terAddis Ababa University

Faculty of Medicine

Department of Microbiology, Immunology and Parasitology

MSc research project Submitted to Addis Ababa University Faculty of Medicine Department of Microbiology, Immunology and Parasitology as a Partial Fulfilment of the Requirement of Master of Science in Medical Microbiology.

BY

Moges Derbe (BSc)

ADDIS ABABA

July 2012

Addis Ababa University

Faculty of Medicine

Department of Microbiology, Immunology and Parasitology

Immunological response among Adult individuals on ART Therapy before and after therapy started for the last 5 years (2006–2010) at Zewuditu hospital Addis Ababa, Ethiopia.

Adviser(s):

Professor Dr. DP Monga, Addis Ababa University Faculty of Medicine Department of Microbiology, Immunology and Parasitology

ADDIS ABABA

July 2012

Abstract

Background: *HIV is isolated in 1983, human immunodeficiency Virus (HIV), the agent that causes acquired immune deficiency syndrome (AIDS), is classified as members of the lentivirus subfamily of retroviruses. There are two main types of HIV: HIV type 1(HIV-1): the most prevalent throughout the world. HIV type 2 (HIV-2) is prevalent in West Africa. They both cause ADIS and the routes of transmission are the same. However, HIV-2 causes AIDS much more slowly than HIV-1. Although HAART is known to profoundly suppress viral replication, it increases CD4 cell count and delays disease progression and death; patients on Highly Active Antiretroviral Therapy (HAART) commonly suffer from side effects of the drug. Each antiretroviral drug is associated with specific adverse effects. Several studies in developed countries have shown that AZT alone and AZT based HAART regimen is associated with significant reduction of hemoglobin (Hb) level and neutrophil number. The impact of its immunological recovery rate / including at what time to start on Treatment is not well known in Ethiopian context. Hence this research was conducted to determine its immunological responses at different interval and at the start.*

Objective: *The aim of this retrospective cohort study was to describe immunological response among HIV-infected individuals receiving highly active antiretroviral therapy (HAART) with long-term follow-up.*

Method: *A Cohort retrospective study design was conducted to assess immunological (the CD_4^+ recovery) among HIV-infected individuals receiving highly active antiretroviral therapy (HAART) with long-term follow-up. Antiretroviral-naive patients with symptomatic HIV disease at baseline (before ART) and after 6 and 9 and 12 months of ART will be collected from records and after start ART in Zewditu Hospital, Addis Ababa, Ethiopia.*

Result: *A total of 887 HIV positive patients in this research; Out of these 472 (53.2%) were female and 415 (46.8%) male patients. The ratio of male to females was almost 1:2. None of them have any opportunistic infection during the time of follow up. The mean age of the study group was 36.76 (17-76). The mean baseline CD4 was 81.40; the mean CD4 count at the 6^{th}, 9^{th} and 12^{th} month was 191.65, 284 and 331 respectively. There was a good immune recovery at the 6^{th} month of therapy from the baseline mean CD_4^+ T cell count of 81 cells /«l to 191.65 cells /«l, which was statically highly significant ($p<0.0001$). This first remarkable rise was continued in the achieving in the mean $CD4^+$ count of 284 cells/«l at the 9^{th} month of visit. Followed by relatively steady lower increase and approaching stable CD4+ T cell count and 12^{th} months of visit.*

Conclusion and Recommendations *In conclusion, in our study, although good CD4 cells recovery in response to ART was documented in more than 81% of follow-up cases, HIV-positive patients were enrolled in ART program at decreased CD4 cells levels. As there is poor recovery of CD4 cell when the start >200 than when they start ART at CD4 count >200 CD4 cell. Therefore, interventions need to be designed to promote early HIV testing and early enrollment of HIV infected individuals into ART services. ART has considerably improved the immune recovery. We strongly recommend underline the need of anti-retroviral therapy in HIV infected patients for immune reconstitution should be started as early as possible. The differential recovery rate between those with base line CD4+ T cell count below 50cells/«l and above 500cells/«l needs further investigation.*

KEYWORDS*:* ***Immunological response, CD^+_4 HIV, Highly Active Antiretroviral Therapy, Zidovudine***

Acknowledgements

First, we would like to Addis Ababa University for giving me this opportunity. Also, I would like to express my express gratitude to my advisor Professor Dr. DP Monga, Addis Ababa University Faculty of Medicine Department of Microbiology, Immunology and Parasitology for the long patience he has shown in checking this paper, the constant guidance and advices given during the conduct of the study.

My special thanks also go to the staff members of Zewuditu Hospital record officers for their constant insight, encouragement, and in providing as necessary materials, references in implementing my paper.

I would like to extend our thanks Hawassa University College of Medicine and Health Sciences, in paying my salary and all other my expenses during my study time. And I would like thank all who helped us in our way to do this study.

Last but not least, we would like to thank all study participants.

Table of contents

Contents **page**

Abbreviations

AAU - Addis Ababa University

Ab - Antibody

Ag - Antigen

AIDs- Acquired immune deficiency syndrome

ART - Anti Retroviral therapy

ARVs - Antiretroviral drugs

CDC - Center for Disease Control

CLD - Chronic liver disease

CVR - Cardio-vascular risk

DMIP - Department of Microbiology, Immunology and Parasitology

ELISA - Enzyme linked immune sorbent assay

FoM - Faculty of Medicine

HAART- highly active antiretroviral therapy

HDL-c- high-density lipoprotein cholesterol

HIV- Human immune deficiency virus

LDL-c- low-density lipoprotein cholesterol

STI- Sexually transmitted infections

TG- triglycerides

TC - total cholesterol

U.S- United States

List of Tables

Chapter– One

Introduction

1.1. Background Information

Human immunodeficiency Virus (HIV), the agent that causes acquired immune deficiency syndrome (AIDS), is classified as members of the lentivirus subfamily of retroviruses. It is isolated in 1983. There are two main types of HIV: HIV type 1(HIV-1): the most prevalent throughout the world. HIV type 2 (HIV-2) is prevalent in West Africa. They both cause ADIS and the routes of transmission are the same. However, HIV-2 causes AIDS much more slowly than HIV-1. (Seoane E, et.al 2008)

Acquired Immunodeficiency Syndrome (AIDS) is one of the most destructive epidemics in the history of mankind. In Ethiopia the adult prevalence of HIV was estimated to be 2.2% in 2008. The total number of People Living with HIV/AIDS (PLHIV) in the same period was estimated to be 1,037,267 adults and 68,136 of them were children. Furthermore the number of deaths due to AIDS for the same period was estimated to be 58,290 for adults and 9,284 among children (UNAIDS, December 2007 Report).

