LONG TERM IMMUNE RECOVERY OF ADULT HIV INFECTED PATIENTS TAKING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AS MEASURED BY CD4+ T CELL COUNTS IN ALERT HOSPITAL, ADDIS ABABA ETHIOPIA

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This is to certify that the thesis prepared by Geremew Gizaw, entitled: *Long term immune recovery of adult HIV infected patients taking highly active antiretroviral therapy as measured by CD4+ T cell counts in ALERT hospital, Addis Ababa Ethiopia* : and submitted in partial fulfillment of the requirements of the degree of masters of science (Clinical laboratory Sciences with a specialty track of hematology and immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

BACKGROUND: More than five years have elapsed since free ART has been available to PLWHA in Ethiopia. The Ethiopian ART guideline follows the ART recommendations for resource limited countries forwarded by WHO in 2006. There are more than 280,000 PLWHA in Ethiopia who have ever been started with ART. The response to treatment is followed using a six monthly determination of CD4+ T cells. However, there is scarcity of information regarding the degree of immune recovery among PLWHA in Ethiopia who have been on ARV since 2005.

OBJECTIVE: To assess immune recovery of adult HIV infected patients who have been on ART from 1-6 years and factors influencing it.

METHOD*:* A retrospective study was conducted in ALERT hospital, Addis Ababa, Ethiopia on adult HIV infected patients who have been taking antiretroviral therapy for more than six months. Data was collected from pre-ART, ART and follow up formats and analyzed using SPSS version 20 and GraphPad Prism 5.

RESULT: Among the total of 4419 reviewed medical records, 61.6% were females and the median age for all study participants was 35 years (IQR, 29-40). The mean (95% CI) weight at baseline was 53(SD=10.4) Kilogram; 57.7 (SD=10.3) for males and 50(SD=9.5) for females (p=0.000). The median CD4 at baseline was 135(IQR, 72-201); 120 (IQR, 62-186) for males and 144 (IQR, 79-209) for females (p=0.000). At baseline, 16.0%, 59.0%, and 25.0% participants had a CD4 cell/ µl of ≤50, 51-200 and ≥201, respectively. Among the total OIs and co-morbidities, Herpes zoster was the most frequently observed one (20.7%). There was a dramatic increment of CD4 cells/ µl across all age groups during the first six months of follow up period. Particularly, the age group

15-24 years had the highest (21.8 cells/µl /month) rate of CD4 cells/ µl increment. The overall rate of increment during the 72 months follow up was 4.4 cells/µl /month. Patients who start antiretroviral treatment with ≤50 cells/ µl had the highest rate (21.2 cells/µl /month) during the first six month of treatment. At the end of the 72 months, the median CD4 difference from the baseline was 314 cells/µl and the overall mean weight increment was 6.2 Kg.

CONCLUSION: The study has shown CD4 increment which is comparable with other developed and developing countries, especially in the first 6 months. In this study, females and younger age groups (15-24 years) have shown a better immune recovery.

KEY WORDS: Antiretroviral therapy; CD4 cell count; HIV infection

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Operational definitions:

- HAART: Highly Active Anti-retroviral therapy. It is a combination of at least three ARVs
- First Line Treatment Regimens: The initial regimen prescribed for patients fulfilling national clinical and laboratory criteria for starting ART. Current Ethiopian treatment guidelines recommend two NRTIs and one NNRTI for initial treatment.
- Adherence
  - ✓ Good Adherence: > 95% of doses are taken (i.e. less than three doses are missed during the month)
  - ✓ Fair Adherence: 85 – 94% of doses are taken (i.e. three to nine doses are missed during the month)
  - ✓ Poor Adherence: <85% of doses are taken (i.e. more than nine doses are missed)
- Treatment-naïve or "drug-naive": Someone who has never used HIV drugs.
- Treatment-experienced: Someone who has used drugs before
- Viral load**:** A measure of the severity of a HIV infection. This can be calculated by estimating the amount of HIV RNA copies per milliliter of blood plasma
- Addiction: A state of physiological or psychological dependence on potentially harmful substances like drug, alcohol and tobacco.

- Working: Able to perform usual work in or out of the house, harvest, go to school, for children, normal activities or playing.

- Ambulatory: Able to perform activities of daily living.

