/dl.

Severe immunodeficiency--CD4+ T cell count < 50 cells/ul.

Severe thrombocytopenia--Individuals who have platelet count < $50 \times 10^9/l$.

Thrombocytopenia--platelet count less than $100 \times 10^9/l$.

ABSTRACT

Background: -Treatment of HIV infected patients with Highly Active Antiretroviral Therapy (HAART) leads to immune reconstitution as shown by increase in CD4+ lymphocyte counts, decreased risk of opportunistic infection and improve survival, however, little is known about its impact on hematological parameters in resource limited settings like Ethiopia.

Objective: -To assess hematological profile among adult HIV infected individuals at the baseline and six month after initiation of Highly Active Antiretroviral Therapy.

Methods: -Retrospective cohort study was conducted among HIV infected adult patients who attended ART clinic and received HAART between September 2005 and August 2010 at ART clinic of Black Lion Specialized Hospital Addis Ababa, Ethiopia. A total of 379 subjects were selected based on convenience sampling. Demographic, clinical and hematological (WBC, CD4+ T cells, Hgb and Platelets) data were collected between July and August 2010 carefully from the existing ART logbook and patient follow up cards accordingly. Selected hematological abnormalities were defined as leucopenia, thrombocytopenia, severe thrombocytopenia, anemia and immunodeficiency. Data collected through a standardized questionnaire were entered into Excel spread sheet and transported into and analyzed by SPSS Version 15.0 soft ware (SPSS INC, Chicago, IL, USA). Median change of different hematological values during follow up time between consecutive visits and each follow up from the base line were compared using Wilcoxon signed rank test.

Results: -At the baseline 379 subjects who started ART from September 2005 to August 2010 were included in the study; 240 of the patients were females. The subjects studied had a baseline CD4+ T cells count ranging from 4-573 cells/ul, median 111 cells/uL IQR (72-176). 329 (86.8%) of the study subjects were having CD4 + T cell count below 200 cells/ul and CD4+ T cell count of <50 cells/ul was seen in 69(18.2%) patients. Moreover, 25 (9.5 %) of the patients were leucopenic, 104 (43.3 %) of females and 60(43.2%) of males were anemic while 60(15.8 %) of the total 379 patients were thrombocytopenic. After six month initiation of HAART, the median CD4+ T cell count was 190 cells/uL IQR (128-278), thrombocytopenia was seen only in 25(6.6%) of the patients and 25 (6.6 %) were leucopenic. The prevalence of anemia was only 22.7 % and 18.8 % in females and males respectively.

Conclusion: -HAART improved CD4+T cell count, Hgb, white blood cells and platelet count of HIV infected individuals. The difference in hematological parameters between baseline and after 6 month initiation of HAART was statistically significant ($P < 0.05$).

1. Introduction

1.1 Background information

Human immunodeficiency virus (HIV) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). The first AIDS cases were reported in 1981. A large part of the infected individuals live in relatively poor and developing countries. Due to the large scale of morbidity & mortality it causes, HIV is fast becoming a major threat in developing/third world countries (1).

At the end of 2009 UNAIDS estimated that there were on average 33.3 million people living with HIV. Annually there were on average an estimated 2.6 million people who became newly infected with HIV. In 33 countries, the HIV incidence has fallen by more than 25% between 2001 and 2009; 22 of these countries are in sub-Saharan Africa. This trend reflects a combination of factors, including the impact of HIV prevention (2) Every day, more than 6800 people become infected with HIV and more than 5700 die per day, mostly because they have no access to HIV prevention, treatment and care services. Despite progress made in scaling up the response over the last decade, the HIV pandemic remains the most serious infectious disease challenge to global public health (3).

HIV is characterized by progressive damage to the body's immune system which results in the development of a number of opportunistic infections and other complications. The haematologic complications of HIV-infected patients include anaemia, neutropenia, lymphopenia and thrombocytopenia. Anemia is the most commonly encountered haematologic abnormalities in HIV patients, occurring with increasing frequency and is a significant predictor of progression to AIDS or death, with more than 70% of patients developing anaemia and requiring transfusion (4).

Multiple hematological abnormalities, including both peripheral blood cytopenias and bone marrow myelodysplastic changes with signs of ineffective hematopoiesis, are commonly observed in patients with AIDS and generally in patients infected with HIV. These abnormalities are frequently multifactorial; however, an important role in their pathogenesis has been ascribed to a defective hematopoiesis. In fact, a reduced in vitro growth of the bone marrow-derived granulocyte-macrophage progenitor cells (colony-forming unit granulocyte-macrophage [CFUGM]), first observed in patients with AIDS and concurrent neutropenia has been reported (5).

ART is treatment for AIDS that helps the body's immune system recover from the damage caused by infection with HIV. Although ART cannot cure AIDS, persons on ART will begin to feel better, eat more, and put on weight. Their bodies will recover the ability to fight infections. As persons on ART treatment become well, they can care for their children and return to household activities and productive life, which benefits the household and national economies. They recover their sense of hope for the future and can become powerful advocates for prevention and mitigation of HIV in their families and communities. The patients may remain well for many years, but must continue to take Antiretrovirals (ARVs) for the rest of their lives. Thus, ART is an important component of the global response to AIDS (6).

Different classes of Antiretroviral drugs act at different stages of HIV cycle. Combination of several, typically three or four drugs are known as Highly Active Anti Retroviral Therapy (HAART). Combination of drugs create multiple obstacles to HIV replication to keep the number low and reduce the possibility of a superior mutation arising which convey resistance to one of the drug being taken in which case, the other drug continue to suppress the reproduction of that mutation. With rare exception no individual drug has been demonstrated to suppress HIV infection for long. These drugs must be taken in combination in order to have lasting effect (7).

HAART produces profound suppression of HIV replication, substantial increase in CD4+ T cells and partial reconstitution of the immune system. All of these result in significant declines of the morbidity and mortality in HIV/AIDS patients. Immune reconstitution in AIDS patients after HAART has been reported in several countries (8, 9, 10, and 11).

Ethiopia has significantly expanded its response to the epidemic since enactment of the National HIV/AIDS Policy in 1998. In 2001, the National HIV/AIDS Prevention and Control Council declared HIV a national emergency; this was followed by various interventions focusing on prevention, risk reduction, and behavioral change. In 2003, the Government of Ethiopia introduced its ART program with the goal of reducing HIV-related morbidity and mortality; improving the quality of life of people living with HIV; and mitigating some of the impact of the epidemic. Ethiopia launched free ART in 2005 and as of February, 2010, a total of 179,183 clients had been enrolled in ART (12, 13).

