ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Comparative study on Changes in Hematological Parameters during the first 6 month of Antiretroviral Therapy initiation among HIV infected pregnant and non-Pregnant women at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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(Hematology and Immunohematology Specialty Track)

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TABLE OF CONTENTS

Contents

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Care
ART	Antiretroviral Treatment.
ARV	Antiretroviral
AZT	Zidovudine
BMI	Body Mass Index
CBC	Complete Blood Count
CCR	Chemokine Receptor
CD4	Cluster of Differentiation 4
DNA	Deoxyribonucleic acid
EMLA	Ehiopian Medical Laboratory Association
EQA	External Quality Assessment
ETB	Ethiopia Birr
GP	Glycoprotein
HAART	Highly active Antiretroviral Therapy.
Hb	Hemoglobin
HCT	Hematocrit
HIV	Human Immunodeficiency Virus
LBW	Low birth weight
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
MTCT	Mother to Child Transmission
NCNC	Normocytic normochromic
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NSHPC	National Study of HIV in Pregnancy and Childhood
NVP	Nevirapine
OI	Opportunistic infection

PCV	Packed cell Volume
PLT	Platelets
PMTCT	Prevention of Mother to Child Transmission
PT	Prophylactic Treatment
PTB	Preterm Birth
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
RNA	Ribonucleic Acid
RPI	Reticulocyte Production Index
SD	Standard Deviation
SPSS	Statistical package for social sciences
UK	United Kingdom
WBC	White Blood Cells
WHO	World Health Organization
ZDVm	Zidovudine monotherapy
ZMHL	Zewditu Memorial Hospital Laboratory

OPERATIONAL DEFINITIONS

Anemia: Hb concentration less than 11 g/dl for pregnant women and less than 12 g/dl for non-pregnant women

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

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

Hypochromic: MCHC<32 gm/dl

Immunodeficiency (Immunosuppression): CD4+T lymphocyte counts < 350 cell/µl

Leucopenia: White blood cell count < 3000 cells/ul.

Macrocytic: Erythrocytes having large size (MCV>100fl).

Microcytic: Erythrocytes having small size (MCV<80fl).

Mild anemia: When the hemoglobin concentration of pregnant women is between 10.0 -10.9g/dl for pregnant and 10.0-11.9 for non-pregnant women.

Moderate anemia: When the hemoglobin concentration of is between 7.0-9.9 g/dl.

Severe anemia: When the hemoglobin concentration is less than 7g/dl.

Thrombocytopenia: Platelet count less than 150,000/µl

Mild thrombocytopenia: When the platelet count is between100,000 to 150,000/µl.

Moderate thrombocytopenia: Platelet count between 50,000 to 100,000/µl.

Severe thrombocytopenia: Platelet count less than 50,000/µl.

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ABSTRACT

Background: The use of ARV prophylaxis during pregnancy can dramatically reduce the risk of vertical transmission of HIV infection. Currently, limited studies have documented the effect of ART in decreasing the risk of opportunistic infection as well as improving survival of HIV infected pregnant women and fetus. However, little is known about its impact on hematological parameters in resource limited settings like Ethiopia.

Objective: To assess changes in hematological parameters after 6 months of ART initiation among HIV infected pregnant and non-pregnant women at Zewditu Memorial Hospital ART clinic, Addis Ababa, Ethiopia.

Methodology: A retrospective comparative study was conducted among HIV infected pregnant women who received ART between September 2008 and January 2014 at ART clinic of Zewditu Memorial Hospital, Addis Ababa, Ethiopia. Data conveniently collected from 384 pregnant women on ART was compared with that obtained from 384 non pregnant women who are on ART as controls. Demographic, clinical and hematological parameters data were collected between November and December 2016 carefully from the existing ART logbook and antenatal follow up cards. Data collected through a standardized format were entered into Excel spread sheet and transported into and analyzed by SPSS Version 20 software. Dependent variable frequencies, mean, standard deviation and range were calculated. Mean change of different hematological values during follow up time from the base line were compared using paired t-test. P value <0.05 was considered as statistically significant.

Results: The results of the study indicated significant difference in RBC Count, Hb, HCT, MCV, MCH,MCHC PLT counts were statistically significantly lower, while CD4 count was significantly higher in HIV infected pregnant women compared to non pregnant women. There was no statistically significant difference between the two groups for PLT and total WBC. Prevalence of anemia, leucopenia, thrombocytopenia and CD4+ T lyphocytopenia at baseline and 6 months of follow up were 49.5% vs 54.4%, 20.7% vs 4.9% , 20.8% vs 18.8% and 88.8% vs 69.8% for HIV infected pregnant women, respectively. The respective values in HIV infected non pregnant women were 37% vs 18% , 8.6% vs 5.8% ,38% vs 7.8% and 58.6% vs 34.1.Microcytic hypochromic anemia was the dominant type in the pregnant women while normocytic hypochromic was the commonest followed by microcytic hypochromic anemia in the non-pregnant women. Thus, monitoring of all hematological parameters (not only Hb) is warranted.

Conclusion: Comparison of hematological parameters of HIV infected pregnant with non pregnant significant changes. Since these parameters are strongly influenced as a result of pregnancy.

Key words: Pregnant women, Hematological parameters, anemia, thrombocytopenia, CD4+ T cells and ART

1 INTRODUCTION

1.1 Background

In Pregnancy, the hematological system undergoes changes in order to meet the demands of the developing fetus and placenta, with major alterations in blood volume. Hemodilution during pregnancy contribute to majority of these changes. Maternal blood volume increase during pregnancy and this involves an increase in plasma volume as well as in red cell and white cell volume. The blood volume increase by 40% to 45 % on average, this increase occurs faster in the second trimester **[1, 2].**

Every stage of pregnancy is unpredictable and each pregnancy comes with different presentation. Some of the hematological profiles showed significant changes in mean values of red blood cells (RBC), packed cell volume (PCV), mean cell volume (MCV),red cell distribution width (RDW), Platelets, white blood cells (WBC),Neutrophils, Lymphocytes, Monocytes, and Eosinophils relative to the non-pregnant women. Moreover, there were significant changes between the trimesters in most of the parameters showing the need for carefulness with pregnant women at any stage of the pregnancy **[5].** Although physiological in nature, but abnormal hematological profile does affect pregnancy and its outcome. The most frequent hematologic complication during pregnancy are anemia **[3,4, 5]** and thrombocytopenia **[3].**

During pregnancy, the total blood volume increases by about 1.5 litter, steadily from as early as 4 weeks of pregnancy to reach a maximum of 35-45 % above the non-pregnant level at 28 to 32 weeks. The plasma volume increases by 40-45 %. Red blood cell mass increases by 30-33 %as a result of the increase in the production of erythropoietin. Erythropoietin levels increase throughout pregnancy, reaching approximately 150% of their pre pregnancy levels at term. Women who take iron supplements have less pronounced Hb changes, as they increase their red cell mass proportionately more than those without dietary supplements **[6].**

The PLT count is slightly lower in pregnant than in non-pregnant women, particularly in the third trimester. This is termed as "gestational thrombocytopenia".It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance (destruction) .The normal level of platelets in pregnancy is 150–400 $x10^9$/L. Thrombocytopenia is classicajmlly defined as a platelet count of less than 150×10^9/L. Counts from 100,000 to 150,000/μL are considered mildly depressed, 50-100×10^9/L are moderately depressed, and less than 50×10^9/μL are severely depressed **[7,8].**

WBC is increased in pregnancy with a typical reference range of 6.0 –16.0 $\times10^9$/L. In the hours after delivery, healthy women have been documented as having WBC 9.0 –25.0 $x10^9$/L. By 4weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (4.0 - 10.0 $\times10^9$/L) Neutrophils are the major type of leucocytes on differential counts **[6].**

The first antiretroviral agent to become commercially available was zidovudine in 1987, followed by didanosine and zalcitabine in1993. Currently 24 different drugs from six distinct classes are available. These are Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non-nucleoside reverse transcriptase inhibitors (NNRTI), Protease inhibitors (PI), Integrase Inhibitor, Fusion Inhibitor and CCR5 Antagonist **[9].**