The goals of treatment with antiretroviral drugs are to inhibit viral replication while minimizing toxicities and side effects associated with the available drugs. The inhibition of virus replication permits restoration of the immune system (suppression of HIV replication, as reflected in plasma HIV concentration, to as low as possible and for as long as possible, the preservation or enhancement of the immune function (CD4 restoration), thereby preventing or delaying the clinical progression of HIV disease. Viral eradication from the host genome is not achievable, thus a cure for HIV is not yet possible. By using highly active antiretroviral therapy (HAART), it is possible to promote growth in children and prolong the survival of all HIV infected patients, reduce their morbidity and improve their quality of life. (Ugandan Antiretroviral Treatment and Care Guidelines for Adults, Adolescents, and Children. 2nd Edition – January 2008).

Enumeration of CD_4^+ T cell count has been useful to initiate and monitor therapy in HIV infected individuals taking potent antiretroviral therapy (ART). The CD_4^+ T cell count recovery shows high variability among patients. (WHO Regional Office for South-East Asia New Delhi, Laboratory Guidelines for enumerating CD4 T Lymphocytes in the context of HIV/AIDS June 2007). The CD4 cell count response to ART varies widely, but a poor CD4

response is rarely an indication for modifying a virologically suppressive ARV regimen. In patients with consistently suppressed viral loads who have already experienced ART-related immune reconstitution, the CD4 cell count provides limited information, and frequent testing may cause unnecessary anxiety in patients with clinically inconsequential fluctuations. Thus, for the patient on a suppressive regimen whose CD4 cell count has increased well above the threshold for opportunistic infection risk, the CD4 count can be measured less frequently than the viral load. In such patients, CD4 count may be monitored every 6 to 12 months, unless there are changes in the patient's clinical status, such as new HIV-associated clinical symptoms or initiation of treatment with interferon, corticosteroids, or anti-neoplastic agent. (Federal HIV/AIDS Prevention and Control Office Federal Ministry of Health July 2007 Guidelines for implementation of the antiretroviral therapy programme in Ethiopia)

HAART may be defined as therapy which is potent enough to suppress HIV viraemia to undetectable levels (<50 copies/mL), as measured by the most sensitive assay available, and which is durable in its virologic effect. HAART conventionally includes three or more drugs from at least two classes. However, as long as there is full and durable suppression of viral load, any regimen should be regarded as HAART. On the other hand, known sub optimal regimens, e.g. monotherpy, double nucleoside, or certain triple nucleoside combinations are not HAART and are contraindicated in HIV disease. (Amolo-Okero F. WHO; 2003)

The Principles of ART Antiretroviral therapy is part of comprehensive HIV care. The guiding principles of good ART include: Not to start ART too soon (when CD4 cell count is close to normal) or too late (when the immune system is irreversibly damaged), Efficacy of the chosen drug regimens, Freedom from serious adverse effects, Ease of administration including no food restrictions, Affordability and availability of drugs and drug combinations, Ongoing support of the patient to maintain adherence.(Federal HIV/AIDS Prevention and Control Office Federal Ministry of Health July 2007 Guidelines for implementation of the antiretroviral therapy programme in Ethiopia)

The Limitations of ART are not only a cure for HIV, but they are expensive; require an adequate infrastructure and knowledgeable health care workers. Training of health care personnel in the use of ARVs is critical to safe and effective use of these drugs. Even when all these are in place, ART has its own limitations in several ways this includes: Drug interactions and drug resistance may decrease the potency of these drugs, patients on ART

may develop adverse drug reactions, the HIV drugs are still expensive even though their prices have significantly come down, patients have to take at least 95% of their pills in order to respond well (adherence is key to successful therapy), the medications have to be taken for life. At present, eradication of HIV in the body is not possible and some patients may not respond (benefit) to treatment and continue to progress with their HIV disease in spite of doing everything right. However, when properly used by both patients and health care providers they are associated with excellent quality of life. (Ugandan Antiretroviral Treatment and Care Guidelines for Adults, Adolescents, and Children. 2nd Edition – January 2008)

Antiretroviral therapy in the developed world has resulted in substantial reductions in HIV-associated morbidity and mortality, changing an HIV diagnosis from a likely death sentence into a manageable chronic infection.

Currently, over 25 antiretroviral drugs and several fixed-doses drug combinations are available in most developed countries.

Individual agents target many of the critical steps in the HIV replication cycle—entry, reverse transcription, integration, and proteolytic processing. Newer regimens offer greater convenience and less toxicity than ones previously used and emerging data suggest that antiretroviral therapy should be initiated earlier during the natural history of HIV infection than was previously recommended. Therefore in order to see whether these treatments are effective or to know their side effect laboratory monitoring is important. (Ugandan Antiretroviral Treatment and Care Guidelines for Adults, Adolescents, and Children. 2nd Edition – January 2008)

Laboratory guidelines for monitoring ART

Basic laboratory tests are important monitoring of immunological response, toxicity & other treatment response of antiretroviral therapy**.** Certain laboratory investigations are recommended as the absolute minimum to manage patients on ART. These should either be available on site or by transportation of specimens to a local reference laboratory. Such tests are needed to identify potential toxic reactions e.g. anemia due to ZDV and then to trigger changes in drug regimes according to recommended protocols; or as adjuncts to monitoring the effectiveness of ART. Increases in total lymphocyte counts are reasonable, though imprecise reflections of immune response to ART (Ugandan Antiretroviral Treatment and

Care Guidelines for Adults, Adolescents, and Children. 2nd Edition – January 2008). Table 1 summarizes the recommended investigations for ART monitoring.

Other tests may be indicated based on the suspicion of a drug toxicity or clinical disease progression. Sometimes it may even be better to refer the patient to a better-equipped facility for more advanced evaluation.

For a patient who has been on ART for **at least six months or a year,** immunologic failure can be defined as;

- A fall in CD4 counts of more than 50% on two or more occasions from the on treatment peak value or
- A return to, or below, the pre-therapy baseline or
- Persistent CD4 levels below100 cells/mm3.