Most studies described the hematologic parameters of HIV infected individuals after receiving HAART, however to our best knowledge there is only few published report in Ethiopia that assessed the hematological parameters after receiving HAART. Therefore, the present study is designed to fill the current information gap by assessing the hematological profile of HIV infected individuals after initiation of antiretroviral treatment (ART) at Black Lion Specialized Hospital ART unit in Addis Ababa Ethiopia.

1.2 Statement of the problem

Hematological abnormalities are among the most common complications of HIV. These involve all lineages of blood cells. HIV associated hematological abnormalities seem to be dependent on the level of virus replication, as these abnormalities are severe in late-stage AIDS patients with high viremia. Bone marrow abnormalities are found in all stages of HIV disease, increasing in frequency as the disease progresses. A number of characteristic but nonspecific, morphologic abnormalities of the bone marrow of AIDS patients have been reported (14). Anemia is a frequent complication that occurs in 20-80% of HIV-infected persons and is associated with faster disease progression, shorter survival times and mortality (15). Neutropenia is common in the advanced stages of AIDS and often caused or exacerbated by concomitant myelosuppressive drugs. Adverse drug reactions and their complications can cause neutropenia in patients with HIV/AIDS. Thrombocytopenia is correlated with low CD4+ T cell count (14). Thrombocytopenic purpura is a common hematologic abnormality occurring in individuals infected with HIV-1 (16).

The pathogenic mechanism of HIV related thrombocytopenia is probably multifaceted. Antiplatelet antibodies and circulating immune complexes would cause peripheral destruction in the spleen, liver, and bone marrow, in that order; and, on the other hand, there would be an ineffective immune thrombopoiesis and direct infection of the megakaryocytes which could cause a change in the function (17).

Several uncommon but more serious adverse effects associated with antiretroviral therapy, including AZT-associated anemia sometimes observed. Soon after the introduction of nelfinavir, Protease inhibitors (PI), several case reports suggested an association between these drugs and increased frequency and severity of bleeding in patients with hemophilia. Although most PIs have been implicated, RTV in particular is associated with this adverse effect. The mechanism is unknown. However, the patients' coagulation parameters are typically normal, and factor VIII replacement is not efficacious in resolving the bleeding. Hemophiliac patients taking PIs should be monitored for increased bleeding, and PI therapy should be discontinued if it occurs. If undergoing surgery, these patients may benefit from temporary cessation of PI therapy during the perioperative period. When possible, a non-PI regimen should be considered for these patients (18).

1.3 Significance of the study

Hematological abnormalities are a common complication of HIV infection. These abnormalities increase as the disease advances. Bone marrow abnormalities occur in all stages of HIV infection (14). The early use of HAART improves clinical, hematological and immunological characteristics, delays the progression of diseases and improve survival in HIV infected individuals. Hemoglobin level have been suggested as useful and inexpensive parameters to indicate need for HAART in settings in which CD4+ T cell counts are unavailable (19). There are limited studies conducted on hematological changes after starting HAART in developing countries especially in Ethiopia there are only few published data showing the change in hematological parameters. This study will provide information about the change of hematological profile which may contribute in improving the management of patient after initiation of ART. Moreover this study provides information for the clinicians whether HIV positive patients need frequent hematological tests

2. Literature review

2.1 Hematologic abnormalities in HIV/AIDS.

Infection of the hematopoietic progenitor cells has been proposed as a potential explanation for HIV related hematological abnormalities. HIV infects CD4+ T cells and can be directly cytotoxic (10). Study in India showed that 89.2% cases at the baseline were with CD4 count < 200 cells/ μ l and CD4 cell count of < 50 cells/ μ l was seen in 18.6% patients (20). In another study conducted at Saint Paul hospital, Addis Ababa Ethiopia by Kassa E et al, 1999 showed that among 79 HIV positive hospitalized patients, 83.5% of them had CD4+ T cell count < 200 cells/ μ l and 35% had CD4+ T cell count < 50 cells/ μ l (21).

Leucopenia is common in patients with HIV infection and seems to correlate with the severity of the disease. Study conducted in the university teaching hospital of Nigeria described that 16% of adult people living with HIV/AIDS were leucopenic (22). Anemia and Thrombocytopenia are also frequent complications of infection with HIV. Study in Iran described that the prevalence of anemia at the baseline was 10% and anemia was associated with female sex (23). Similarly in another study conducted in Iran showed that anemia was frequent in HIV positive patients (24). Another study conducted in India showed that anemia was seen in 65.5% of patients and Thrombocytopenia was seen in 7% (14/200) cases (14).

2.2 Response of Hematological parameters during HAART

Study in Nigeria showed that the use of ARVDS therapy increased PCV, Hgb, WBC and CD4+T cell count of the subjects. Subjects on ARVDS therapy showed insignificant increase from 31.56 ± 0.65 % to 32.26 ± 0.49 % for PCV, from 10.30 ± 1.170 to 10.44 ± 0.17 gm/dl for hemoglobin concentration, from 412.53 ± 15.23 cells/ μ l to 422.93 ± 25.73 cells/ μ l for CD4+T cell and a significant increase in WBC (total) from 4070 ± 250 to 4760 ± 170 cells/ μ l. The result showed that ARVDS therapy has the ability to improve hematological parameters (PCV, Hgb, WBC and CD4+ T cell count) and as a result boost the immune system of the body (7).

2.2.1 Platelet response during HAART

A retrospective study in Italy showed that 15 patients with HIV-associated STP (platelet count < 50,000 cells/ μ l) mostly antiretroviral experienced (13/15), underwent HAART for at least 6 months (median 21; range 6-41 months) during which the platelet (PLT) count was monitored. The platelet response was compared to that observed in 19 patients previously treated with zidovudine (AZT) monotherapy. The result indicated, HAART induced a significant increase in the PLT count within the third month which was sustained up to the sixth month of therapy. No (severe thrombocytopenia) relapse was observed among eight PLT responders followed for longer than 6 months (median 27; range 7-41 months) (25).

2.2.2 Response of CD4 + T cells during HAART

A study carried in India involving forty three drug naive AIDS patients. Mean baseline CD4+ T cell count was 112 ± 60 cell/ μ l. During follow up, a CD4 cell count increased by ≥ 50 cells/ μ l in 84.6 % cases. Mean CD4 cell count increased from 126 ± 16.6 cells/ μ l at baseline to 278 ± 196.7 cells/ μ l (20). Another study in the Netherlands showed that in all children and adults with relatively high CD4+ T cell counts at start of therapy (>200 cells/ μ l), total CD4+ T cell numbers normalized within 1 year of therapy. After long-term HAART (4.4–9.6 years), naive CD4+ T cell counts had normalized in both groups (26). Similarly a prospective study conducted at Zewditu hospital Ethiopia described that CD4+ T cells after 6 month of initiation of HARRT were statistically higher compared to baseline readings (11).