For HIV infected pregnant women, the initiation of ART for their own health is recommended for all women who have CD4 counts of ≤350 cells/mm^3, irrespective of WHO clinical staging, and for all women in WHO clinical stage 3 or 4, irrespective of the CD4 count. These criteria for initiating ART for pregnant women are the same as for non-pregnant women. The available data show that maternal ART during pregnancy and continued during breastfeeding is the most effective intervention for maternal health. It is also efficacious in reducing the risk of HIV transmission and infant death in this group of women with the highest risk of mother to child transmission (MTCT). Therefore, HIV infected pregnant women in need of treatment for their own health should start ART irrespective of gestational age and should continue with it throughout pregnancy, delivery, during breastfeeding (if breastfeeding) and thereafter **[10].**

In 2013, WHO guidelines changed the CD4 count for HIV therapy initiation from less than 350 cells/ μL to 500 cells/μL and recommends ART in one simplified regimen to all pregnant and breastfeeding women regardless of CD4 count during the period of risk of mother to child transmission. This continuation of life-long ART is recommended, particularly in generalized epidemic settings with high rates of fertility, and limited access to CD4 testing. The guideline recommends two options: the first option is providing lifelong ART to all pregnant and breastfeeding women living with HIV regardless of CD4 count or clinical stage. The second option is providing ARV drugs for pregnant and breast feeding women with HIV during the mother to child transmission risk period and then continuing lifelong ART for those women eligible for treatment for their own health **[11].**

However, the gap between pregnant women and all adults is declining as new guidelines are being rolled out on the importance of starting pregnant women on antiretroviral therapy **[11, 12].**

1.2 Statement of the problem

HIV has created an enormous challenge worldwide. Globally, an estimated 35.3 (32.2–38.8) million people were living with HIV in 2012. There were 2.3 (1.9–2.7) million new HIV infections globally, showing a 33% decline in the number of new infections from 3.4 (3.1–3.7) million in 2001. At the same time the number of AIDS deaths is also declining with 1.6 (1.4–1.9) million AIDS deaths in 2012, down from 2.3 (2.1–2.6) million in 2005. The sub Saharan Africa remains the most heavily affected region, with 67% of the global burden and 90% of children living with HIV worldwide and 75% of AIDS deaths **[12].**

Mother to child transmission of HIV is a devastating consequence of HIV infection during pregnancy and is largely preventable. Evidence-based interventions such as universal antenatal screening, provision of antiretroviral therapy, delivery by elective caesarean section and avoidance of breastfeeding have ensured reduced rates of MTCT **[13].**

Ethiopia is one of the seriously affected countries in sub Saharan Africa. According to HIV related estimates and projections for Ethiopia, national prevalence of HIV infection in 2014 is 1.2 % (1.6% for female and 0.8% for men) among adult population. The prevalence of HIV infection in the same year was 3.3 % (male 2.3%, female 4.4% female) in urban and 0.5 (male 0.3%, female 0.6%) in rural area. As of October 2013, total number of patients ever started on treatment was 499,412 out of 822,531 patients ever enrolled in the 880 health facilities (Hospital and Health center). Currently, 98.5% of the patients are on the first line regimens **[14,15].**

The coverage and uptake of PMTCT of HIV services has remained very low in Ethiopia. Only 9.3% of the estimated numbers of HIV positive pregnant women were provided with ARVs for PMTCT **[16].** Currently among HIV tested positive pregnant Women, 60 percent did not receive ARV prophylaxis for PMTCT **[17]**. Many physiological hematological changes occur during pregnancy to accommodate the demands of the developing fetus, while it is also possible that the pregnant women may also show treatment failure. Despite both HIV and its treatment cause hematological complications, the change in hematologic profiles in the face of physiologic changes is not well studied.

1.3 Significance of the study

The study focuses on changes of hematological parameters after initiation of ART in HIV+ pregnant women as compared to their non-pregnant counterparts. The study has significant direct benefits for maternal health as well as decreasing infant mortality. As the study assesses change in hematological parameters after initiation of ART at two different intervals (baseline and at 6^{th} month), it provides information to clinicians for improving management of HIV infection during pregnancy. The result can also be utilized as an input by policy makers for improving HIV positive women's care.

2 LITERATURE REVIEW

Studies have shown that, both HIV and its treatment bring hematological changes during pregnancy in addition to the expected associated physiologic changes. The most common changes associated with HIV as well as pregnancy are anemia and thrombocytopenia. CD4+ T cell lymphocytopenia is a well-documented consequence of HIV infection. Antiretroviral therapy, on the other hand, have both benefits (viral suppression, CD4+ T cells reconstitution, and improvement in hematological abnormalities) as well as side effects related to some antiretrovirals like in ZDV related anemia **[9].** Apart from directly benefiting the mother, the use of antiretrovirals can dramatically reduce the risk of vertical transmission of HIV infection **[18]** and hematological complications in the child **[3]** as well as associated with better pregnancy outcome **[19,20].**

A study carried out in Abidjan, Coˆted'Ivoireto to assess and characterize all hematological and immunological parameters of a population during HIV infection. Included 30 men, 108 non and 164 pregnant women. The study indicated a significant alteration of hematological and immunological parameters in study population. This deterioration was during pregnancy. Thus, no pregnant woman revealed a normal hematological status. An analysis of erythrocyte parameters showed that all women (pregnant and non pregnant) were anemic respectively 99.1 % and 100 %. However, men also reported a high prevalence of anemia (80 %). The hematological and CD4 Count of the pregnant women showed that hypochromic microcytic anemia (HMA) , normochromic normocytic anemia (NNA) hypochromic normocytic anemia (HNA), amacrocytic hypochromic anemia (MHA),WBC(< $4x0^9$/l), platelet count(< $150X10^9$/l) (47.6%, 32.3% , 19.5 % , 0.6% , 9.8% and 29.3%) respectively **[21].**

Thorne *et al* (2014) compared ART response of women with previous use of ZDV monotherapy (ZDVm) in pregnancy with women who were ART naïve by linking two UK studies: the UK Collaborative HIV Cohort (UK CHIC) and the National Study of HIV in Pregnancy and Childhood (NSHPC). The result suggested that ART-naïve (n = 1937) and ZDVm experienced (n = 91) women had similar increases in CD4 count; both groups had a low risk of AIDS. ZDVm experienced and ART-naïve women started therapeutic ART at similar baseline CD4 counts (ZDVm-experienced: 226 (162 –339)) cells/ mm^3; ART-naïve: 225 (150 – 302) cells /mm^3. The median CD4 count change after 6 month of ZDVm treatment was 106 (41 – 171) cells/mm^3

while that of ART-naïve women was 106 (34- 147) cells /mm^3. At 12 month the changes of the median CD4 count in ZDVm experienced and ART-naïve women was 150 (61 – 233) and 160 (70 -256) cells/mm^3, respectively **[22].**

Another study conducted in the United Kingdom and Ireland to explore pregnancy and prenatal outcomes in diagnosed HIV infected women receiving antiretroviral therapy. As the uptake of HAART increased, significant overall improvements of CD4 count in pregnancy was reported for 75.9% (4901/6459) of births between 1998 and 2006, at a median of 29 days before delivery. In later years, CD4 tests tended to be closer to delivery, at a median of 28 days before delivery in 2005-2006, compared with 37 days in 1998-1999. Median CD4 count increased significantly, from 315 cells/µl in 1998 to 395 cells/µl in 2006 **[19].**

A Cohort study conducted by Rudin *et al* in Swiss mothers and children in 2008 G.C, compared virologic failure and the frequency of ART changes in 372 pregnant and 324 non-pregnant women. The result showed that the associated physiological changes occurring during pregnancy did not put pregnant mothers at higher risk of virologic failure. After ART, the median CD4 count and viral load copies of pregnant women were 452 cells/mm^3 and 380 copies per milliliter, respectively. For non-pregnant women the respective median CD4 count and viral load copies were 188 cells/mm^3 and 480 copies per milliliter. Women starting HAART during pregnancy changed the treatment less often than non-pregnant women. The risk of virologic failure tended to be lower in pregnant than in non-pregnant women, respectively **[23].**