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

Table 1 Definitions of treatment failure in adults and adolescents (6):

	Definition
Clinical Failure [a]	New or recurrent WHO stage 4 condition [b,c]
Immunologic failure [d]	Fall of CD4 count to pre-therapy baseline(or below) 50% fall from the on-treatment peak value (if known) Persistent CD4 levels below 100 cells/mm^3
Virologic Failure	Plasma viral load above 10,000 copies/ml in duplicates after six months on ART

a. Should be differentiated from Immune Reconstitution Inflammatory Syndrome (IRIS).
b. Certain WHO clinical conditions (e.g. pulmonary TB, severe bacterial infections), may indicate treatment failure and should be investigated.
c. Some WHO clinical stage 4 conditions (lymph node TB, uncomplicated TB pleural disease, esophageal candidiasis, recurrent bacterial pneumonia) may not be indicators of treatment failure and thus do not require consideration of second-line therapy.
d. Without concomitant infection to cause transient CD4 cell decrease. If patient is asymptomatic and treatment failure is being defined by decreased CD4 cell criteria alone, consideration should be given to performing a repeat CD4 cell count before establishing diagnosis of treatment failure.

Acronyms

µl: micro liter

AHRI: Armauer Hansen Research Institute

AIDS: Acquired Immune Deficiency Syndrome

ALERT: All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre

ANC: Antenatal Care

ART: Anti-Retroviral Treatment

BP: Bacterial Pneumonia

BSS: Behavioral Surveillance Surveys

CASCADE: Concerted Action on Seroconversion to AIDS and Death

CD4: Cluster designation 4

CI: Confidence Interval

CM: Cryptococcal Meningitis

CT: Central nervous system Toxoplasmosis

DC or DA: Diarrhoea Chronic/Acute

DHS: Demographic and Health Survey

EMA: Ethiopian Medical Association

EMLA: Ethiopian Medical Laboratory Association

EPHA: Ethiopian Public Health Association

ETB: Extra Pulmonary Tuberculosis

FACS: Fluorescent Activated Cell Sorter

HAART: Highly Active Antiretroviral Treatment

HAPCO: HIV/AIDS Prevention and Control Office

HIV: Human Immune Deficiency

IQR: Inter-quartile Range

ISS: Immune Suppression Syndrome

LMIC: Low and Middle Income Countries

$Mm^{3:}$ millimeter cub

NNRTI: Non- Nucleotide Reverse Transcriptase

NRTI: Nucleotide Reverse Transcriptase

OARAC: Office of AIDS Research Advisory Council

OIs: Opportunistic Infections

PCP: Pneumocystis Pneumonic

PEPFAR: President's Emergency Plan for AIDS Relief

PI: Protease Inhibitor

PLWHA: Peoples Living with HIV/AIDS

PMTCT: Prevention of Mother-to-Child Transmission (of HIV)

PTB: Pulmonary Tuberculosis

QASI: Quality Assessment and Standardization for Immunological measures

RH: Relative Risk

RNA: Ribonucleic Acid

TB: Tuberculosis

UNAIDS: Joint United Nations Program on HIV/AIDS

WHO: World Health Organization

1. Introduction

1.1 Background information

In the year 2009 an estimated 33.3 million adults and children worldwide were living with HIV and of these 2.6 million were newly infected and 1.8 million died of HIV/AIDS. [1] In the same year, sixty seven percent of HIV infection worldwide, 68% new cases among adults and 72% of AIDS related deaths were estimated in sub Saharan Africa even though this region contains only 11 percent of the global population. According to the estimates of the World Health Organization (WHO), for the year 2009 nearly 5.2 million people living in low and middle-income countries (LMIC) were receiving Antiretroviral Therapy (ART) [2]. According to the UNIAIDS/WHO update data the number of AIDS-related deaths has declined by over 10% over the past five years due to antiretroviral treatment [1, 2] .

With a population estimated at over 77 million, Ethiopia is the second most populous nation in Africa. About 85% of the population lives in rural areas, and approximately one-fifth are aged 15- 24 years. HIV/AIDS was first recognized in the country in the mid-1980's, at about the same time as in other countries in the region. Efforts to collect epidemiological data began shortly thereafter, and there are many studies from the late 1980s and 1990s reporting prevalence data and risk factors in a number of high-risk groups. In recent years, the main source of information about HIV has been antenatal clinic (ANC) based sentinel surveillance, with surveys being conducted and published at two year intervals, most recently in 2005. In addition, useful epidemiological information at national level and for specific communities can be extracted from the Demographic

and Health Surveys (DHS) and Behavioral Surveillance Surveys (BSS), the most recent versions of which were also completed in 2005[3].