Table 1. The recommended investigations for ART monitoring

	Tests	**Level available**	**Objective**	**Frequency**
Absolute minimum tests	HIV antibody test	All levels	Diagnose HIV and initiate ART	Once before ART
	Haemoglobin or hematocrit	All levels	Monitor degree of anaemia – if severe transfuse before ART or use d4T instead of ZDV	When indicated or if on AZT, at 4, 8 & 12 weeks and thereafter when indicated
Basic recommended tests	Total WBC + differential	All levels	Monitoring neutropenic side effects	6-12 monthly & when indicated
	LFTs: alanine or aspartate aminotransferases	District hospitals	Monitor hepatitis co-infection and hepatotoxicity	When indicated. For women who start ART with CD4 250-350, that include NVP 2, 4, 8, 12 wks
	Serum creatinine and/or blood urea	District hospitals	Monitor renal function	When indicated. For pts on TDF, before start and every 6 months
	Serum glucose	District hospitals	Monitor hyperglycaemia in patients on Protease Inhibitors	When indicated
	Pregnancy test	District hospitals	Change therapy to appropriate regimen	When indicated
Desirable tests	Bilirubin	District hospitals	Monitor hepatitis co-infection and hepatotoxicity	When indicated

	Serum lipids	Referral hospitals	Monitoring hyperlipideamia for those on Protease Inhibitors	When indicated
	CD4 cell count	District or Referral hospitals	Monitoring immune response to therapy	6 monthly or when suspect failure
	Serum lactate	Referral Hospitals	Diagnosing lactic acidosis when on NRTI e.g. d4T or ddI	When symptoms suggest lactic acidosis
Optional tests	Viral load	Referral Hospitals & Research Centres	Monitoring viral response to therapy & diagnosing HIV in children <18 months	Every 12 months or when suspect failure

1.2 Statement of the problem

ART results in improvement in clinical status, immunity recovery and brings about effective reversal of the clinical stage in patients with symptomatic disease. However, the value of monitoring the efficacy of ART, defining ART failure and determining when to switch ART is less clear. Studies are urgently needed to address the use of clinical criteria (clinical stage on treatment) in deciding when to switch ART in the absence of CD4 cell counts or viral load testing.

The optimum time to commence ART is before patients become unwell or present with their first opportunistic infection. Immunological monitoring (CD4 testing) is the ideal way to approach this situation. A baseline CD4 cell count not only guides the decision on when to initiate ART but is also essential if CD4 counts are to be used to monitor ART.

Enumeration of CD_4^+ T cell count has been useful to initiate and monitor therapy in HIV infected individuals taking potent antiretroviral therapy (ART).

The first six months on ART are critical. Clinical and immunological improvement should be manifested but are not always apparent and drug toxicities may emerge. Some patients fail to respond as expected or may even exhibit clinical deterioration initially. These issues combine to present specific challenges for simplified clinical management. Complications in the first few weeks following the initiation of ART are seen most commonly when therapy is started in patients with severe immunodeficiency. The apparent failure of a patient with advanced HIV disease to improve initially does not necessarily reflect a poor response to ART. It takes time for HIV viral replication to be controlled by ART and for the patient's immune system to strengthen. It also takes time for reversal of the catabolism associated with HIV infection, particularly in patients with significant HIV-associated wasting. Additionally, as a patient with advanced disease recovers immune function, exacerbation of previously subclinical coexisting infections (e.g. tuberculosis) may occur, resulting in an apparent worsening of disease. This is not attributable to failure of the therapy but to its success and the resulting immune reconstitution. Such symptoms might be interpreted as an initially poor response to ART. It is important to allow sufficient time on therapy before judging effectiveness and to consider the possibility of the immune reconstitution inflammatory syndrome (IRIS) in patients with worsening disease in the first few months of ART. In such cases, the switching of ART would be inappropriate.

In most patients, CD4 cell counts rise with the initiation of therapy and immune recovery. This may continue for many years into effective therapy, although this may be blunted if the baseline CD4 count is very low. However, even patients with CD4 counts below10 cells/mm^3 can achieve an effective CD4 recovery, given sufficient time after the initiation of ART. Some patients may never have CD4 counts that exceed 200cells/mm^3 and thus never leave the zone of severe immunosuppression. In those who achieve a substantial peak response, a subsequent progressive decline in CD4 counts in the absence of intercurrent illness indicates immunological failure. The baseline CD4 count and the trend of the CD4 response assessed by regular six monthly CD4 counts are needed to best characterize and define immunological failure. In a minority of patients with advanced disease and low CD4 counts when therapy is initiated, the CD4 counts may not rise or may fall slightly, even with clinical improvement.

The immune reconstitution inflammatory syndrome (IRIS) is a spectrum of clinical signs and symptoms resulting from the restored ability to mount an inflammatory response associated with immune recovery. It can present with the signs and symptoms of a previously subclinical and unrecognized opportunistic infection, as a paradoxical worsening of treatment response several weeks into therapy, or as an autoimmune disease such as Graves disease (hyperthyroidism) in the context of immune recovery on ART. Typically, IRIS occurs within two to twelve weeks of the initiation of ART, although it may present later. The incidence of IRIS is estimated to be 10% among all patients initiating ART and up to 25% among patients initiating ART with a CD4 cell count below50 cells/mm3. However, the clinical syndromes associated with IRIS in resource limited settings have been relatively poorly described and it is not known whether there are any important regional variations in the clinical spectrum.
Haematological toxicity could occur due to drug-induced bone marrow suppression, most commonly seen with AZT (anaemia, neutropenia).

The value of immunological monitoring in defining ART failure largely depends on having a baseline CD4 count before commencing ART and on having longitudinal CD4 measurements on ART. One-off (spot) CD4 counts on ART are difficult to interpret when making decisions about treatment success or failure. CD4 count as a sign of immunological treatment failure, the CD4 cell count remains the strongest predictor of HIV-related complications; even after the initiation of therapy.
The baseline pretreatment value is informative: lower CD4 counts are associated with smaller and slower improvements in counts. However, precise thresholds that define treatment failure

in patients starting at various CD4 levels are not yet established. As a general rule, new and progressive severe immunodeficiency as demonstrated by declining longitudinal CD4 cell counts should trigger a switch in therapy.

Patients starting with low CD4 counts may demonstrate slow recovery, but persistent levels below100 cells/mm3 represent significant risk for HIV disease progression. Caveats to be noted are that intercurrent infections can result in transient CD4 count decreases, and that, with relatively infrequent monitoring (e.g. every six months), the true peak of the CD4 cell count may be missed. As a general principle, intercurrent infections should be managed, time should be allowed for recovery and the CD4 cell count should be measured before ART is switched.

Plasma viral load as an indicator of treatment failure, although viral load testing is not yet widely available, it is a sensitive and informative way to identify treatment failure. Therefore diagnosing treatment failure based on clinical or CD4 criteria alone will provide a greater opportunity for the selection of drug resistance mutations before regimen change and may compromise particularly the NRTI component of the second-line regimen through increasing class-wide drug resistance. This provides another strong argument for moving towards the wider availability of plasma viral load testing in resource-constrained settings. In particular, simple point-of-care assays are needed which identify, qualitatively or semi quantitatively, viral load thresholds that inform clinical management decisions.