Multivariate analysis was conducted at 6 sites in the United States and Puerto Rico in 2006 by McIntosh *et al* to evaluate the effect of prenatal antiretroviral drug exposure on hematological values in HIV uninfected Children. The study included 1820 HIV uninfected and ARV exposed children and compared them with those in 351 ARV unexposed. Hemoglobin concentrations, platelet, neutrophil, lymphocyte, and CD4+ T cell counts were analyzed at birth and at ages 2, 6, 12,18, and 24 months. The results showed that Hemoglobin concentrations and neutrophil, lymphocyte, and CD4+ T cell counts were significantly lower at age 0–2 months in infants exposed to ARV drugs than in those who were not. At 6–24 months, differences in hemoglobin concentrations and neutrophil counts were no longer significant whereas differences in platelet, lymphocyte, and CD4+ T cell counts persisted and CD8+ T cell counts became significantly lower. In comparison with ARV monotherapy, combination therapy was associated with larger

decreases in neutrophil, lymphocyte, and CD8+ T cell counts at age 0–2 months but with only differences in CD8+ T cell counts at age 6–24 months **[24].**

Ahir *et al* in Mumbai, India assessed whether mother to child transmission is the most significant route of HIV transmission in children below the age of 15 yr by enrolling 58 pregnant women. Of them, 18 women during pregnancy were eligible for initiation of combination of NVP as first line of therapy (CD4 count < 350 cells/µl). The remaining 40 women were given NVP (200mg) prophylaxis at the onset of labor. The median CD4 count in women on prophylactic treatment (PT) was slightly higher than those on ART (483 vs.289 cells/µl). But higher viral load was found in women on PT. At the end of 18 months follow up, only two children whose mothers were on PT were HIV positive, while infants of all the mothers who were on ART were negative **[25].**

In addition, study by Ichipi-Ifukor PC et al. found that PCV (32.58 ± 4.01), Hb (10.00 ± 1.28), granulocytes (59.91 ± 7.71) and PLT(202.177 ±48.75) were significantly decreased whereas lymphocytes increased (29.10 ± 8.2) significantly in pregnant women when compared to the controls (PCV 37.07 ± 3.19, Hb 11.71 ± 1.32, granulocyte 64.78 ± 11.45,PLT 224 ±75.21, and lymphocyte 23.4 ± 6.9). WBC showed no significant difference. The study concluded that pregnancy in women has the tendency to alter hematological indices **[26].**

Many studies have identified the hematological profile of the pregnant women as one of the factors affecting pregnancy and its outcome Low hemoglobin (Anemia) is a widely identified hematological abnormality followed by thrombocytopenia in pregnant women**[27].**

Das S. et al in their study conducted at Bankura` Samilani Medical College, Bankura showed d that Pregnant women exhibited statistically significant lower values of Hb, PCV, monocyte and lymphocyte while WBC, eosinophil and erythrocyte sedimentation rate (ESR) were significantly elevated compared to non-pregnant women **[28].**

A prospective observational cohort study was conducted in Nigeria on CD4 changes in 126 HAART naïve and HAART experienced HIV positive pregnant women. The overall mean CD4 change in the 2 months of study in the three trimesters groups were 217, 192.93 and 110.12 cells/µl respectively, while the percentages differences of mean of the CD4 counts in between points of evaluation for each trimester were: 33.09% and 15.19% (1^{st} trimester), 33.33% and 14.51% (2^{nd} trimester) and 12 .38% and 7.13% (3^{rd} trimesters), respectively.

The findings suggest that better immunologic response and fewer neonatal infections in group of naïve women initiated on HAART while in the first 26 weeks of pregnancy and lesser response in those commenced in the third trimester of pregnancy. The study recommended that concerted efforts should be directed towards the initiation of HAART prophylaxis before end of 2nd trimester of pregnancy **[29].**

Another study conducted in Nigerian to evaluate the CD4 count and determines the relationship between CD4 count and packed cell volume (PCV). The study population included 130 ethnic Nigerian women. The mean (SD) percentage for the PCV of HIV seropositive pregnant women was 30.1% (1.6%) with a median of 30%, a maximum of 33%, and a minimum of 26%. The corresponding PCV of HIV seronegative pregnant women was 34.9% (2.3%) with a median of 35%, a maximum of 39%, and a minimum of 29%. The mean (SD) CD4 count of HIV seropositive pregnant women was 323.7 (170.7) cells/μL with a median of 287 cells/μL while that of HIV seronegative pregnant women was 578.3 (196.7) cells/μL, with a median of 534 cells/μL. Based on their finding they concluded that pregnancy may partially deplete CD4 cells because a significant difference was observed in mean (SD) CD4 count in HIV seropositive and HIV seronegative pregnant women at various gestational ages. Also PCV in HIV seropositive pregnant women was directly proportional to their CD4 counts **[30]**.

Improvements on CD4 count as well as anemia parameters have implication on pregnancy outcomes. For example, a case control study conducted Nigeria, in university of Ilorin Teaching Hospital to evaluate pregnancy outcomes in pregnant women on HAART have shown that commencement of HAART before pregnancy appears to improve pregnancy outcomes. A total of 268 HIV positive women were enrolled. Women initiating HAART in pregnancy were taken as study population (n=54) whereas those who initiated it before pregnancy were used as controls (n=214). Pregnancy outcomes were worse with statistical significance in participants who initiated HAART in pregnancy as compared to those who initiated before pregnancy: miscarriage (11.1% vs. 0.9%), preterm delivery (31.5% vs. 1.9%), term pregnancy (42.6% vs. 96.3%), and anemia (35.2% vs.0.9%). Mother to child transmission of HIV was recorded in only 3 (1.1%) babies **[31].**

Additionally, a case control study that was conducted in Nigeria, university of Benin Teaching Hospital to evaluate effect of HAART on some hematological parameters on 50 HIV infected pregnant women and 50 HIV seropositive pregnant women not on HAART as controls at different trimesters. The study which was carried out by Osime*et al* revealed that there were no significant changes in Hb, and WBC between test and control subjects, while in the second trimester there was a significant decrease in platelet counts in the control subjects. In the third trimester of gestation WBC also increased significantly though these increases were within normal ranges **[32].**

In a cross sectional study conducted in Cameroon, Yaoundé, in 2014, Zeudja *et al* assessed the impact of antiretroviral treatment on birth outcome according to the timing of antiretroviral initiation in relation to pregnancy. A total of 617 newborn babies were enrolled. Almost 96% of their mothers were taking antiretroviral drugs free of protease inhibitor. Overall rate of LBW was at 11.6% and preterm birth (PTB) 9.7%. In bivariate analysis, PTB were similarly rated in preconception HAART (8.1%) versus during pregnancy (10.1%), in ART started during pregnancy, the PTB frequency was analogous, irrespective of the time of ART initiation before 28 weeks (10.9%), or after (9%). In addition, LBW rates were registered at 11.7% prior to pregnancy versus 11.6% after conception. Commencing ART during pregnancy that is <28 weeks of pregnancy was almost twice associated to a higher risk of LBW. In this study, it was observed that ART prior to conception mainly free of protease inhibitor does not increase the risk of PTB or LBW **[33].**

On the other hand, pregnancy has been shown to disengage women from having HIV treatment and care. A cohort study conducted in South Africa in 2014 by Thebus *et al* have shown disengagement of HIV positive pregnant and postpartum women from antiretroviral therapy services. A total of 358 women initiated ART during pregnancy, of whom 142 women started ART in the general adult ART service while 216 women initiated ART within the ANC clinic. By six months postpartum, 24% of women (n = 86) had missed at least one visit and an additional 32% (n = 115) had disengaged from care; together, 49% of women had either missed a visit or had disengaged by six months postpartum. A among women starting ART in the ANC clinic, 61% had been newly diagnosed with HIV in the current pregnancy compared to 53% of women referred out to start ART. Women starting ART in the ANC clinic also tended to have a lower WHO clinical stage at ART initiation **[34].**

Sub-analysis of the data from a prospective trial was conducted in south Africa in University of KwaZulu Natal on 669 HIV infected and 642 HIV uninfected at baseline, and for 897 of the HIV infected and 436 of the HIV uninfected pregnant women at 6 weeks to compare prevalence of thrombocytopenia. The result showed that the prevalence of thrombocytopenia was 5.3% during pregnancy and 1.2% at 6 weeks after delivery. The prevalence was comparable among HIV-infected (6.0%) and HIV-uninfected women (4.7%). Among the HIV-infected women, who were not receiving antiretroviral therapy (mean CD4 cell count of 453 cells/μL), there was no significant association between immunosuppression and the severity of thrombocytopenia **[35].**