2.2.3 Response of WBC during HAART

After starting HAART, there was a significant improvement in chemotaxis and fungicidal activity of phagocytic cells. Values of chemotaxis reached normal ranges in 13 out of 18 patients (72%) for neutrophils and eight out of 18 (44%) for monocytes, whereas phagocyte killing was rarely restored to normal values (3/18 cases for monocytes and 0/18 for neutrophils). HAART was also associated with significantly increased phagocyte chemiluminescence production in response to phorbol-12-myristate-13-acetate or opsonized *C.albicans*. The functional improvement of two critical components of innate antimicrobial immunity, such as neutrophils and monocytes, may contribute to the improved cell-mediated immune responses against opportunistic infections in HAART-treated patients (27). Study conducted in Markurdi, Benue state of Nigeria indicated that ART brings statistically significant increment in WBC from 4070 ± 250 cells/ μ l at baseline to 4760 ± 170 cells/ μ l after antiretroviral drugs treatment (7).

2.2.4 Response of hemoglobin during HAART

In a retrospective evaluation of medical records of 32,867 HIV-infected persons followed in nine cities in the United States showed that the 1-year incidence of anemia, defined as a hemoglobin level <10 g/dl or a physician's diagnosis of anemia, was approximately 37% for patients with a clinical AIDS-defining condition; 12% for those with immunologic AIDS, defined as a CD4 count <200; and 3% for persons without either of these conditions. Use of ZDV either currently or in the past 6 months was associated with anemia. A total of 41.5% of those with a history of ZDV in the past 6 months and 27.7% of those without such history were anemic at baseline. The strong statistical associations between worsening parameters of HIV disease and increased likelihood of anemia suggest that effective antiretroviral therapy may be associated with improvement in Hb levels (29).

In a cohort study of HIV-positive injection drug users (IDUs) in Baltimore Maryland showed that at baseline revealed an overall prevalence of anemia of 40%. During mean follow-up of one year, among 102 subjects who received HAART, there was a mean increase in hemoglobin of 3.6 ± 1.7 g/l. Among 103 control subjects who were not receiving antiretroviral medications; there was a mean decrease in hemoglobin of 4.2 ± 1.1 g/l (29).

Treatment with AZT was the only independent risk factor for developing anemia under HAART. Prospective study was conducted in Germany, on HIV-infected treatment-naïve patients seen between 01/2001 and 08/2002. Identification of two groups of patients (no anemia, pre-existing anemia) and observation of hematological parameters after initiation of HAART was carried out. Anemia was differentiated as mild (Hb-level: women 10-12g/dl; men 10-14g/dl), moderate (8-9.9g/dl) or severe (<8g/dl). The results showed that at baseline 138 (61%) out of 221 patients demonstrated anemia (114 mild (51%), 23 moderate (10%), 1 severe). Within 12 months of HAART the median hemoglobin level increased significantly in pts with mild and moderate anemia (30).

Retrospective study was conducted on 230 HIV infected patients who were taking ART and had follow up at Minilik II hospital ART clinic, Addis Ababa Ethiopia. The result showed that the prevalence of anemia before ART was 52.6% and after the initiation of antiretroviral therapy was 37.3%. Of this prevalence of anemia was higher in female than in males in both cases before (70.25% vs 29.75%) and after treatment (69.23 % vs. 30.77%) (31).

All grades of anemia and neutropenia events were consistently more common with AZT- based regimes relative to d4T- based therapy. Studies in United Kingdom showed that, treatment efficacy as measured by changes in CD4⁺ T cell counts and viral load did not differ significantly between regimes. Hgb levels decreased with AZT treatment by a mean (SE) 0.4(0.05) gm/dl and 0.2(0.06) g/dl at weeks 24 and 48, respectively, but increased with d4T treatment by 0.05 (0.03)g/dl and 0.58(0.04) gm/dl, respectively (32).

Zidovudine-related anemia usually occurs within 3 months after therapy initiation. Risk factors include high zidovudine dosage, increased treatment duration, low CD4+ T cell count, and preexisting anemia. Studies from Nigeria, Cote d'Ivoire, Haiti, and India have found rates of zidovudine-related anemia of 3%–12%. A study from Cote d'Ivoire suggested synergistic toxicity between zidovudine and cotrimoxazole. Among 498 patients already receiving cotrimoxazole prophylaxis, the introduction of zidovudine-containing HAART resulted in one-half of the cohort developing severe neutropenia. Complete recovery occurred in nearly all patients after discontinuation of cotrimoxazole therapy, suggesting that this toxicity was attributable to a drug-drug interaction between these 2 myelosuppressive drugs (33).

3. Study Objectives

3.1 General objective

To assess hematological profile among HIV infected adult individuals after initiation of Highly Active Anti Retroviral Therapy (HAART) at Black Lion Specialized hospital Addis Ababa, Ethiopia.

3.2 Specific objectives

-To describe selected hematological profile(WBC, Hgb, Platelets and CD4+ T cell counts) at the base line and after six month initiation of HAART

-To compare the median differences of selected hematological profile(WBC, Hgb, Platelets and CD4+ T cell counts) between the baseline and after six month initiation of HAART

-To describe median change of Hgb, CD4+ T cells, platelet count and WBC count during follow up

3.3 Hypothesis

HAART improves hematological profile (WBC, Hgb, Platelets and CD4+ T cell counts) of HIV infected adult individuals after six month initiation of the treatment when compared with baseline values.

4. Materials and methods

4.1 Study design- Retrospective study was conducted to assess hematological profile among HIV infected individuals after initiation of Highly Active Anti Retroviral Therapy (HAART) at ART clinic of Black Lion Specialized Hospital Addis Ababa.

4.2 Study site- The study was conducted in Addis Ababa Black Lion Hospital (BLH) ART unit. The hospital is found in the capital city, Addis Ababa in the Lideta sub city. This institution is selected based on the availability of patients from all parts of the country as it is referral and general specialized teaching hospital in Ethiopia as well as the ease of access and sufficient availability of the data in this unit. As of February 2010, 2619 clients were on ART at Black Lion hospital (13).

4.3 Study period- The data of HIV/AIDS patients attended at the ART unit of Black Lion Hospital between September 2005 and August 2010 was collected from July to August 2010.

4.4 Population

4.4.1 Source population - All HIV/AIDS patients attending Black Lion Specialized Hospital ART clinic were the source population.