The HIV pandemic created unprecedented burden on the economies and health care systems of affected countries, particularly in Sub-Saharan Africa, where prevalence is highest. In Ethiopia, HIV/AIDS has become a major public health concern, leading the Government to declare a public health emergency in 2002. In 2007, the estimated adult HIV/AIDS prevalence in Ethiopia was 2.1 percent. Although the epidemic is currently stable, HIV/AIDS remains a major development challenge for Ethiopia. Poverty, food shortages, and other socio-economic factors amplify the impact of the epidemic. According to data from the Joint United Nations Program on HIV/AIDS (UNAIDS), approximately 980,000 Ethiopians were living with HIV/AIDS in 2007, and 67,000 individuals have died as a result of infection with the virus. National projections estimate approximately 1.1 million Ethiopians are living with HIV and prevalence increased slightly to 2.3 percent by 2009 [4- 6].

Ethiopia's HIV/AIDS epidemic pattern continues to be generalized and heterogeneous with marked regional variations. At the national level, the epidemiologic trend over the past eight years has been stable. However, HIV prevalence appears to be declining in urban areas, according to analysis of data from ANC sites that collected data consistently for more than ten years. For example HIV prevalence among pregnant women attending ANC in Addis Ababa has declined from 23% in 1996 to 10% in 2007. Periurban and

small market town residents, young females are the most at risk individuals and affected segments of the population by the epidemic [7].

HIV infection causes progressive depletion of CD4+ T cells, which leads to immunodeficiency syndrome with a wide range of opportunistic infections and malignancies that eventually lead to death. Several studies indicated that proper use of antiretroviral treatment substantially reduces morbidity, mortality and prolongs life expectancy of HIV/AIDS patients. [1, 8-10] However, there are numerous factors that could limit its effectiveness especially in resource limited settings. Studies indicated that the response of ART varies among population due to differences in viral subtype, host factors, co-infections, such as tuberculosis (TB), other bacterial diseases or socioeconomic status and environment [11-13].

Access to antiretroviral therapy continued to expand rapidly. At the end of 2008, more than 4 million [3 700 000– 4 360 000] people were receiving antiretroviral therapy in low- and middle-income countries, an increase of more than 1 million (36%) compared with the end of 2007 and a 10-fold expansion in 5 years. The greatest expansion in the number of people receiving treatment in 2008 was in Sub-Saharan Africa, where about 2 925 000 [2 690 000– 3 160 000] people were receiving antiretroviral therapy at the end of 2008 versus 2 100 000 [1 905 000–2 295 000] people at the end of 2007. The estimated coverage of antiretroviral therapy in low and middle-income countries reached 42% [40–47%] in 2008, and coverage in Sub-Saharan Africa was 44% [41–48%]. Despite progress, more than 5 million of the estimated 9.5 million [8 600 000–10 000 000] people

needing antiretroviral therapy were still without access to treatment, making it absolutely critical to accelerate programme delivery to reach universal access goals [14].

In early 2005, 211,000 men, women and children in Ethiopia needed ART but only 16,400 were receiving it, some free and others on a co-payment basis. The government's objective was to put 58,500 people on ART by March 2006, 31,000 through the global Fund and 27,500 through President's Emergency Plan for AIDS Relief (PEPFAR). ART was first offered in July 2003 through 12 government hospitals on a co-payment basis. Around 3,000 HIV-positive people accessed services in one hospital in Addis Ababa from two doctors and two nurses who had received no more than three weeks' training in counseling and ART. Most patients were men between 25 and 44 years old. In January 2005, free ART through the Fund and PEPFAR became available in 22 hospitals [15].

Since the advent of the ART program, more than 200,000 people have started on treatment in about 500 facilities throughout Ethiopia. ART service expansion has been recent and fast from only four facilities in 2003 to 517 in 2009. Parallel with this, the number of people who have accessed ART has also increased substantially from 900 in 2003 to 211,000 in 2009. The impact of the program on the survival and quality of life of patients has also been demonstrated. A recent population-based study revealed significant decline in adult AIDS mortality as a result of the scale-up of ART in Addis Ababa. But despite the recent gains, universal access to ART is still far from being achieved. The number of patients ever started on ART represents only 54 percent of the population needing ART in the country. Although ART is being provided free-of-charge, with a

rapid expansion of facilities providing the service, population access to treatment cannot be deemed equitable and universal due to a number of deterrents that operate at individual, community, and facility levels [16, 17].

1.2 Statement of the Problem

Until recently, the main argument for delaying HAART related to the toxicities and inconvenience of these drugs and the fact that treatment was likely to be life-long. It was felt that patients would be unable to maintain the high levels of adherence that are required for successful outcomes [18] and, as a result, would develop resistant strains of HIV, possibly resulting in the exhaustion of treatment options [19,20]. Indeed, Wood *et al.* [21] concluded that starting HAART at higher CD4+ T cell counts would not provide protection against the effects of nonadherence if patients were not able to maintain these strict regimens. Given the perceived low risk of AIDS and mortality at CD4+ T cell counts more than 350 cells/μl, it was thought that little would be gained by exposing patients to antiretroviral therapy too soon. There was also a concern that toxicities may differ among those starting HAART at different CD4+ T cell levels, possibly resulting in a higher discontinuation rate among those starting with high CD4+ T cell counts. However, despite some findings of a higher hepatotoxicity rate in individuals starting nevirapine with high CD4+ T cell counts [22, 23], there is limited evidence to support an increased frequency of toxicities in those starting HAART with higher CD4+ T cell counts[24, 25].