A study was done in Burkina Faso, Kenya and South Africa, 2013 by Cherish *et al* to assess the effect of triple zidovudine containing regimens. ART eligible pregnant women were followed from 28 weeks of pregnancy until 12–24 months after delivery (n = 1070). At enrolment (corresponded to a median of 32 weeks gestation), the median hemoglobin was 10.3 g/dl . Severe anemia occurred subsequently in 194 (18.1%) women, mostly in those with low baseline hemoglobin, lowest socio-economic category, advanced HIV disease, prolonged breastfeeding (≥6 months) and shorter ARV exposure. Severe anemia incidence was similar in the randomized arms. After 1–2 months of ARV's, severe anemia was significantly reduced in all groups, though remained highest in the low CD4 cohort **[36]**

Another multicenter study was conducted at four study sites in Malawi (2 sites), Tanzania, and Zambia to compare selected hematological and biochemical measurements in 2,292 HIV infected and 367 HIV uninfected pregnant women and their infants. All the HIV infected mothers received nevirapine prophylaxis at the time of labor, as did their infants after birth (always within 72 hours of birth). The results showed that HIV infected pregnant women had lower red blood cell counts, hemoglobin, hematocrit, and white blood cell counts than HIV uninfected pregnant women. Platelet and monocyte counts were higher among HIV infected pregnant women at both time points. At the 4–6-week visit, HIV-infected infants had lower hemoglobin, hematocrit and white blood cell counts than uninfected infants. Platelet counts were lower in HIV infected infants than HIV uninfected infants, both at birth at 4 – 6 weeks **[37].**

A cohort study carried out in Kenya in 2014 by Wandabwa *et al* evaluated the effectiveness of HAART in PMTCT among 50 pregnant women before and 6 months after administration of option B HAART and 50 children 6 weeks after HAART. The study showed the mean absolute CD4 cell counts of mothers after 6 month administration of HAART was 496 counts/µl, which was significantly higher than the value before the administration (435 counts/µl). The study also determined changes in the viral load in mothers before and 6 months after the administration of HAART. Following the HAART administration in mothers, up to 90% of children were confirmed to be HIV negative. According to this study administration of HAART to mothers and children demonstrated an effective mechanism of PMTCT **[18].**

A case control study that was conducted in Sudan by Elgari to evaluate hematological parameters of pregnant women. The study revealed that there were significant decreased in RBCs count, Hb, PCV, MCV, MCH and MCHC of pregnant women compared to non-pregnant women. WBCs count was increased significantly while in contrast, platelets count was significantly lower than the control values. On the bases of blood picture, classified anemia's of pregnancy as normocytic normochromic 37% with reticulocyte production index (RPI) mean value of 0.49±0.2, microcytic hypochromic 52(52%) with RPI mean value of 0.76±0.6, and dimorphic picture11 (11%) with RPI of mean value 2.1±0.8 **[38].**

In Ethiopia, as far as my literature search goes, there is no published study assessing the changes in hematological values in HIV infected pregnant as well as non-pregnant women on antiretroviral treatment. Studies focused on access and utilization of PMTCT services and MTCT rates of HIV. According to a five year national level PMTCT data collected from series of publications of the FMOH and HAPCO from 2006 to 2010, it examines the progresses and unaddressed needs in access and utilization of PMTCT services in Ethiopia. The data showed that only 53% of known HIV-positive mothers and 48% of known HIV exposed infants have received ARV prophylaxis **[39].**Studying antiretroviral treatment associated hematological changes in HIV infected pregnant women could provide information on the benefits of HAART for evidence based decision to seeking the service.

3 STUDY OBJECTIVES AND HYPOTHESIS

3.1 General Objective

To assess changes of hematological parameters in HIV infected pregnant and non-pregnant women at base line and at 6^{th} months of ART initiation at Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To describe hematological parameters at the base line and at six month initiation of ART in pregnant and non-pregnant women
- To determine mean change of WBC, Hgb, RBC count, red blood cell indices, Platelets and CD4+ T at base line and at 6 months.
- To determine the prevalence and severity of anemia among HIV infected pregnant women

3.3 Hypothesis

There is no change in hematological parameters in an HIV infected pregnant women who commence ART during their pregnancy.

4 MATERIALS AND METHODS

4.1 Study design

A retrospective comparative study was conducted to assess changes in hematological parameters among HIV infected pregnant and non-pregnant women after six months of initiation of Anti-Retroviral Therapy (ART) at Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

4.2 Study period

The data of HIV infected pregnant and non-pregnant women attending the ART unit of Zewditu Memorial Hospital between September 2008 and January 2014 was collected from November to December 2015.

4.3 Study Site

The study was conducted in Addis Ababa at Zewditu Memorial Hospital ART clinic. The Hospital which is located in Kirkose sub-City was established in 1925 E.C. renovated in 1963 E.C.by American missionaries and owned by the Seventh-day Adventist Church until nationalized during the Derg regime around 1976. It is managed underAddis Ababa City Administration Health Bureau. The total number of staffs in the hospital is 705 (with 459 health professional and 246 administrative staffs). The ART clinic of the Hospital is a model center which was established in July, 2003 G.C. It has four ART Physicians, eight nurses, five druggists and five data clerks to give full service for attending 50 – 60 patients per day.

4.4 Study population

4.4.1 Source of population

All HIV positive women attending Zewditu Memorial Hospital ART clinic were the source population

4.4.2 Study population

4.4.2.1 Cases

Cases were HIV infected pregnant women who had hematology (CBC and CD4) tests at baseline and at 6^{th} month after initiation of ART (defined as two or more antiretroviral drugs for at least 6 months) by WHO clinical and immunological criteria **[39].**

4.4.2.2 Control Population

Controls were HIV infected non-pregnant women who had hematology (CBC and CD4) tests at baseline and at 6th month after initiation of ART by WHO clinical and immunological criteria **[39].**

4.5 Inclusion and Exclusion criteria

4.5.1 Inclusion criteria

- HIV infected pregnant and non-pregnant women who had baseline (Pre-ART) and at least six months follow up data with complete hematological values (CBC and CD4).

4.5.2 Exclusion criteria

- HIV infected pregnant and non - pregnant women who were not eligible for ART

4.6 Study variables

4.6.1 Dependent variables

- Hematological parameters(CBC and CD4) of HIV infected pregnant and non - pregnant women

4.6.2 Independent variables

- Age, level of education, marital status, WHO clinical stage, gestational age, type of ART drug regime, and follow up time.

4.7 Sample size determination and Sampling

4.7.1 Sample size determination

The minimum required sample size is determined by using two population proportion formula.

Sample size calculation considers the following criteria:

Two-sided confidence level (1-alpha) ---------- 95%

Power (% chance of detecting) ---------- 80

Percentage exposure among control ---------- 30% **[40]**

Least extreme odds ratio to be detected ---------- 1.9

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

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

where;

n is the sample size estimate for one group

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

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

Calculated sample size for cases ----------154

Calculated sample size for control ----------154

Estimated non-response rate ----------10%

Ratio of control to cases----------1

Therefore the maximum sample size used:

Total cases ----------384

Total control ----------384

Total sample size ----------**768**

4.7.2 Sample technique

A total of 384 HIV infected pregnant women and 384 non pregnant women who were enrolled at Zewditu Memorial Hospital ART unit from September 1, 2008 to January 1, 2014 were included in the study using convenience sampling method.

4.8 Data collection and Measurement

4.8.1 Data Collection tools

A WHO standardized data extraction format was used to extract socio-demographic characteristics, clinical information and hematological parameters of the study participants from Zewditu Memorial Hospital ART log book and patient follow up cards at different time interval from the 0 month (baseline) and 6 month followi1ng initiation of ART.

4.8.2 Data collection procedure

Two ART nurses and three card room staffs together with the principal investigator were involved in data collection. Data collectors were trained for one day with the objective of standardizing the data collection instrument and providing them with basic skill of extracting the data both from the ART log book as well as patients follow up cards. Data on demographic, clinical including ART regimen type and hematological (WBC, CD4+ T cells, RBC count, Hgb, MCV, MCH, MCHC, and Platelets) values were carefully extracted from ART log book and patient follow up cards by using standardized data extraction form which was prepared based on the information from ART log book.