Viral load testing is already available in some centers and programmes. However, the viral load threshold triggering a switch in ART is not defined, but according to some literatures
Virological failure is defined as plasma HIV-1 RNA level above 10,000 copies/ml in a person who has been on a regimen for more than six months and in whom drug adherence is determined to be sufficient. This level has been chosen on the basis of the association of viral load levels greater than 10 000 copies/ml with subsequent clinical progression and appreciable CD4 cell count decline. Virological success is defined as a plasma HIV-1 RNA level below the limit of detection of the assay being used (e.g. values below 400 or below 50 copies/ml after six months of treatment).

Clinical and laboratory monitoring of HIV-infected patients serves two purposes. Firstly, for patients under care who are not yet eligible for ART, regular monitoring is essential for the identification of the point at which they become eligible for ART.

Secondly, once patients have been initiated on ART, regular monitoring is necessary to assess efficacy, manage side-effects and identify treatment failure. Regular monitoring is also essential for reinforcing ARV adherence, the most critical parameter in the success of ART programmes.

For patients who are to be initiated on AZT-containing regimens, haemoglobin should be measured before initiation and at weeks 4, 8 and 12 on therapy.

Immunological failure is measured by the level of CD4 cells. The CD4 cell count remains the strongest predictor of HIV-related complications, even after the initiation of therapy.

Patients starting with low CD4 counts may demonstrate slow recovery, but persistent levels below100 cells/mm3 represent significant risk for HIV disease progression.

Caveats to be noted are that intercurrent infections can result in transient CD4 count decreases, and that, with relatively infrequent monitoring (e.g. every six months), the true peak of the CD4 cell count may be missed. As a general principle, intercurrent infections should be managed and time allowed for recovery before the CD4 cell count is done to guide on the need to switch ART.

More over some patients may not respond (benefit) to treatment and continue to progress with their HIV disease in spite of doing everything right and there could be exaggerated immune response which may lead to autoimmune disease

1.3 Significance of the study

Patients on ART need close monitoring to assess their adherence to the prescribed regimen, tolerance and side effects of the medications and efficacy of the treatment.

Once someone starts on ART a schedule for follow-up and monitoring should be drawn up. It usually includes a first visit two weeks or earlier after initiation (which may be useful to also evaluate and reinforce adherence to ART), then monthly for 6 months and thereafter every three months. Monthly visits should be combined with those of drug dispensing, as they provide useful opportunities to reinforce adherence. However, after 6 months, the drug dispensing visits may not correspond with those for clinical follow-up (3)

Use of highly active antiretroviral therapy (HAAT) has been linked to dyslipidemia and increased risk of cardiovascular disease (CVD) as well as hepato toxicity in HIV-infected patients and anemia, neutropenia in industrialized countries. The effects of HAART on its Immunological response rate and hematological disorders among sub-Saharan Africans, for whom access to antiretroviral therapy is expanding, remain largely unknown. (Seoane E, et.al 2008 & Buchacz K, et.al 2008)

Therefore this study will help to provide information on ART at what time it could show good Immunological responses as well as its other effects and the impact of HAART on the hematological profile of Ethiopian HIV/AIDS patients /which still not known/ and at what time should start ART. Hence this research was conducted to determine the magnitude of hematological abnormalities and its immunological responses at the start and at different interval.

It can also use as information by other bodies that are responsible for promotion of health activities; individuals who use ART and can be used as base line information for further studies.

1.4 Literature Review

Highly active antiretroviral therapy confers several benefits, including reduction in viral load & longevity in HIV positive patients. However, Metabolic and morphological complications have been increasingly reported among patients in the advanced industrialized countries receiving chronic HAART up to 10-20 years (Seoane E, et al 2008 & Buchacz K, et al 2008).

Although 25.8 million people are living with HIV/AIDS in Sub Saharan Africa, few studies tried to assess the safety and efficacy of HAART. In one multi centred study conducted in Uganda, Kenya and Zambia 12% of patients on AZT-based HAART regimen switched drug because of drug related severe anemia or GI toxicity (Amoroso A, et al 2007).

A research carried on 1281 HIV-infected patients initiating HAART were enrolled in the Anti PROtease (APROCO) cohort to investigated determinants of increase in CD4 count using longitudinal mixed models in patients who maintained a plasma HIV RNA <500 HIV-1 RNA copies/mL. Mean estimated increases in CD4 count in patients with persistent virological response were 29.9 cells/muL/ month before month 4, 64 cells/microL/month between months 4 and 36 (Le Moing V, et .al 2007).

A related study which is done to examine the long-term impact of adherence on virologic, immunologic, and dual response stratified by type of HAART regimen in treatment-naive patients starting HAART in British Columbia, Canada; and to assess the degree of virologic and immunologic response associated with emergence of drug resistance, progression to AIDS, and mortality and found to be patient responses was 394 (44.9%) for CD4+/pVL+ (best), 350 (39.9%) for CD4-/pVL+ or CD4+/pVL- (incomplete) and 134 (15.3%) for CD4-/pVL- (worst). They found a positive correlation between adherence and virologic and immunologic responses ($P < 0.01$). Having worst compared with best response (reference group) was associated with higher odds of mortality (odds ratio: 6.09; 95% confidence interval: 2.57-14.42) and emergence of drug resistance (odds ratio: 10.56; 95% confidence interval: 5.93-18.81) even after adjusting for adherence and HAART regimen. (De Jesus E, Herrera G et al. Clinical Infectious Disease, 2004; 39:1038-46)

While ART significantly decreases mortality, the latter is higher in the first six months than during the subsequent time on therapy, particularly when patients start with stage 4 clinical

events, severe immunosuppression and very low CD4 counts. The ART-LINC collaboration (18 treatment programmes in Africa, Asia and South America) recorded a 4% mortality rate in 2725 patients under active follow-up six months after starting therapy but noted that mortality fell to 2% in the subsequent six months of therapy. The DART trial reported that 39 of 62 deaths (63%) in a cohort of over 1000 adults followed for two years occurred in the first six months of therapy (WHO Antiretroviral Therapy for HIV infection in adults and adolescents: Recommendations for a public health approach 2006 revision)

A cohort study in the Médecins Sans Frontières (MSF), of over 6000 patients treated with a generic FDC of d4T + 3TC + NVP, almost 70% of deaths occurred during the three first months after ART initiation. This greater risk of death is seen especially in patients with disseminated TB (and other severe OIs) and a pre-ART CD4 cell count <50 cells/mm3. (WHO: Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach. 2006).

Although HAART is known to profoundly suppress viral replication, it increases CD4 cell count and delays disease progression and death; patients on Highly Active Antiretroviral Therapy (HAART) commonly suffer from side effects of the drug (D'Arminio A, et al 2000, De Jesus E, et al 2004 & Eron J, et al 2000). Each antiretroviral drug is associated with specific adverse effects. Among the antiretroviral drugs, Zidovudine (AZT) remains to be the most widely used drug resulting in myelosuppression (De Jesus E, et al 2004 & Moyle G, et al 2004).