4.4.2 Study population - The study participants were HIV infected individuals who had started HAART (defined as two or more antiretroviral drugs for at least 6 months), by WHO clinical and immunological criteria (34), and who had WBC, CD4 + T cell, Hgb and platelet values taken at the time of ART initiation and at least 6 months after initiation of the treatment

4.4.3 Sample size & Sampling techniques

4.4.3.1 Sample size: determination

Sample size for patients based on the parameter with the greatest standard deviation in relation to its size, the CD4+ T cell percentage. A formula for difference between means used (35)

A total of 379 paired subject were included giving 90% power to detect a difference in mean CD4+ T cell percentage of 10%, given a standard deviation of 10.0 (36).

$$n = \frac{2(SD)^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{\diamond^2}$$

$$\frac{2(10)^2 7.9}{50} = 316, 20\% \text{ non response rate} = 379$$

$$\alpha = 0.05$$

$$\beta = 0.20$$

$$\text{Power} = 1 - \beta = 0.80$$

\diamond -- Clinically significant difference

Total sample size = 379

4.4.3.2 Sampling technique

379 HIV infected individuals who had enrolled at Black Lion hospital ART unit from September 1 2005 to August 31 2010 were included in the study using Convenience sampling method.

4.4.4 Inclusion criteria

-Baseline (Pre-HAART) and at least six months follow up data with complete hematological values (WBC, CD4+ T cells, Hgb and Platelet count).

-Subjects with age above 18 yrs.

4.4.5. Demographic and clinical data- Participants demographic variables, prophylaxis taken and type of ART regimen, hematological (White blood cells, CD4+ T cells, hemoglobin and Platelet) values were carefully extracted from ART log book and patient follow up cards by using standardized data extraction form which was prepared from ART log book.

4.5 Study variables

4.5.1 Dependent variables-Hematological parameters (CD4+Tcells, Hgb, WBC and Platelets)

4.5.2 Independent variables- Sex, Type of ART drug regime, WHO clinical stage and Follow up time

4.6 Measurement and Data collection

4.6.1 Data collection tools

A WHO standardized Data Extraction format was used to extract sociodemographic characteristics, clinical information and hematological parameters of the study subjects from Black Lion hospital ART log book and patient follow up card at different time interval from the baseline (September 2005). The data were censored at 31 August 2010, or at the last clinic visit for a patient lost to follow up (defined as those who were not known to have died, but who had not been seen at any time during the year 2010). All covariates for each patient was collected at 0 month (baseline), 6 month, 12 month, 18 month, 24 month, 30 month, 36 month, 42 month, 48 month, 54 month and 60 month following initiation of HAART.

4.6.2 Data collection procedure

Five staff nurses and two ART trained nurses together with the principal investigator were involved in data collection. The two ART trained nurses acted as supervisors. Both the data collectors and supervisors were trained for one day with the objective of standardizing the data collection instrument and providing them with basic skill of extracting the data both from the ART log book as well as patients follow up cards. Before the actual data collection, a pre-test of the instruments and the procedure was conducted and corrective measures were taken.

4.6.3 Hematological Abnormalities

Anemia defined as hemoglobin < 13 gm/dl for men and < 12 gm / dl for women (14). Thrombocytopenia defined as platelet count below 100,000 cells/ μ l(36). Severe thrombocytopenia defined as platelet count < 50,000 cells/ μ l (25). Leucopenia defined as WBC count less than 3,000 cells/ μ l (37). Mild anemia is defined as hemoglobin values of 10-12gm/dl for women and 10-13 gm/dl for men, moderate anemia is hemoglobin values of 8-9.9 gm/dl and severe anemia defined as hemoglobin values less than 8 gm/dl(24). Immunosuppression defined as a CD4+ T cell count <200 cells/ μ l (28).

4.6.4 Quality Control and Quality Assurance

The quality of data were controlled at different levels for completeness and consistency; first by data collectors at the end of each day, then by supervisors every day, then by principal investigator, and finally during data entry. And every third day of data collection, the investigator and supervisors revisited 5% of the extracted data from ART log book and from the patient follow up card to check whether the data collected as planned or not. Whenever an error found in any level, supervisors traced and corrected it.

4.7 Data processing and analysis

Data collected through a standardized questionnaire were entered into Excel spread sheet and transported into and analyzed by SPSS Version 15.0 soft ware (SPSS INC, Chicago, IL, USA) Values are presented as mean \pm SD and median \pm Q (interquartile range) as appropriate. Percentages were used to describe the proportions of the discrete variables. A P-value <0.05 was considered statistically significant. Median change of different hematological values during follow up time from the base line and between consecutive follow up were compared using Wilcoxon signed rank test.

4.8 Ethical consideration

The study was approved after it is ethically reviewed by the research ethical committee of Addis Ababa University Department of Medical Laboratory sciences and the Institutional Review Board (IRB) of Addis Ababa University Medical Faculty. Then a letter informing the hospital administrators was written from the Institutional Review Board (IRB) and Permission obtained from Black Lion Specialized hospital to access ART laboratory and clinical data. All the information obtained from the study subjects were coded to maintain confidentiality.

5. Results

5.1. Demographic, clinical, ART regimes and prophylaxis description

A total of 379 HIV infected individuals aged 21 to 87 years who had started antiretroviral treatment at Black Lion hospital ART unit from September 1, 2005 to August 31, 2010 were included in the present study. The median age of the study subjects was 35 years. Of these enrolled individuals 63.3 % (n=240) were females. At the baseline 177(46.7%) infected individuals were classified as WHO HIV/AIDS clinical stage III and 269 (71 %) classified as functional status of work. Regarding the eligible reasons to start ART, 81.5% of patients started the treatment based on CD4+ T cell count < 200 cells/ul. The most widely used ART regimen in this study was 1a (30), d4T (30)-3TC-NVP, 139 (36.6 %) followed by 1c (AZT-3TC-NVP), 84(22.2%) and 347 (91.4%) of individuals were taken cotrimoxazole as prophylaxis (table 1).

Table 1. Baseline demographic, clinical characteristics and drugs taken by HIV infected individuals who started antiretroviral treatment at Black Lion Hospital ART unit Addis Ababa Ethiopia (n=379) from September 2005 to August 2010.

| Variable | Frequency | Percent |
|--|------------------|----------------|
| Age group (years) | | |
| 21-30 | 120 | 31.7 |
| 31-40 | 156 | 41.2 |
| 41-50 | 76 | 20.0 |
| > 50 | 27 | 7.1 |
| Sex | | |
| Male | 139 | 36.7 |
| Female | 240 | 63.3 |
| WHO clinical stages | | |
| Stage I | 15 | 4 |
| Stage II | 79 | 20.8 |
| Stage III | 177 | 46.7 |
| Stage IV | 108 | 28.5 |
| Eligible reason to start ART | | |
| CD4 | 309 | 81.5 |
| Clinical | 26 | 6.9 |
| Both clinical and CD4 | 42 | 11.1 |
| Other | 2 | 0.5 |
| Functional status of the study subjects | | |
| Ambulatory | 94 | 24.8 |
| Bedridden | 14 | 3.7 |
| working | 269 | 71 |
| Other | 2 | 0.5 |
| Type of ART | | |
| 1a(30) | 139 | 36.6 |
| 1c | 84 | 22.2 |
| 1b(30) | 73 | 19.3 |
| 1d | 62 | 16.4 |
| 1e | 21 | 5.5 |
| Prophylaxis | | |
| Cotrimoxazol | 346 | 91.4 |
| No prophylaxis | 25 | 6.5 |
| Cotrimoxazole + INH | 8 | 2.1 |