4.8.3 Quality Control and Quality Assurance

The hospital is a model ART center which has dedicated data management team and quality system to ensure quality of data. To ensure the quality of instrument performance, running of controls was done and preventive maintenance was performed according to standard operating procedures. The hematological and CD4 analyses were done by senior laboratory technician and technologists. Based on an annual onsite assessment performed in 2005 E.C. and 2006 E.C. as per WHO AFRO checklist, Zewditu Memorial Hospital Laboratory (ZMHL) scored 3 stars, which indicated that the laboratory had a good performance. ZMHL also participates in External Quality Assurance (EQA) program which all in all earned it the three stars.

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

4.9 Data Processing and Analysis

Data collected through a standardized questionnaire were entered into Excel spread sheet and transported into and analyzed by SPSS Version 20.0 software. Percentages were used to describe the proportions of the discrete variables. Mean change of different hematological values during follow up time from the base line were compared using paired t-test. Dependent variable frequencies, mean, standard deviation and range were calculated. P value < 0.05 was considered as statistically significant.

4.10 Ethical consideration

The study was approved by Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences, Addis Ababa University. Written letter had been obtained from the department before the actual work is started. In addition, ethical clearance was also obtained from Addis Ababa City Administration Health Bureau Ethical Reviewing Committee. Permission had been obtained from the ZMH administration. Full explanation about the purpose of the study was made to persons in charge of the health facility. All the information obtained from the ART center was coded to maintain confidentially throughout the study.

5 RESULTS

5.1 Demographic, clinical, and ART regimes

A total of 768 HIV infected women (384 pregnant with age mean 32 years and 384 non pregnant women (control) with age mean of 36 years) all taking ART at baseline and 6th months of follow up were included in this study. The majority of the study groups 166 (43.5%) were in the age range of 26-30 years and 143 (37.2%) were >36 years for the control group. At start of ART intake 123 (32%) HIV infected pregnant women were having gestation age less than 7 weeks, while the remaining 261 (68%) were in the range of 8 -17 weeks (Table 1).

Majority 292 (75.5%) of the pregnant women and 245 (64%) of controls were living with a sexual partner (married); whereas 92 (24.5%) pregnant women and 139 (36%) non pregnant women were living alone (never married, separated, divorced or widow). The proportion of pregnant women whose educational status was secondary and tertiary level among cases was 282 (73.4%), while smaller proportions of controls were seen in this group 227(59.1%). Almost similar proportions of employment status were seen in both groups (Table 1).

At the baseline 169(44%) pregnant women were classified as WHO HIV clinical stage I and 136 (35.4%) classified as Stage II for cases while controls were relatively in more advanced stages (29.7% stage I and 40.4% stage II). Regarding the eligible reasons to start ART, pregnant women started the treatment based on CD4+ T cell count < 350 cells/ul while for infected non pregnant women < 200 cells/ul. The most widely used ART regimen for cases and controls were 1c, AZT+3TC+NVP, 133(34.6%) vs 128 (33.6%) followed by 1a (30), d4T+3TC+NVP, 108 (27.9%) vs 126(32.5%) and 1b d4T+3TC+EFB, 51(13.0%) vs 67(17.2%) respectively (Table 1).

Table 1.Baseline demographic and clinical characteristics of HIV infected pregnant and non-pregnant women who started antiretroviral treatment at Zewditu Memorial Hospital ART clinic, Addis Ababa, Ethiopia (Study subject, n = 384) and (control, n = 384) from September 2008 to January 2014.

Type of Variables		**Study Subject (n= 384)**		**Control (n= 384)**	
		Frequency	**Percent**	**Frequency**	**Percent**
Age (Years)	≤ 20	13	3.4	13	3.1
	21 – 25	68	17.4	27	7.1
	26 – 30	166	43.5	121	31.8
	31 – 35	98	25.5	80	20.8
	>36	39	10.2	143	37.2
	Mean ± SD	32 ± 7.0		36 ± 4.0	
Marital Status	Married	292	75.5	245	64.0
	Never Married	80	20.8	78	20.3
	Divorced &Window	12	3.7	61	15.7
Educational Status	Non Educated	29	7.6	54	13.8
	Primary	73	18.8	103	26.8
	Secondary	221	58	171	44.6
	Tertiary	61	15.6	56	14.8
WHO clinical staging	I	169	44.0	114	29.7
	II	136	35.4	154	40.4
	III	69	18.0	89	23.2
	IV	10	2.6	27	6.7
Gestational age (Weeks)	≤ 7	123	32.0	-	-
	8 - 17	261	68.0		
Type of ART	1a (30)	108	27.9	126	32.5
	1b (30)	51	13.0	67	17.2
	1c	133	34.6	128	33.6
	1d	24	6.3	35	9.1
	1e	68	18.2	28	7.6

5.2 Baseline hematological parameters among study participants and controls enrolled at Zewditu Memorial Hospital ART clinic.

The overall base line mean (±SD) WBC count, RBC Count, Hb, HCT, MCV, MCH, MCHC, PLT and CD4 count were 5.7 ± 2.5 x 10^9/L, 4.07 ± 0.9 x 10^{12}/L,10.8 ± 1.1 gm/dL 29±5.1%,77 ± 14fL , 26 ± 6 pg, 28 ± 5.8gm/dl, 226 ± 93 x 10^9/L and 220 ± 129 cells/µl respectively for HIV infected pregnant women and 5.2 ± 2.3 x 10^9/L,4.09 ± 1.3x 10^{12}/L,12 ± 2.2 g/dL,34 ±10%, 84 ± 16fL, 29 ±9pg, 29 ± 9.7 gm/dl, 230 ± 106x10^9/L and 205 ±112 cells/µl, respectively for HIV infected non pregnant women. RBC Count, Hb, HCT, MCV, MCH,MCHC and PLT counts were statistically significantly lower, while CD4 count was significantly higher in HIV infected pregnant women compared to controls. There was no statistically significant difference between the two groups for WBC and 3 part differential counts (Table 2)

Table 2. Baseline Hematological parameters (Mean ± SD) in HIV infected pregnant and non-pregnant women at Zewditu Memorial Hospital ART clinic, Addis Ababa Ethiopia from September 2008 to January 2014.

Parameter	Pregnant Women (n=384)	Non pregnant Control (n=384)	P – Value
RBC (x10 ^{12}/L)	4.07 ± 0.9	4.09 ± 1.3	< 0.05
HB (gm/dL)	10.8 ± 1.1	12 ± 2.2	< 0.05
HCT (%)	29 ± 5.1	34 ±10	< 0.05
MCV (fL)	77 ± 14	84 ± 16	< 0.05
MCH (pg)	26 ±6	29 ±9	< 0.05
MCHC (gm/dL)	28 ± 5.8	29 ± 9.7	< 0.05
WBC (x10^9 /L)	5.7 ± 2.5	5.2 ± 2.3	> 0.05
Platelet (x10^9 /L)	226 ± 93	230 ± 106	< 0.05
Neutrophil (x10^9/L)	3.2 ±3.1	2.8 ± 3	> 0.05
Lymphocyte (x10^9/L)	1.9 ± 1.1	1.6 ± 1.6	> 0.05
MID Cells (x10^9 /L)	0.7 ± 0.6	0.5 ± 0.4	> 0.05
CD4 Count (cells/µL)	220 ±129	205 ±112	< 0.05

5.3 Hematological profile at 6th month after initiation of ART

Table 3 summarizes hematological profiles at 6th months of ART initiation. The mean (±SD) WBC count, RBC Count, Hb, HCT, MCV, MCH, MCHC, PLT and CD4 count were 6.1 ± 2.3 x 10^9/L,4.05 ± 0.9 x 10^{12}/L,10.4 ± 1.1gm/dL,28 ± 5.4%,75 ± 14fL, 27 ± 6.1pg, 28 ± 5.9 gm/dl, 224 ± 93x10^9/L and 258 ± 133cells/µl in HIV infected pregnant women. RBC parameters and CD4 count were significantly lower in pregnant compared to non-pregnant women. The respective values were RBC count 4.28 ± 1.3 x 10^{12}/L, Hb 13 ± 2 gm/dL, HCT 37 ± 9%, MCV 86 ± 16fL, MCH 28±10pg, MCHC 30 ± 9.7 gm/dL, CD4 + T cell 262 ± 129 cells/µL and platelet count 254 ± 10210^9/L). Whereas WBC counts did not statistically significantly differ between the two groups ($p > 0.05$).