Several studies in developed countries have shown that AZT alone and AZT based HAART regimen is associated with significant reduction of hemoglobin (Hb) level and neutrophil number (Sullivan S, et al 1998, Eron J, et al 2000 , and Moyle G, et al 2004). Though most of the studies on hematological abnormalities are on adults, one randomized comparative trial done to assess the safety and efficacy of AZT and d4T in symptomatic HIV infected children showed a prevalence of anemia to be 5% among the AZT group whereas 2% among the d4T group. Similarly the prevalence of neutropenia was higher in AZT group (Mark W, et al 1998).

At the end of February, 2007, 72,127 HIV patients were started on Antiretroviral Therapy (ART) at 234 facilities across the country (Ministry of Health of Ethiopia – HAAPCO March 22, 2007). Regardless of the fact that Ethiopians' normal immunohematologic profile is

known to be lower than the white population by 2 to 3% (Kassu A, et al Nov. 2001). Therefore using the western cut of point may not guaranty to suggest clinical cases in Ethiopia. AZT-based HAART is one of the first line regimens in the guideline (Ministry of Health of Ethiopia, Guideline for Paediatric HIV/AIDS Care and Treatment in Ethiopia. July 2007).

Chapter – Two

Objectives

2.1 General objective

☞ The aim of this investigation was to determine the CD_4^+ response among HIV-infected individuals receiving highly active antiretroviral therapy (HAART) with long-term follow-up.

2.2 Specific objectives

- To describe the baseline mean CD_4^+ T cell count
- To compare CD4 level before and after treatment and different intervals
- To locate at what time of ART therapy the patients show exaggerated immunological response
- To describe at what point of follow up the patient show good immunologic recovery

Chapter- 3

Methodology

3.1 Study design

A retrospective cohort study design was conducted to assess the immunological response mainly of their CD^+4/ before and after ART and their data was obtained from registration book. All patients, antiretroviral-naive patients with symptomatic HIV disease at baseline (before ART) and exactly at $6^{th,}$ 9^{th} and 12^{th} months of ART which were free from any infection were included. And their initial their CD^+4 were collected from records and after start ART in the Zewuditu Hospital, Addis Ababa, Ethiopia.

Inclusion criteria

- Patients with full information
- ART patients whose CD^+4 is monitored at the start and exactly 6^{th} , 9^{th} & 12^{th} months of therapy
- Free from any other infection during the study time

Exclusion criteria

- Patients partial information
- ART patients whose CD^+4 are not monitored at the start and exactly 6^{th}, 9^{th} & 12^{th} months of therapy.
- Patients not having any other infection during the study time

3.2. Study area & period: 5 years data was obtained from Zewuditu Hospital, Addis Ababa city from May, 2011 to Nov.30, 2011.

3.3 Variables

3.3.1 Dependent variables

CD^+_4 T cell count

3.3.2 Independent variables

Age

Sex

3.4 Sampling technique

All patients data were included who started ART for the last five years that do have complete information on CD4 count. (2006 - 2010)

3.5 Data collection

Data were collected in predesigned dummy table

3.6 Data analysis and interpretation

The collected, cleared raw data were compiled and analysed using SPSS version 16.0 based on set variables or objective of the study and the results was presented using descriptive measures, graphs, tables.

We evaluated CD4 cell responses in the following ways: i) whether patients failed to attain mean CD4 cell count increase from baseline of at different interval months (defined as immunological non-response); ii) whether patients achieved an absolute CD4 count of 200 cells/µl at the 12^{th} months visit; iii) and whether patients had achieved an absolute CD4 cell count of 500 cells/µl at 12 months (super-responders).

Mean and the median were compared using found to be normally distributed therefore the mean was used in the evaluation of the response. Baseline CD4 cell counts were categorised as follows: <50, 50–99, 100–149 and >150 cells/µl.

Finally interpreted into variable information and comparisons was made, results were displayed in tables, graphs and expressed in words. We calculated mean CD4 cell counts during three intervals (from baseline to 6 months of ART, 6 to 9 months, and 9 to 12 months)

3.7 Pre test

The method was tested on quality control for its reliability & validity before it is used for actual work this was help us to be more familiarized with methods and to thing the following points.

- The acceptability of different approaches during data collection
- The acceptability of the method which will be used.
- To have base line information about what information's should be included.

3.8 Quality control (QC)

The data was therefore be checked constantly by QC program. This was considered as part of the procedure itself. Thus QC program was designed to effectively evaluate the precision & accuracy of the data collection monitor reliability and evaluate the performance of the collector done. Therefore during the experiences the quality was checked.

3.9 Ethical consideration

The study protocol was be submitted to the DMIP scientific and ethical committee before the study starts. After getting ethical clearance, letter of support was written to hospital (Zewuditu Hospitals) by the department of Microbiology, Immunology and Parasitology. To ensure confidentiality of data, study subjects was identified using codes and unauthorized persons did not have access to the collected data. The findings were utilized for proper management of the patients.

3.10 Communication of results: The outcomes of the result will be informed to concerned bodies.

Chapter- Four

Results

A total of 887 HIV positive patients in this research; Out of these 472 (53.2%) were female and 415 (46.8%) male patients. The ratio of male to females was almost 1:2. None of them have any opportunistic infection during the time of follow up. The mean age of the study group was 36.76 (17-76).

The mean baseline CD4 was 81.40; the mean CD4 count at the 6^{th}, 9^{th} and 12^{th} month was 191.65, 284 and 331 respectively. There was a good immune recovery at the 6^{th} month of therapy from the baseline mean CD_4^+ T cell count of 81 cells /μl to 191.65 cells /μl, which was statically highly significant ($p<0.0001$). This first remarkable rise was continued in the achieving in the mean $CD4^+$ count of 284 cells/μl at the 9^{th} month of visit. Followed by relatively steady lower increase and approaching stable CD4+ T cell count and 12^{th} months of visit. The pick recovery was noted in those patients having a base line CD_4^+ of >200cells/μl, while patients with a base line CD_4^+ count <200cells/μl showed less recovery rate. In general, the result indicated that the recovery was significantly in those patients who started therapy at the base line CD_4^+ < 200cells/μl.

Table 2: **Distribution of study subjects by sex & age characteristic, Zewuditu Hospital from 2006-2010.**

Age Category	SEX		Total
	FEMALE (No/%)	MALE (No/%)	
<20	1 (33.3%	2 (66.7%)	3(100)
20-29	127(76.5)	39 (23.5)	166 (100)
30-39	234(56.2)	182(43.8)	416(100)
40-49	81(37.3)	136 (62.7)	217(100)
50-59	25(36.8)	43(63.2)	68(100)
>=60	4(23.5)	13(76.5)	17(100)
Total	472(53.2)	415(46.8)	887(100)

Table 3 Means Comparison at different time intervals and sex at Zewuditu Hospital from 2006-2010.