5.2 Baseline hematological profile among study subjects enrolled at BLH ART clinic, Addis Ababa

The subjects studied had a baseline CD4+ T cells count ranging from 4-573 cells/ μ l, median IQR of CD4+ T cells , hemoglobin ,white blood cell count and platelet count were 111 cells/ μ l IQR (72-176) cells/ μ l, 12.5gm/dl IQR (11.3-14) gm/dl, 4700 cells/ μ l IQR (3700-6000) cells/ μ l and 192,000 cells / μ l IQR (121,000-264,000) cells / μ l respectively (Table 2).

Table 2. Baseline hematological parameters in HIV infected males and females at Black Lion Hospital ART unit Addis Ababa Ethiopia from September 2005 to August 2010.

| Hematological Parameters | No of patients n= | Total Median (IQR) | Male Median (IQR) | Female Median (IQR) | P value | **Reference values |
|--------------------------------|----------------------|--------------------------|--------------------------|---------------------------|---------|--------------------|
| Hgb(gm/dl) | 379 | 12.5 (11.3-14) | 13.2(11.5-14.5) | 12.2(10.9-13.6) | 0.000 | 12.2 – 18.3 |
| WBC (cells / μ l) | 379 | 4700(3700-6000) | 4,900 (4,000-6,400) | 4,600(3,500-6,000) | 0.093 | (3,000-10,200) |
| Platelets (cells / μ l) | 379 | 192,000(121,000-264,000) | 202,000(130,000-267,000) | 183,000(114,500-263,400) | 0.278 | (98,000-337,000) |
| CD4+T (cells / μ l) | 379 | 111(72-176) | 102(61-165) | 112(72.2 -170) | 0.358 | 366-1235 |

** Taken from Immunohematological reference ranges for adult Ethiopians (37)

5.3 Comparison of hematological parameters between base line and 6 month after initiation of HAART

At time of ART initiation , the median CD4 + T cell, platelet count, WBC count and Hgb were 111 cells / μ l IQR (72-176) cells / μ l, 192,000 cells / μ l IQR (121,000-264,000) cells / μ l, 4700 cells/ μ l IQR (3700-6000) cells/ μ l and 12.5gm/dl IQR (11.3-14) gm/dl respectively. After 6 months of follow up, there marked increase in CD4+T cell count by a median of 89 cells/ul, platelet count 72,000 cells / μ l, WBC count 600 cells / μ l and Hgb 1.5 gm/dl. The observed difference is statistically significant ($p < 0.05$) using Wilcoxon signed rank test. In addition to that subjects who were classified at the baseline as leucopenic, thombocytopenic, severely thrombocytopenic, anemic, with imunosuppresion and in severe imunosuppresion were 9.5%, 15.8%, 2.9%, 43 % (in both sexes), 86.8% and 18.2% respectively. After 6 month follow up 6.6%, 6.6%, 0.5 %, (females-22.7%, males-18.8%), 52.5% and 2.1% respectively (Table 3).

Table 3. Comparison of hematological parameters between baseline and 6 month initiation of HAART at Black Lion Hospital ART unit Addis Ababa Ethiopia from September 2005 up to August 2010.

| | Baseline | After 6 month initiation of HAART | |
|---|---------------------------|-----------------------------------|----------------|
| <u>Hematological parameters</u> | <u>Median (IQR)</u> n=379 | <u>Median (IQR)</u> n=379 | <u>P value</u> |
| WBC (cells / μ l) | 4,700 (3,700-6,000) | 5,300 (4,200-6,900) | <0.05 |
| **Hgb (gm/dl) | 12.5 (11.3-14) | 14(12.4-15.1) | <0.05 |
| Platelet count (cells / μ l) | 192,000 (121,000-264,000) | 264,000(179,000-317,000) | <0.05 |
| CD4 + T (cells / μ l) | 111(72-176) | 190(128-278) | <0.05 |
| <u>Hematological Abnormalities</u> | <u>n (%)</u> | <u>n (%)</u> | |
| Leucopenia (WBC < 3000 cells / μ l) | 36 (9.5%) | 25 (6.6%) | |
| Anemia | | | |
| Mild anemia | | | |
| Female (10-12 gm/dl) | 81 (33.7%) | 38 (16%) | |
| Male (10-13 gm/dl) | 54 (38.9%) | 25 (18.3%) | |
| Moderate anemia (8-9.9 gm/dl) | 25 (6.6%) | 12 (3.2%) | |
| Severe anemia(<8 gm/dl) | 4 (1.1%) | 5 (1.3%) | |
| Thrombocytopenia | | | |
| (Platelet count<100,000 cells / μ l) | 60 (15.8%) | 25 (6.6%) | |
| Severe thrombocytopenia | | | |
| (Platelet count < 50,000 cells / μ l) | 11(2.9%) | 2 (0.5%) | |
| Immunosuppression | | | |
| (CD4+T count< 200 cells / μ l) | 329 (86.8%) | 199 (52.5%) | |
| Severe immunosuppression | | | |
| (CD4+T count< 50 cells / μ l) | 69 (18.25) | 8 (2.1%) | |

Key -- **Hgb – n=376

5.4. Hematological parameters change at different follow up time

The median change of hematological parameters from the baseline were compared using Wilcoxon Signed rank test, the result showed that, median CD4+ T cell, Hgb, WBC and Platelet were having a statistically significant as increased trend at 6,12,18,24,30,36,42,48 and 54 months to the baseline. The median change in hematological parameters from baseline to last visit of CD4 + T, Hgb, white blood cell count and platelet count was *+ 208 cells / μ l, *+1.8gm/dl , *+1140 cells/ μ l and *+ 35,000 cells/ μ l respectively. Analysis of every six month median change between consecutive follow up time was done by Wilcoxon Signed rank test and revealed that CD4 +T cell count and hemoglobin were showed significant increment at 6 and 12 months of ART ($P < 0.05$), however statistical significant change was not observed as the course of treatment continued. The median changes of WBC and platelet count from baseline were significant at 6 month of ART but did not show significant change as the treatment continued. Trend of hematological parameters of patients following antiretroviral treatment showed that the parameters increased at 6 month of ART at significant level but somehow stable as the treatment continued (figure 1).