Table 3. Hematological parameters (Mean ± SD) of HIV infected pregnant and non-pregnant women at 6 month initiation of ART at Zewditu Memorial Hospital ART Clinic, Addis Ababa, Ethiopia from September 2008 up to January 2014.

Parameter	Pregnant Women (n=384)	Non pregnant Control (n=384)	P – Value
RBC (x10 ^{12}/L)	4.05 ± 0.9	4.28 ± 1.3	< 0.05
HB (gm/dL)	10.4 ± 1.1	13 ± 2	< 0.05
HCT (%)	28 ± 5.4	37 ± 9	< 0.05
MCV (fL)	75 ± 14	86 ± 16	< 0.05
MCH (pg)	27 ± 6.1	29 ± 10	< 0.05
MCHC (gm/dL)	28 ± 5.9	30 ± 9.7	< 0.05
WBC (x10^9 /L)	6.1 ± 2.3	5.7 ± 2.9	> 0.05
Platelet (x10^9 /L)	224 ± 93	254 ± 102	< 0.05
Neutrophil (x10^9/L)	3.5 ± 3.2	2.8 ± 2.3	> 0.05
Lymphocyte (x10^9/L)	1.9 ± 1.4	2.1 ± 0.9	< 0.05
MID Cells (x10^9 /L)	0.7 ± 0.7	0.6 ± 0.4	< 0.05
CD4 Count (cells/µL)	258 ± 133	262 ± 129	< 0.05

5.4 Hematological parameters change between base line and 6 month follow up time

The mean change of hematological parameters at 6 month from the baseline were shown in Figure 1.The mean Hb, MCV, MCH and MCHC, and platelet counts decreased and RBC slightly increased from baseline to 6 month visit. The respective mean changes for Hb, RBC count, MCV, MCH, MCHC, and platelet count were, -0.4 gm/dl,*+0.02x10 ^{12} cells/l , *-2fl, *-+1pg, 1gm/dl and *-2x10^{9} cells/l on HIV positive pregnant women respectively (Figures 1.1and 1.2)

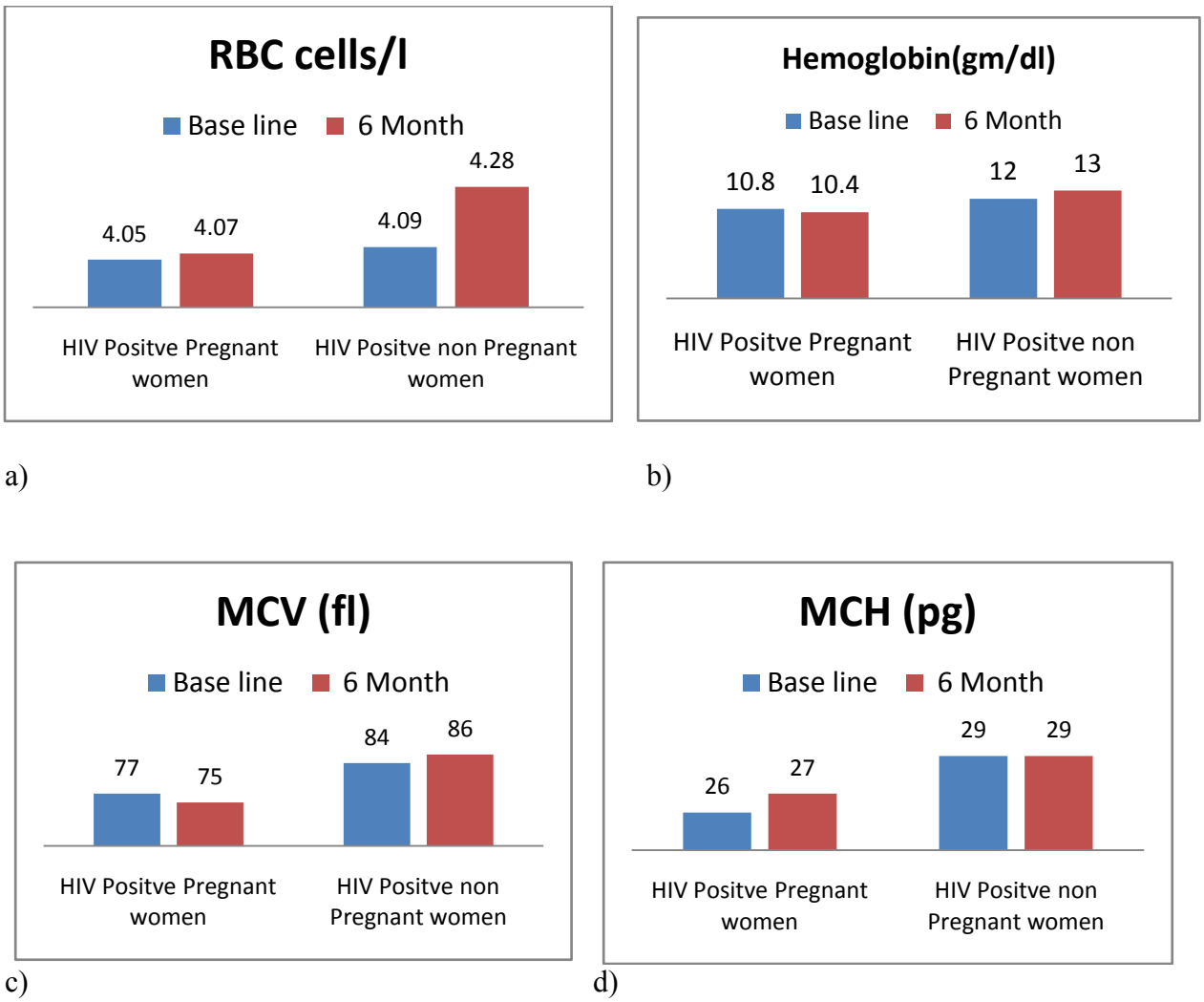
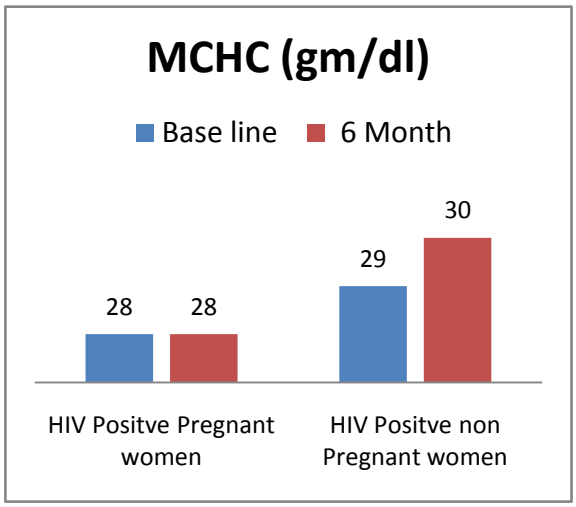
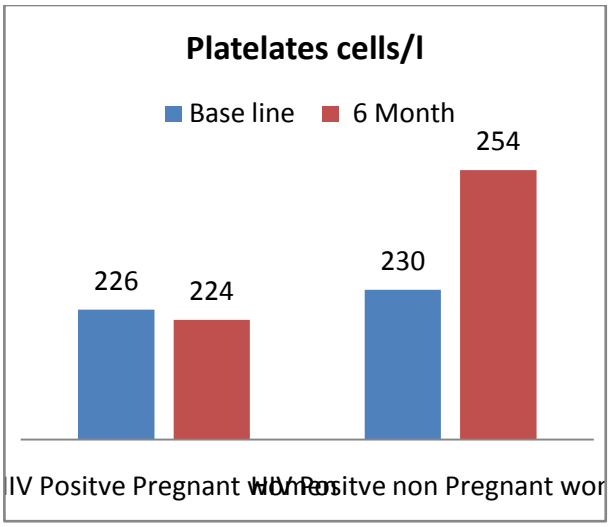