SEX		BaseCD4	CD46	CD49	CD412
FEMALE	Mean	87.96	207.49	304.278	344.63
	N	472	472	472	472
MALE	Mean	73.94	173.98	260.843	315.46
	N	415	415	415	415
Total	Mean	81.40	191.81	283.956	330.98
	N	887	887	887	887

Table 4: **CD4 cell count among ART naïve HIV patients by age at Zewuditu Hospital Ethiopia from 2006-2010**

Baseline CD4 Count Category		Age Category						Total
		<20	20-29	30-39	40-49	50-59	>=60	
<50	Count	0	86	185	90	25	10	396
	% within Baseline CD4	.0%	21.7%	46.7%	22.7%	6.3%	2.5%	100.0%
50-99	Count	1	25	82	52	14	2	176
	% within Baseline CD4	.6%	14.2%	46.6%	29.5%	8.0%	1.1%	100.0%
100-149	Count	0	25	58	39	11	2	135
	% within Baseline CD4	.0%	18.5%	43.0%	28.9%	8.1%	1.5%	100.0%
150-199	Count	0	24	68	30	16	3	141
	% within Baseline CD4	.0%	17.0%	48.2%	21.3%	11.3%	2.1%	100.0%
200-249	Count	2	4	15	3	2	0	26
	% within Baseline CD4	7.7%	15.4%	57.7%	11.5%	7.7%	.0%	100.0%
250-299	Count	0	2	8	3	0	0	13
	% within Baseline CD4	.0%	15.4%	61.5%	23.1%	.0%	.0%	100.0%
Total	Count	3	166	416	217	68	17	887
	% within Baseline CD4	.3%	18.7%	46.9%	24.5%	7.7%	1.9%	100.0%

Table 5: Mean baseline CD4 cell count of ART-naïve HIV positive patients by gender at Zewuditu Hospital Ethiopia from 2006-2010

Mean CD4 cells	
Sex	**CD4 cells/µl**
Female (N = 472)	87.96
Male (N = 415)	73.94
Total	81.40

Table 6: CD4 cell counts at baseline and after six month ART of HIV patients at Zewuditu Hospital Ethiopia from 2006-2010,

CD4 cell/µl	At baseline (N=887) N (100%)	At six month follow- up (N = 887 N (100%)
<50	396(44.65)	58(6.56)
50-99	176(19.84)	119(13.42)
100-149	135(15.22)	199(22.44)
150-199	141(15.89)	190(21.42)
200-249	26(2.93)	101(11.39)
250-299	13(1.47)	87(9.81)
300-349	0	51(5.75)
350-399	0	22(2.48)
>=400	0	60(6.77)

Table 7: **CD4 cell counts at baseline and after nine month ART of HIV patients at Zewuditu Hospital Ethiopia from 2006-2010,**

CD4 cell/µl	At baseline (N = 887) N (100%)	At nine month follow- up (N=887)* N (100%)
<50	396(44.65)	10 (1.13)
50-99	176(19.84)	13(1.47)
100-149	135(15.22)	121(13.64)
150-199	141(15.89)	168(18.94)
200-249	26(2.93)	113(12.74)
250-299	13(1.47)	134(15.12)
300-349	0	110(12.40)
350-399	0	65(7.33)
>=400	0	153(17.25)

Table 8: CD4 cell counts at baseline and after 12 month ART of HIV patients at Zewuditu Hospital Ethiopia from 2006-2010

CD4 cell/µl	At baseline (N = 887 N (100%)	At 12 month follow- up (N= 887)* N (100%)
<50	396(44.65)	3 (0.34)
50-99	176(19.84)	10 (1.13)
100-149	135(15.22)	61 (6.88)
150-199	141(15.89)	114 (12.85)
200-249	26(2.93)	124 (13.98)
250-299	13(1.47)	127 (14.32)
300-349	0	98 (11.05)
350-399	0	102 (11.50)
>=400	0	248 (27.99)

Chapter- Five

Discussions

In this study, the majority of ART-naïve HIV patients were female. A similar finding was reported by Braitsein *et al*; (14) from South Africa who stated that ART-naïve patients in low-income countries were more likely to be females. This is because females are biologically and socially more vulnerable to HIV infection in the developing countries (16).

Most of the HIV infected patients enrolled in our study were young age between 20 and 40 years old who were sexually more active and thus have a higher risk of infection compared to the other age groups (16). These findings could conform as previous reports from elsewhere in Ethiopia which reported that HIV prevalence decreases significantly to increasing level of education as well as their socio economic status (17).

At baseline, the mean CD4 cell count of ART-naïve HIV infected patients was lower 153 cells/µl than the reports from other countries (19). This could be due to delayed presentation and/or testing, differences in educational and socio-economic levels. Moreover, Tsegaye *et al*; (20) reported that healthy HIV-negative Ethiopians had lower mean CD4 cell counts (775/µl) than other Africans and individuals from Western countries.

In our study, female HIV patients had higher mean CD4 cell counts than male ($p < 0.002$) before ART was initiated. This is consistent with Kumarasamy *et al*; (21) report from India. This difference could be due to several Bibliography on HIV/AIDS in Ethiopia and Ethiopians in the Diaspora: The 2009 Update 7 reasons; HIV associated TB could be the contributing factor for the low CD4 count in males as the proportion of patients having TB was significantly higher in male HIV positive patients than females ($p=0.003$). In addition, it may be due to a sex-related difference in the overall CD4 counts among males and females as reported by Tsegay *et al*; (20). HIV sero-negative Ethiopian females had relatively higher CD4 cell counts than HIV sero negative males.

Our data indicates that the majority of HIV patients started antiretroviral treatment with more advanced immunodeficiency status. Since the majority (95.6%) of HIV patients had AIDS as defined by their CD4 cell counts < 200 cells/µl, as shown in indicating advanced immune suppression at initiation of ART. This was significantly higher when compared to the studies

conducted in Nigeria, south eastern United States and Thailand which reported a lower rate of AIDS at the initiation of ART (22-24).

Therefore, in the study hospital, delayed enrollment in ART program could be attributed by several factors. The other possible factor may be due to fear of stigma. In Ethiopia, only one third of HIV infected persons disclosed their HIV status to their partner (25) further compromising the utilization of the counseling and testing and ART services. A similar observation was made among South Africans where patients stared ART program with advanced immunodeficiency status (27). These findings indicate urgent need to promote early and enhanced HIV testing to enable HIV/AIDS patients to benefit from the expanding ART services.

The limitation of this study was no socio economic status as well as educational level was included because of improper registration in the log books.