Key-- *+ indicates increment in hematological parameters

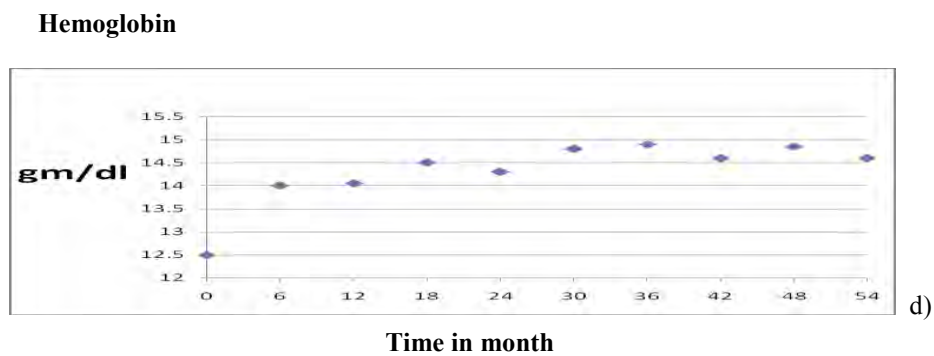
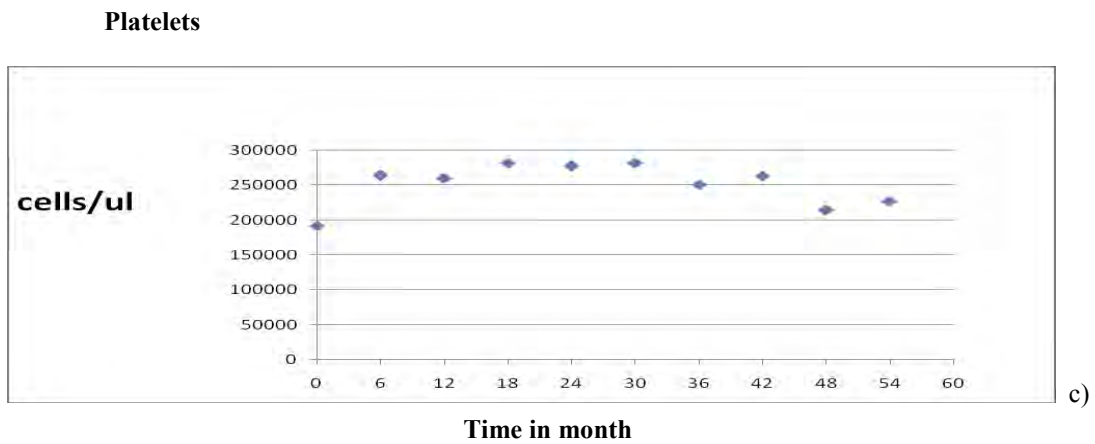
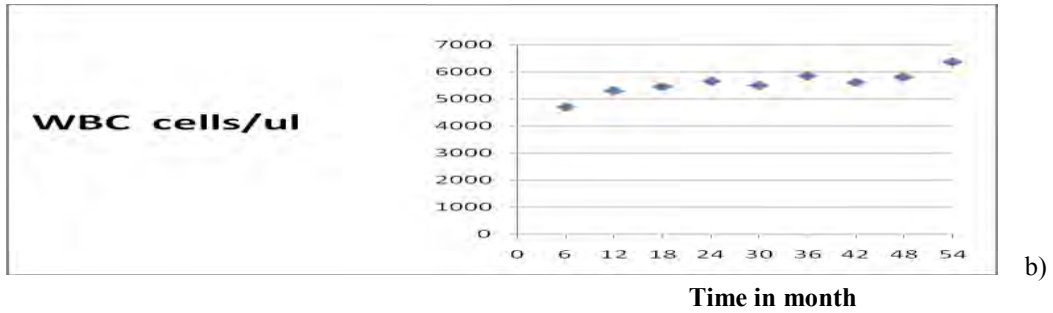
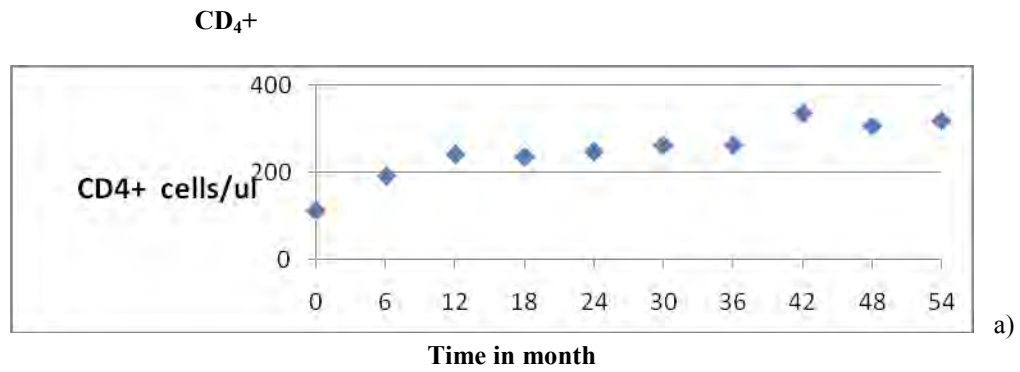


Figure1. Trend of hematological parameters (a-CD4⁺ T , b- WBC count, c- platelet count, d-Hgb) of patients following antiretroviral treatment at 0, 6, 12, 24, 30, 36, 42, 48 and 54 months of follow up, at Black lion hospital ART unit from September 2005 up to August 2010.

6. Discussion

Hematological parameters showed statistical significant change after six month of HAART initiation. Of 379 HIV infected adult individuals during the initiation of antiretroviral therapy revealed that the median hemoglobin, white blood cell count, platelet count and CD4+ T cell count were 12.5gm/dl IQR (11.3-14) gm/dl, 4700 cells/ μ l IQR (3700-6000) cells/ μ l , 192,000 cells/ μ l IQR (121,000-264,000) cells/ μ l and 111 cells/ μ l IQR (72-176) cells/ μ l respectively.

Multiple hematological abnormalities including peripheral cytopenias usually observed in individuals infected with HIV (5). Out of 379 patients, 86.8 % (n=329) cases were those with CD4+T cell counts < 200 cells/ μ l and CD4+ T cell count of < 50 cells/ μ l was seen in 18.2% (n=69) of the patients, indicating the stage of severe immunosuppression. In agreement with this other study conducted in India showed that 89.2% cases at the baseline were with CD4 count < 200 cells/ μ l and CD4+ T cell count of < 50 cells/ μ l was seen in 18.6% of the patients(20). This study also supported by another study conducted at Saint Paul hospital, Addis Ababa Ethiopia that described among 79 HIV positive hospitalized patients , 83.5% of them had CD4+ T cell count < 200 cells/ μ l and 35% had CD4+ T cell count <50 cells/ μ l (21). In the present study, Leucopenia was observed in 9.5 % of patients before starting the treatment. This was lower compared to what has been reported from a study conducted in the university teaching hospital of Nigeria that described 16 % leucopenia in people living with HIV/AIDS (22).