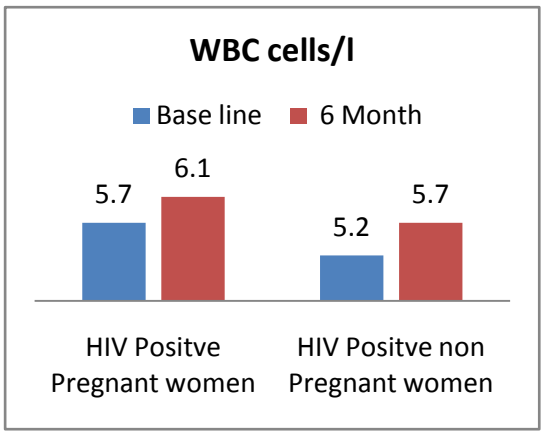
Figure 1 Hematological parameters (a-Hb,b-RBC count,c- MCV and d-MCH) of HIV positive pregnant and non-pregnant women at 0, and 6 months of follow up, at Zewuditu Memorial Hospital ART unit from September 2008 up to January 2014



a)



b)



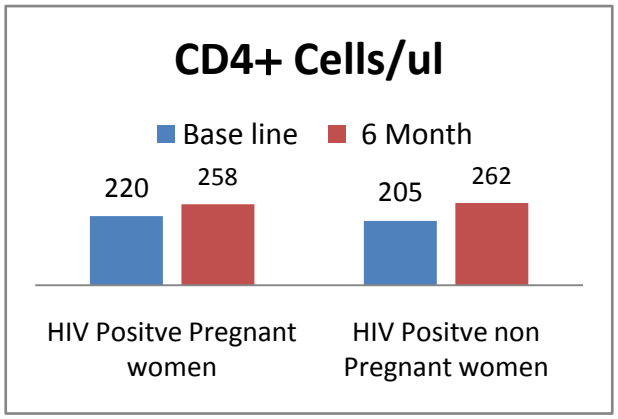


Figure 2. Hematological parameters (a-MCHC, b-Platelet count, c- WBC count and d-$CD4^+$ count) of HIV positive pregnant and non-pregnant women at 0, and 6 months of follow up, at Zewuditu Memorial Hospital ART unit from September 2008 up to January 2014.

(CD4 change: +38 vs +57; WBC change: +0.4vs +0.5)

In contrast for non-pregnant women mean Hb, RBC count, MCV, MCH and MCHC, and platelet from baseline to 6 month visit were increased by mean Hb, RBC count, MCV, MCHC, and platelet count of*+1gm/dl,*+0.19x10^{12}cells/l ,*+2fl,+1pg,*+1gm/dl and *+24x10^9 cells/l.For both group mean WBC count andCD4 count were higher by mean WBC count and CD4 count of *+0.4x10^9 cells/l and *+38 cells /µl for pregnant group respectively, while for the non-pregnant control group higher by mean WBC count and CD4 count of,*+0.5x10^9 cells/l and *+57 cells /µl for the pregnant study group, respectively (Figures 1.1and 1.2).

5.5 Hematological abnormality

Using the WHO criterion of hemoglobin less than 7.0 g/dl as indicative of severe anemia and platelet count less than 50,000/µl indicative of severe thrombocytopenia. At base line classified as leucopenic (20.7% vs 8.6%), thrombocytopenic (20.8% vs 38%), severely thrombocytopenic (0%, in both groups), anemic (49.5% vs 37%), CD4+ T lyphocytopenic(82.0% vs 50.3%) and sever CD4+ T lyphocytopenic(6% vs 8.6%), respectively for pregnant and non-pregnant women. After 6 month of ART initiation the prevalence of leucopenic (4.9% vs 5.8%), thombocytopenic, (18.8% vs 7.8%), severe thrombocytopenia (0% in both group), anemia (54.4 %vs18%), CD4+ T lyphocytopenic (67.0% vs 32.7%) and severe CD4+Tlyphocytopenia (0.5% vs 1.6%), respectively for pregnant and non-pregnant women (Table 4).

Table 4: Hematological disorders in HIV infected pregnant women (n= 384), and Control group women (n= 384) at base line and at 6 month initiation of ART at Zewditu Memorial Hospital ART clinic Addis Ababa Ethiopia from September 2008 up to January 2014

Hematological Abnormalities	**Pregnant**		**Non-pregnant (Control group)**	
	Base line	**6 Month**	**Base line**	**6 Month**
	n (%)	**n(%)**	**n(%)**	**n(%)**
Leucopenia	79(20.7)	19(4.9)	33(8.6)	22(5.8)
Anemia	21(11.1)	134(64.0)	124(87.3)	58(84.1)
Mild anemia	165(86.8)	75(36.0)	15(10.6)	6(8.7)
Moderate anemia	4(2.1)	0	3(2.1)	5(7.2)
Severe anemia	**190(49.5)**	**209(54.4)**	**142(37.0)**	**69(18.0)**
Thrombocytopenia	65(81.2)	57(79.2)	57(39.0)	8(26.7)
Mild Thrombocytopenia	15(18.8)	15(20.8)	89(61.0)	22(73.3)
Moderate Thrombocytopenia	0	0	0	0
Severe thrombocytopenia	**80(20.8)**	**72(18.8)**	**146(38.0)**	**30(7.8)**
CD4+ T lyphocytopenia	318(82.0)	266(67.0)	192(50.3)	125(32.7)
Severe CD4+ T lyphocytopenia	23(6.0)	2(0.5)	3(8.6)	6(1.6)
	341(88.8)	**268(69.8)**	**195(50.8)**	**131(34.1)**

Based on red blood cell morphologic classification of anemia, most of the anemic HIV pregnant women had Microcytic-hypochromic 45.7% vs 48.8%, type of anemia respectively, at baseline and 6 months of follow up. In contrast, the dominating morphologic type of anemia in HIV infected non pregnant women was of Normocytic hypoochromictype (50% vs 69.5%), respectively, at baseline and 6 months of follow up (Table 5).

Table 5. Distribution of Morphologic type of Anemia among HIV infected women at Zewditu Memorial Hospital ART clinic, Addis Ababa, Ethiopia from September 2008 up to January 2014.

Type of Anemia	**Case**		**Control**	
	Base line n(%)	**6 Month n (%)**	**Base line n (%)**	**6 Month n (%)**
Microcytic-hypochromic	87(45.7)	102(48.8)	37(26.1)	11(15.9)
Normocytic hypochromic	52(27.6)	54(25.8)	71(50)	48(69.5)
Normocytic normochromic	42(22)	52(24.9)	29(20.4)	8(11.2)
Dimorphic	9(4.7)	1(0.5)	5(3.5)	2(2.9)
Total	**190(49.5)**	**209(54.4)**	**142(37.0)**	**69(18.0)**

6 DISCUSSION

Both antiretroviral therapy and pregnancy cause dramatic changes on a wide variety of hematological parameters. The study included 384 ART taking pregnant women and 384 ART taking non pregnant women as controls to assess the effect of the double burden of ART and pregnancy on hematological parameters and immune status. The study revealed significantly lower RBC parameters in pregnant women compared to non-pregnant women (P .value <0.05). The findings are consistent with previous study which reported that the decreases in hemoglobin and red cell indices concentration are common findings during pregnancy and results from increased plasma volume combined poor iron intake **[13, 41].**

In the present study, reduced hemoglobin and hematocrit values were noted at 6 month of ART therapy compared to baseline in the pregnant group. Similar observation was noted in Jamaican pregnant women which showed a decline in hemoglobin concentration and hematocrit in HIV infected pregnant women **[41,42].**Others study conducted shown that decrease in hemoglobin concentration(from 9.05 from 9.05 ± 0.35 to 8.05 ±0.29) after 6 month follow up in Nigeria ART pregnant women **[33].**The decrease in hemoglobin concentration and HCT may be due to increase in plasma volume during pregnancy causing hemodilution, hormonal changes that increases fluid retention and iron deficiency **[43].**

Other studies also demonstrated significantly lower RBCs count, HB, HCT MCV, MCH and MCHC in pregnant women compared to non-pregnant women all taking ART. The findings are consistent common findings during pregnancy and results from increased plasma volume combined with poor iron intake **[39,44].**

In this study the leucocyte count was significantly higher compared to that of control and remained elevated throughout pregnancy at 6 month of ART therapy. This is consistent with the findings of other study **[45]** Neutrophil is higher in the studied group than the control group, but the difference did not reach to a statistically significant level. In this present study lymphocyte counts were similar while MID cell count was significantly lower in the pregnant group than in control. This is consistent with the observation of previous studies **[46].**