The mean CD4 cell count for 887 follow-up cases increased from 81.4 to 191.8 cells/ µl (95% CI) after 6 month of treatment, from 81.4 to 284. Cells/ µl at 9^{th} month and from 81.4 to 331 cells/ µl (95% CI) on the 12^{th} month follow up. This was comparable with report of Gautam *et al*; (30) in India.

However, among treatment-naïve HIV patients, 566 (63.8%) failed to attain CD4 cell count above 200 cells/µl at 6 months. Lower CD4 cell counts (< 200 cell/µl) before staring ART had significantly associated with failure to attain CD4 cell count recovery as the majority of the patients whose CD4 cell count remained < 200 cells/µl at 6 month were from those groups with low baseline CD4 cell count. A higher proportion of patients with baseline CD4 count > 200 cells/µl had increased CD4 cells count after 6 months of treatment than those with a lower baseline CD4 counts. Lower baseline CD4 cell counts therefore may correlate with poor immune responses and thus determine the degree of morbidity and mortality related to HIV/AIDS as reported by other studies too (31-32).

None of the patients showed exaggerated immunological response which could lead to autoimmune disorder.

CHAPTER- 6

Conclusions and Recommendations

In conclusion, in our study, although good CD4 cells recovery in response to ART was documented in more than 81% of follow-up cases, HIV-positive patients were enrolled in ART program at decreased CD4 cells levels. As there is poor recovery of CD4 cell when the start >200 than when they start ART at CD4 count >200 CD4 cell.

Therefore, interventions need to be designed to promote early HIV testing and early enrollment of HIV infected individuals into ART services. As socio-demographic factors and lack of awareness about ART services, fear of stigma and discrimination compromise the utilization of ART program, improving public awareness by advocacy and social mobilization should be included in the ART service.

ART has considerably improved the immune recovery. We strongly recommend underline the need of anti-retroviral therapy in HIV infected patients for immune reconstitution. The differential recovery rate between those with base line CD4+ T cell count below 50cells/μl and above 500cells/μl needs further investigation

Annex-1 Data collection format

Patient Code	Age	Sex	Educational status	Occupational	Marital status	Ethnicity	Religion	CD_4 count			
								At Start	2nd	3 rd	4th

Annex – 2 THERAPY OF HIV

Several distinct classes of drugs are now used to treat HIV infection

Table: 5 Drugs Used in the treatment of HIV/available Antiviral agents

References

1. Adish A, Esrey S, Gyorgis T and John T. Risk factors for iron deficiency anemia in preschool children in northern Ethiopia. Public Health Nutrition. 1999; 2(3): 243-252.
2. Amolo-Okero F, et al: Scaling up antiretroviral Therapy: Experience in Uganda. WHO; 2003.
3. Amoroso A, Sheneberger R, Edozien A et al. Antiretroviral-associated Drug Toxicities Leading to a Switch in Medication: Experience in Uganda, Kenya and Zambia. 14th Conference on Retroviruses and Opportunistic Infections Los Angeles. California Feb 25-28, 2007.
4. Battegay M, Nuesch R, Hirschel B, Kauffman R: Immunological recovery and antiretroviral therapy in HIV infection. *Lancet Infect Dis* 2006, 6(5):280-287.
5. Buchacz K, et al. Changes in lipid profile over 24 months among adults on first-line highly active antiretroviral therapy in the home-based AIDS care program in rural Uganda. J Acquir Immune Defic Syndr. 2008 Mar 1;47 (3):304-11.
6. Colafigli M, et al: score change in HIV-1-infected patients switched to an atazanavir-based combination antiretroviral regimen. HIV Med. 2008 Mar;9 (3):172-9.
7. D'Arminio A, Lepri C and Rezza G. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. AIDS, 2000; 14:499-507.
8. De Jesus E, Herrera G and Teofilo E. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults. Clinical Infectious Disease, 2004; 39:1038-46.
9. Egger M, May M, et al; ART Cohort Collaboration. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet 2002 Jul 13; 360 (9327):119-29.
10. Eron J, Yetzer S and Ruane J. Efficacy, safety, and adherence with a twice-daily combination lamivudine/zidovudine tablet formulation, plus a protease inhibitor, in HIV infection. AIDS, 2000; 14:671-81.
11. Gange SJ, et al: Rapid declines in total lymphocyte count and hemoglobin in HIV infection begin at CD4 lymphocyte counts that justify antiretroviral therapy. *AIDS* 2003, 17(1):119-121.

12. Kassu A, Tsegaye A, Petros B, et al. Distribution of Lymphocyte Subsets in Healthy Human Immunodeficiency Virus-Negative Adult Ethiopians from Two Geographic Locales. Clinical and Diagnostic Laboratory Immunology. Nov. 2001, p. 1171–1176.
13. Lau B, Gange SJ, Phair JP, Riddler SA, Detels R, Margolick JB: Use of total lymphocyte count and hemoglobin concentration for monitoring progression of HIV infection. *Jaids-Journal of Acquired Immune Deficiency Syndromes* 2005, 39(5):620-625.
14. World Health Organization (WHO). TB/HIV a Clinical Manual 2nd ed. Geneva: WHO, 2004.
15. Lawn S, Bekker L, Robin R: How effectively does HAART restore immune responses to Mycobacterium tuberculosis? *AIDS* 2005, 19:1113-1124.
16. UNAIDS. HIV Epidemic a global update United Nations World AIDS day report. *Health Millions*.1998.
17. Bradley H, Bedada A, Brahmbhatt H, Kidanu A, Gillespie D, Tsui A. Educational attainment and HIV status among Ethiopian voluntary counseling and testing clients. AIDS Behav 2007; 11(5):736-42.
18. Dabis F, Balestre E et al. Cohort Profile: Antiretroviral Therapy in Lower Income Countries (ART-LINC): international collaboration of treatment cohorts. Int J Epidemiol 2005; 34(5):979-86.
19. Saul J, Erwin J, Bruce JC, Peters B. Ethnic and demographic variations in HIV/AIDS presentation at two London referral centers 1995-9. Sex Transm Infect 2000; 76(3):215.
20. Tsegaye A, Messele T, Tilahun T, Hailu E, Sahlu T, Doorly R, et al. Immunohematologic al reference ranges for adult Ethiopians. Clin Diagn Lab Immunol 1999; 6(3):410-4.
21. Kumarasamy N, Venkatesh KK, Cecelia AJ, Devaleenol B, Saghayam S, Yepthomi T, et al. Gender-based differences in treatment and outcome among HIV patients in South India. J Women Health (Larchmt) 2008; 17(9):1471-5.
22. Chasombat S, McConnell MS, Siangphoe U, Yuktanont P, Jirawattanapisal T, Fox K, et al. National expansion of antiretroviral treatment in Thailand, 2000-2007: program scale-up and patient outcomes. J Acquir Immune Defic Syndr 2009; 50(5):506-12.
23. Gay CL, Napravnik S, Eron JJ, Jr. Advanced immunosuppression at entry to HIV care in the Southeastern United States and associated risk factors. AIDS 2006; 20(5):775-8.
24. Nwokedi EE, Ochicha O, Mohammed AZ, Saddiq NM. Baseline CD4 lymphocyte count among HIV patients in Kano, Northern Nigeria. Afr J Health Sci, 2007; 14:4.