HIV infection may lead to anemia in many ways, some of them are changes in cytokine production, decreased erythropoietin concentrations, opportunistic infectious agents(5). The present study has shown that from the total of 139 males; 60(43.2%) and from the total of 240 females;104(43.3%) were anemic. The most occurred anemia in this study was mild anemia and this is also supported by study conducted in Iran described that mild anemia occurred in 46% of study subjects while severe anemia was not observed (24) In another study conducted in India showed that anemia was seen in 65.5% of patients (14).

In another report from Iran demonstrated a prevalence of 10 % at the baseline and anemia was associated with female sex (23) while in our study and study conducted in Nigeria showed that no significance association was observed in the prevalence of anemia with sex (4). In agreement with our study another study conducted at Zewditu memorial hospital showed that from 155 HIV/AIDS patients at the baseline 35.5% were anemic (11).

HIV affects platelets in several ways including antiplatelet antibodies and circulating immune complexes would cause peripheral destruction in the spleen, liver, and bone marrow, ineffective immune thrombopoiesis and direct infection of the megakaryocytes which could cause a change in the function (17). In this study from the total of 379 study subjects, thrombocytopenia was seen in 60(15.8%) of the patients at baseline. which was higher than the study in India that showed thrombocytopenia was seen in 7% (14/200) of the cases (14).

The present study compared hematological profile of HIV infected adult individuals at baseline and 6 month after initiation of HAART revealed that the median CD4+ T cells count at the baseline for 379 patients was 111 cells/ μ l IQR (72-176) cells/ μ l and after 6 month initiation of HAART, increased to 190 cells/ μ l IQR (128-278) cells/ μ l ($P < 0.05$). In another retrospective study conducted at Zewditu memorial hospital Addis Ababa Ethiopia that described at the time of HAART initiation, the mean \pm SD of CD4+ T-cell count of the total 1166 was 113.6 ± 71 cells/ μ l and after 6 months initiation of HAART the mean \pm SD of CD4+ T-cell count increased to 230 ± 118 cells/ μ l (38).

The present study also supported by a prospective study at Zewditu Memorial hospital Ethiopia showed that mean \pm SD CD4+ T cells of 185 patients was 123 ± 73 cells/ μ l and after 6 months follow up increased significantly to 212 ± 112 cells/ μ l (11). In another study conducted in Newdelhi, India showed that the change in CD4+ T cell count after ≥ 3 months of HAART (follow up) indicates mean CD4+ T cell count increased from 126 ± 16.6 cells/ μ l at baseline to 278 ± 196.7 cells/ μ l (20). In agreement with the present study another study conducted in china showed CD4+ T cell count \pm SD at baseline was 106 ± 85 and after 12 months follow up the CD4+ count increased to (168 ± 51) cells/ μ l (8).

Our study showed that the median white blood cell count at the baseline was 4700 cells/ μ l IQR (3700-6000) cells/ μ l and after 6 month initiation of HAART, increased to 5300 IQR (4200-6900)

cells/ul ($P < 0.05$). Antiretroviral drugs described improvement of leucopenia, at the baseline from the total of 379 patients 36 (9.5 %) were leucopenic and after HAART only 25 (6.6 %) were leucopenic. A retrospective study done at Zewditu memorial hospital Addis Ababa Ethiopia indicated that during initiation of HAART, the mean \pm SD of WBC count of the total 1166 was 4767 ± 1824 and after 6 months initiation of HAART increased to 6409 ± 1998 (38). In agreement with our findings, a study conducted in Markurdi, Benue state of Nigeria indicated that ART brings statistically significant increment in WBC from (4070 ± 250) cells/ μ l at baseline to (4760 ± 170) cells/ μ l after antiretroviral drugs treatment (7).

Antiretroviral therapy decreases the prevalence of anemia in HIV infected patients and it is essential for the hemoglobin restoration of these patients. The present study compared hemoglobin values of 376 HIV infected patients at baseline and after 6 months initiation of HAART and revealed that the median hemoglobin was 12.5 gm/dl IQR (11.3-14) gm/dl and 14 gm/dl IQR (12.4-15.1) gm/dl at the baseline and after 6 month initiation of HAART respectively ($P < 0.05$), the prevalence of anemia at the baseline was 43.3. % (104/240) and 43.2 % (60/139) in females and males respectively. And after HAART only 22.7 % (54/238) and 18.8 % (26/138) in females and males respectively.

A study conducted in the United States shown that HAART improved anemia in women, hemoglobin showed significance difference when compared baseline with 1 year follow up, the anemia prevalence at the baseline and after follow up was 38.3% and 26.1% respectively (39). In another retrospective study at Minilik II hospital, Ethiopia reported that HAART improved the hemoglobin value after initiation of the treatment, prevalence of anemia before ART was 52.6% and after the initiation of antiretroviral therapy was 37.3%. Of this prevalence of anemia was higher in female than in males in both cases before (70.25% vs 29.75%) and after treatment (69.23 % vs. 30.77%) (31). The present study also supported by another study conducted at Zewditu memorial hospital Addis Ababa Ethiopia that indicated at the base line, the mean \pm SD of Hgb value of the total 1166 was 12 ± 2.4 gm/dl and after 6 months initiation of HAART increased to 13.2 ± 3.8 gm /dl (38).

HAART induces a sustained platelet response in HIV infected individuals (25). In the present study Platelet count was increased significantly after 6 months initiation of the treatment ($P < 0.05$). The median + IQR platelet cells count at the baseline for 379 patients was 192,000 cells/ μ l

IQR (121,000-264,000) cells/ μ l ,while 60(15.8 %) of them were Thrombocytopenic and severe thrombocytopenia was observed in 2.9% of the patients and after six month treatment with HAART, the median platelet count was increased to 264,000 cells/ μ l IQR (179-317) cells/ μ l. In addition to that only 25(6.6%) cases and 2(0.5%) of the patients were with thrombocytopenia and Severe thrombocytopenia respectively. Similarly in a retrospective study in Italy reported that HAART induced a significant increased in the platelet count with in the third month following initiation of the treatment which was sustained up to the sixth months of therapy (25).