The finding of increased mean white blood cells at 6 month of therapy in pregnant women is also consistent with findings in Nigeria (from 6.42± 0.68 to 6.45 ± 0.20)**[3].**Similar study conducted in India on the physiological Changes in hematological parameters indicated that leukocytosis is

common during pregnancy **[41,47].** This may be as a result of the body building the immunity of the fetus and it is achieved by a state of selective immune tolerance, in the presence of a strong antimicrobial immunity. Similarly to the previous study reported that pregnancy leukocytosis, primarily related to increased circulation of neutrophils in the 6 month of therapy **[44, 48].**

Significant decreases in platelet count of ART pregnant women compared to ART non pregnant women (P value <0.05) in the first 6 month therapy is in agreement with other studies. Although platelet counts remain in the normal pregnant range in most women during uncomplicated pregnancies mean platelet counts of pregnant women may be slightly lower than in healthy non pregnant women. Platelet count does decrease during pregnancy. This is termed as ‘‘gestational thrombocytopenia.’’ It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance **[44].**

Anemia, leucopenia and thrombocytopenia were common findings in the present study. The prevalence of anemia in the present study was 54.7% in HIV infected pregnant women taking ART at 6 month therapy. HIV infection and pregnancy may lead to anemia in many ways, some of them are changes in cytokine production, decreased erythropoietin concentrations, opportunistic infectious agents **[49].** Hormonal changes during pregnancy cause a release of renin from the kidneys. The increase in plasma volume is relatively greater than the increase in red cell mass, which results in a fall in maternal Hb and HCT value causing physiological anemia **[41,50].**Another study was conducted in south Africa in university of KwaZulu Natal showed that the prevalence of thrombocytopenia was 5.3% during pregnancy and 1.2% 6 weeks after delivery **[35].**

Our study also reported a high rate of thrombocytopenia in pregnant women as compared to those who are non-pregnant but the mean platelet difference between the base line and 6 month follow up in both groups was not statistically significant (P>0.05).This study suggests that platelet count does decrease during pregnancy. This is termed as ‘‘gestational thrombocytopenia.’’ It is partly due to hemodilution and partly due to increased platelet activation and accelerated clearance **[41,44].**

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 could cause a change in the function **[51].**

CD4 cell count were higher in pregnant women taking ART than the non-pregnant control group. This may be consistent with the 2010 WHO adult ART guidelines which recommend and prioritize starting ART in all HIV Positive pregnant women with CD4 counts ≤350 cells/ul, as compared to HIV positive non pregnant women who start ART when the CD4 count drops below 200 cells/ ul. This showed that non-pregnant HIV infected women are enrolled in the treatment program at an advanced stage as compared to the pregnant women. This is because pregnancy by itself is considered to be a physiologically immunocompromised state. Any alteration in any parameter of the immune system can affect the health of the pregnant woman as well as outcome of pregnancy. This fact has been established in many collaborative studies and therefore formed the basis of the recommendation for the HAART prophylaxis in HIV positive pregnant women, irrespective of the CD4 count levels **[20,52].**

The current WHO guidelines recommended all pregnant and breastfeeding women regardless of CD4 count during the period of risk of mother to child transmission. This continuation of life-long ART is recommended, particularly in generalized epidemic settings with high rates of fertility, and limited access to CD4 testing. The guideline recommends two options: the first option is providing lifelong ART to all pregnant and breastfeeding women living with HIV regardless of CD4 count or clinical stage. The second option is providing ARV drugs for pregnant and breast feeding women with HIV during the mother to child transmission risk period and then continuing lifelong ART for those women eligible for treatment for their own health. In settings that are not implementing lifelong ART for all pregnant and breastfeeding women living with HIV **[12].**

In summary, this study was conducted having an objective of identifying changes in hematological parameter among pregnant women as compared to non-pregnant women during the first 6 month of ART in Zewditu Memorial Hospital. As shown in this study, pregnant mothers on ART have significantly higher rates of anemia, thrombocytopenia, and CD4+ T lymphocytopenia but low rate of leucopenia as compared to non-pregnant controls.

7 STRENGTH AND LIMITATION OF THE STUDY

7.1 Strength of the study

- The study is carried out in the first and model ART center of the country with ample experience in ART service.
- ART data managed by data management team dedicated to the center
- The hospital participates in EQA and earns three stars based on WHO-Afro checklist

7.2 Limitation of the study

- The study is conducted based on secondary data which might have incomplete and biased information.
- The study was restricted to 6 month period as the subsequent visits values will be influenced by delivery and post-delivery situations of the women.
- This study was facility based study; thus, was not representing a particular population except the clients who visited the health facility

8 CONCLUSION AND RECOMMENDATION

8.1 Conclusion:

In conclusion, there were significant decrease in RBC count, Hb, HCT, Red cell indices, MID cells, lymphocyte, platelet count and rate of recovery in CD4 count of ART taking pregnant women relative to ART taking non pregnant women with 6 month of ART period. Whereas there were significant increment in WBC count, and neutrophil of both groups within 6 month ART period. The commonest hematological abnormalities in the study participants were anemia and thrombocytopenia. Prevalence of anemia and thrombocytopenia were commonest finding in pregnant women than non-pregnant women after 6 months of ART therapy. Anemia in both groups was of mild and moderate type.

8.2 Recommendations

- Hematological tests should be performed regularly to follow the effectiveness of the treatment and take appropriate measures.
- The laboratory results should be handled and appropriately recorded on ART log book and antenatal follow up card.
- All hematological parameters (complete blood count) should be routinely performed during pregnancy instead of screening for hemoglobin only.
- Anemic HIV infected pregnant women's red blood cell blood picture (peripheral blood morphology) should be reviewed to characterize the type of anemia and administer the correct therapy.
- A large community based study should be done to determine the prevalence and associated factors of anemia and thrombocytopenia in the general population of HIV infected pregnant women.
- In our study thrombocytopenia is found in HIV infected pregnant women during the first 6 month of therapy .Therefore, platelet count should be routinely performed during antenatal visit to differentiate the cause of thrombocytopenia and to avoid excessive bleeding during or after childbirth early by diagnosing timely.

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10 Annexes

10.1 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 parameter of HIV infected pregnant women 6 month after receiving Active Antiretroviral Therapy. I am going to review your hospital ART logbook and ART pregnant women and non pregnant women follow up card to analyze the Hematological profile of HIV infected pregnant women and non pregnant women at 6 month after receiving 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 and 6months 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 JenberuAschalew

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10.2 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 is provided by our ART unit greatly improves the life of our HIV infected pregnant women who start ART and management of ART which further lead to improve the survival of HIV infected pregnant women 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_____ Signature ____

Supervisor Name _____ Date_____ Signature _____

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

Jenberu Aschalew

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10.3 Annex III – Data extraction form

Identification Code:		Date of data collection:	
Data collector name:		Supervisor:	
Pregnant: ☐		Non pregnant: ☐	
Characteristics	Tick	Characteristics	Tick
Age		Level of education	
≤ 20		Non – Educated	
21 – 25		Primary	
26 – 30		Secondary	
31 – 35		Tertiary	
>36			
Marital status		ART Regimen	
Single		1a(30)(d4T+ 3TC +NVP)	
Married		1b(30))(d4T+3TC +EVP)	
Divorced/Windowed		1c(AZT+3TC+NVP)	
WHO Staging		1d)(AZT+3TC +EVP)	
I		1e)(TDF+3TC +EVP)	
II		1f (TDF+3TC +NVP)	
III		Others specific:_____	
IV			
Gestational age ,Weeks			
≤7weeks			
8 -17 weeks			

Section 2: Hematological parameter at the start of ART (Baseline) and at 6 month of therapy

Identification Code:			Date of data collection:	
Data collector name:			Supervisor:	
Hematological Parameters	Pregnant women		ART Non pregnant women	
	0 Month	6 Month	0 Month	6 Month
RBC (x10 ^{12}/l)				
HB (gm/dl)				
HCT (%)				
MCV(fl)				
MCH(Pg)				
MCHC(gm/dl)				
WBC(X10^{9} /l)				
Neutrophil(X10^{9}/l)				
Lymphocyte(X10^{9}/l)				
MID Cells (X10^{9} /l)				
Platelet (X10^{9} /l				
CD4 count (cells/µl)				

10.4 Annex IIII: Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate: Jenberu Aschalew (BSc)

Signature_____

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

Date of submission_____/_____/_____

This thesis has been submitted with my approval as university advisor.

Name of advisor: Dr. Aster Tsegaye (MSc, PhD)

Signature _____

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

Date of submission_____/_____/_____