25. Degefa A, Sanders EJ, Mekonnen Y, et al. Knowledge and attitudes towards antiretroviral therapy among factory workers participating in a cohort on HIV and AIDS, Addis Ababa, Ethiopia. Ethiop Med J 2003;41 Suppl 1:75-87.
26. Le Moing V, Thiébaut R, Chêne G, Sobel A, Massip P, et al. Long-term evolution of CD4 count in patients with a plasma HIV RNA persistently <500 copies/mL during treatment with antiretroviral drugs. HIV Med. 2007 Apr; 8 (3):156-63.
27. Burns FM, Fakoya AO, Copas AJ, French PD. Africans in London continue to present with advanced HIV disease in the era of highly active antiretroviral therapy. AIDS 2001; 15(18):2453-5.
28. Lederman HM, Williams PL, Wu JW, Evans TG, Cohn SG, McCutchan JA: Incomplete immune reconstitution after initiation of highly active antiretroviral therapy in human immunodeficiency virus-infected patients with severe CD4+ cell depletion. *J Infect Dis* 2003, 188:1794-1803.
29. Lima VD, Harrigan R, Murray M, Moore DM, Wood E, Hogg RS, Montaner JS. Differential impact of adherence on long-term treatment response among naive HIV-infected individuals. AIDS. 2008 Nov 12; 22 (17):2371-80.
30. Burns FM, Fakoya AO, Copas AJ, French PD. Africans in London continue to present with advanced HIV disease in the era of highly active antiretroviral therapy. AIDS 2001; 15 (18):2453-5.
31. Falster K, Petoumenos K, Chuah J, Mijch A, Mulhall B, Kelly M, et al. Poor baseline immune function predicts an incomplete immune response to combination antiretroviral treatment despite sustained viral suppression. J Acquir Immune Defic Syndr 2009; 50(3):307-13.
32. Lawn SD, Myer L, Bekker LG, Wood R. CD4 cell count recovery among HIV-infected patients with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa. BMC Infect Dis 2006; 6:59
33. Ludwig H and Strasser K. Symptomatology of Anemia. Semin Oncol. 2001; 28: 7-14.
34. Mark W, Russel B, Jane C, et al. A Randomized trial of Stavudine versus Zidovudine in Children with Human Immunodeficiency Virus Infection. Pediatrics 1998; 101: 214-220.
35. Ministry of Health of Ethiopia – HAAPCO. Monthly HIV Care ART Update released on March 22, 2007.
36. Ministry of Health of Ethiopia. Guideline for Pediatric HIV/AIDS Care and Treatment in Ethiopia. Addis Ababa, July 2007.

37. Moyle G, Sawyer W, Law M, Amin J and Hill A. Changes in the Efficacy of Thymidine Analogue-based HAART: A Meta-analysis of Six Prospective, Randomized, Comparative Studies. Clinical Therapeutics, 2004; 26:1.
38. Moyle G. Anemia in Persons with HIV Infection: Prognostic Marker and Contributor to Morbidity. AIDS Rev, 2002; 4:13-20.
39. Muyingo SK, Walker AS, Reid A, Munderi P, Gibb DM, Ssali F, Levin J, Katabira E, Gilks C, Todd J: Patterns of individual and population-level adherence to antiretroviral therapy and risk factors for poor adherence in the first year of the DART trial in Uganda and Zimbabwe. *J Acquir Immune Defic Syndr* 2008, 48(4):468-475.
40. Nguemaïm NF, Mbuagbaw J, Nkoa T, Alemnji G, Této G, Fanhi TC, Asonganyi T, Samé-Ekobo A. Serum lipid profile in highly active antiretroviral therapy-naïve HIV-infected patients in Cameroon: a case-control study. HIV Med. 2010 Jul 1; 11(6):353-9.
41. Patterson K, Napravnik S, Eron J, Keruly J, Moore R: Effects of Age and Sex on Immunological and Virological Responses to Initial Highly Active Antiretroviral Therapy. *HIV Med* 2007, 8(6):406-410.
42. Rahman AP, Eaton SA, Nguyen ST, Bain AM, Payne KD, Bedimo R, Busti AJ, Safety and efficacy of simvastatin for the treatment of dyslipidemia in human immunodeficiency virus-infected patients receiving efavirenz-based highly active antiretroviral therapy. Pharmacotherapy. 2008 Jul; 28 (7):913-9.
43. Seoane E, Resino S, et al. Lipid and apoprotein profile in HIV-1-infected patients after CD4-guided treatment interruption. J Acquir Immune Defic Syndr. 2008 Aug 1; 48(4):455-9.
44. Sullivan S, Hanson L, Chu Y, Jeffrey J and John W. Epidemiology of anemia in human immunodeficiency virus (HIV) infected persons: Results from multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project. Blood. 1998; 91: 301-308.
45. Turcinov D, Stanley C, et al: Dyslipidemia and adherence to the Mediterranean diet in Croatian HIV-infected patients during the first year of highly active antiretroviral therapy. 2009 Jun; 33 (2):423-30.
46. Uganda: *National Antiretroviral Treatment Guidelines for Adults, Adolescents and Children.* Ministry of Health; 2008.
47. UNAIDS, December 2007 Report.
48. Ungsedhapand C, Srasuebkul P, et al Three-year durability of dual-nucleoside versus triple-nucleoside therapy in a Thai population with HIV infection. *J Acquir Immune Defic Syndr* 2004, 36(2):693-701.

49. Weidle PJ, Malamba et al: Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance. *Lancet* 2006, 360(9326):34-40.
50. WHO: Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach. 2006.
51. Yamashita TE, et al: Immunologic and virologic response to highly active antiretroviral therapy in the Multicenter AIDS Cohort Study.
52. WHO Regional Office for South-East Asia New Delhi, Laboratory Guidelines for enumerating CD4 T Lymphocytes in the context of HIV/AIDS June 2007

Declaration

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

MSc. candidate: Moges Derbe (BSc)

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

1. Supervisor: Professor Dr. DP Monga

   Signature: _____

   Date and place _____

   Addis Ababa, Ethiopia.

2. Supervisor:

   Signature: _____

   Date and place _____

   Addis Ababa, Ethiopia.