The median changes of WBC and platelet count from baseline were significant at 6 month of ART but did not show significant change as the treatment continued. Trend of hematological parameters of patients following antiretroviral treatment indicated that the parameters increased at 6 month of ART at significant level but somehow stable as the treatment continued. HAART results in immunologic improvement, even among persons with low pretherapy CD4+ lymphocyte counts. Long term treatment with HAART restore the CD4 level of the patients, in our study patients data were observed for median change of 30 months (6-54 months). The median change from baseline to last visit (54 month) of CD4 + T cell count, hemoglobin, white blood cell and platelet count was *+ 208 cells/ μ l, *+1.8gm/dl, *+1140 cells/ μ l and *+35,000 cells/ μ l respectively. In another study conducted in Baltimore, Maryland described that by 6 years of follow up the median change of CD4 + T cell count observed, from baseline to 6 year follow up was *+ 274 cells/ μ l (40).

Key = *+ indicates the increment of hematological parameters

7. Strength and limitation of the study

7.1 Strength

- ✓ Long follow up data were included

7.2 Limitations

- Lack of complete information on some hematological parameters(RBC indices, diff count, RBC morphology, Coagulation tests)
- The laboratory method (type of hematological analyzer) used to analyze hematological parameters was not clearly stated on the ART log book and Patient's card
- The study had the usual limitation of hospital based studies(in appropriate keeping of record, service access ,budget constrain etc)

8. Conclusion and Recommendation

8.1 Conclusion

→ Significant hematological changes observed after 6 and 12 months of HAART for CD4 + T cell count and hemoglobin while significant change at 6 month for white blood cells and platelet count but there was no significant change observed for the rest follow up time when median change assessed between 2 consecutive follow up.

8.2 Recommendations

- ➔ Hematological tests should be performed regularly to follow the effectiveness of the treatment
- ➔ The laboratory results should be handled and appropriately recorded on ART log book and patient's card.
- ➔ Further research is needed on hematological parameters including Red cell indices, differential count, Red blood cell morphology, Coagulation tests.

9. References

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Annex I- information sheet

Hello! My name is I am here on behalf of the School of medical laboratory Sciences, Addis Ababa University; to assess Hematological profile of HIV infected individuals after receiving Highly Active Antiretroviral Therapy

I am going to review your hospital ART logbook and ART client follow up card to analyze the Hematological profile of HIV infected individuals after receiving Highly Active Antiretroviral therapy which will contribute in the improvement of ART management.

The details of the questions will be around: the socio-demographic characteristics, Clinical information, and hematological values in study subjects at 0, 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 months initiation of ART

I have received permission from the Hospital administration and respective health offices to conduct this study.

The information from this study will not be used for other purposes by any of the institutions and individuals without your agreement and the information will be completely confidential.

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

Jemal Alemu

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Addis Ababa University

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For additional information, please contact AAU, Medical Faculty Institutional Review Board

(IRB) office at Tel +251-11-5-53-87-34

Fax +251-11-5-51-1-51-30-99

P.O.Box 9086, Addis Ababa, Ethiopia

Email- aaumfirb@yahoo. Com

Annex II. Consent form

I the undersigned individual being oriented about the relevance of this study was well informed; our ART unit participation in this study is crucial, all the information is kept confidential and will be used solely for this study.

In addition, we have been well informed that the name will not be asked and unique identification is not required

Our agreement to participate in this study is with the assumption that, the information that provided by our ART unit greatly improves the life of our HIV infected individuals who start ART and management of ART which further lead to improve the survival of HIV infected individuals after initiation of ART.

Generally, we have been explained about this research study and we have understood the same. And, we hereby agree to participate in this research study and give our voluntary consent.

Institution head Sign _____ Date _____

Interviewer Name _____ Signature. _____

Date of interview _____

Witness Signature _____ Date _____

Supervisor Name _____ Date _____ Signature _____

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

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Annex III – Data extraction form for Retrospective observational Cohort

I am ----- working as data collector in this study that asses the hematological profile of HIV infected individuals after receiving highly active antiretroviral therapy (HAART) in black lion specialized hospital, Addis Ababa Ethiopia ART unit. On this Data extraction form names of the individuals will not be written and I am going to extract information from ART Log book about demographic, clinical and hematological values at the start of ART and every six month visit, in which all the extracted data will be kept completely confidential.

Identification Code _____

Date of data collection _____

Data collector name _____

Supervisor _____

Section I: Background characteristic

Sex Age (In complete years)

| | | |
|----------------------------------|-----------------------------------|-------|
| <input type="checkbox"/> Male | <input type="checkbox"/> Femal | _____ |
|----------------------------------|-----------------------------------|-------|

Section II. Clinical variable at the start of ART (Baseline information)

| | | | |
|--|----------------------------|--------------------------|--------------------------|
| Date of starting ART | | Date. (DD/MM/YY) [/ /] | |
| Reason to be eligible for starting ART (why eligible) | <input type="checkbox"/> | <input type="checkbox"/> | Other (specify) _____ |
| | Clinical only | CD4 | |
| Where dose the ART started | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | With in the Hospital | Transfer in | Other _____ |
| Name and dose of ART taken by the study subject Dose (optional) → | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | 1a(30) | 1a(40) | 1b(30) |
| | _____ | _____ | _____ |
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | 1b(40) | 1c | 1d |
| | _____ | _____ | _____ |
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | 1e | 1f | |
| | _____ | _____ | |
| Functional status of the study subject | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | Ambulatory | Bedridden | Working |
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | Other _____ | | |
| prophylaxis (with dose) taken at start of ART | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | Bacterium _____ | Isoniazide _____ | Fulconazole _____ |
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | Bactrium+Fluconazole _____ | Bactrium + | |
| | | INH _____ | |
| WHO clinical stage of HIV disease at start of ART | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | Stage I | Stage II | Stage III |
| | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| | | Stage IV | None |

Hematological variable at the start of ART (Baseline information)

| | | | |
|------------|----------------|------|--|
| CD4 | _____ cell/μl | Diff | (Neut -----,Lymp-----,Mono-----,Eos-----,Bas-----) |
| WBC | _____ cell/μl. | MCV | _____ |
| Platelet | _____ cell/μl. | MCH | _____ |
| Hemoglobin |gm/dl | MCHC | _____ |
| | | RDW | _____ |

Declaration

I the undersigned, declare that this thesis is my original work, has never been presented in this or any other university, and that all resources and materials used herein, have been duly acknowledge.

Name: Jemal Alemu (Bsc)

Signature _____

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission: _____

This thesis has been submitted with my approval as a University advisor.

Name: Dr. Aster Tsegaye(MSc , PhD)

Signature _____

Dr. Wondwosen Amogne (MD,Internist)

Signature _____

Ato Bineyam Taye (BSc,MPH)

Signature _____

Ato Asaye Birhanu (BSc,MSc)

Signature _____