Antiretroviral drugs have developed rapidly in the last few years, as has clinicians' ability to manage HIV-infected patients. Not only are the drugs more potent, easier to take, and have fewer and less serious toxicities than those that made up original HAART regimens

[26], but clinicians are aware of the problems associated with incomplete adherence and are better able to prevent and manage toxicities. Improved pharmacokinetic profiles and fixed dose combinations mean that drugs are easier to take and may be more forgiving to minor deviations from full adherence, resulting in less resistance [27].

Several large studies have provided important data on the risk of AIDS and death among individuals with high CD4+ T cell counts. Not only are these events more common than previously thought, but there is a continual increase in the risk of an event as the CD4+ T cell count declines, even when the count is high. For example, in the CASCADE collaboration [28], although the 6-month risk of AIDS varied according to patient age, a risk factor for disease progression [29], and viral load, it ranged from 2 to 10% in many individuals with a CD4+ T cell count more than 350 cells/μl. Podlekareva *et al.* [30] described risk factors for the development of opportunistic infections at higher than expected CD4+ T cell count levels; with the exception of pulmonary and extra-pulmonary tuberculosis, the strongest predictor for other opportunistic infections was a low CD4+ T cell count, even when the opportunistic infection occurred at a CD4+ T cell level previously thought to be protective. Data from the SMART trial, in which patients were randomized to receive either uninterrupted or episodic HAART [31], suggested that episodic treatment was associated with a higher risk of clinical disease at all CD4 levels.

Although not designed to answer the question directly, among patients who were antiretroviral-naïve or who had not received HAART for at least 6 months prior to trial entry, major clinical events (opportunistic infections, serious non-AIDS events, and deaths from causes other than opportunistic infections) were more common in those with a lower CD4+ T cell count [32], with event rates of 8.4, 5.3, and 0.9 per 100 person-years

among those with CD4+ T cell counts of 250-349, 350-499, and ≥500 cells/μl, respectively. Finally, in the Swiss HIV Cohort Study, the risk of non-Hodgkin's lymphoma was more than twice as high (adjusted hazard ratio of 2.28) in non-HAART users with CD4+ T cell counts of 200-349 cells/μl compared to those with counts ≥350 cells/μl [33].

In general, the decision of when to start treatment in an HIV-infected individual has always been problematic. On the one hand, treatment should be initiated at an early point in the individual's course of disease, prior to a time when CD4+ T cell loss is such that there is substantial risk of clinical progression. On the other hand, the original antiretroviral drugs were often inconvenient to take, of limited efficacy, and were associated with substantial toxicities. Thus, clinicians balanced the risks of delaying treatment (potentially placing the patient at risk of serious illness and death from AIDS) with the inconvenience and possible long-term effects of taking treatment. On the basis of evidence that clinical progression rates were low while the CD4+ T cell count remained above 200 cells/μl but increased rapidly at lower levels, most early treatment guidelines recommended that treatment be delayed until the CD4+ T cell count had fallen below 200 cells/μl. Over time, however, as treatments have improved and the number of treatment options available to patients has increased, this threshold has increased; most treatment guidelines now recommend that all individuals with a CD4+ T cell count less than 350 cells/μl should be treated [34, 35].

1.3 **Rationale of the study**

Measuring CD4+ T cell count and HIV RNA viral load levels to monitor response to antiretroviral therapy (ART) are important measures of the efficacy of ART in individual patients and of the effectiveness of ART in populations of patients enrolled in HIV care and treatment programs. However, few data exist on long term CD4 response to ART among patients receiving care in resource-limited settings, like Ethiopia where HIV RNA testing is not generally available or conducted. Several studies in Europe and North America have reported robust improvements in CD4 cell counts following ART initiation in clinical trials and in observational studies. In addition, CD4+ T count at the time of ART initiation is an important determinant of the degree of immunologic and virologic response, as well as subsequent risk of morbidity and mortality. Among those patients who are able to remain on ART, robust immunologic responses can be maintained for long periods, and the risk of serious morbidity and mortality may eventually diminish to levels observed in the general population.

Although data from resource-limited settings are less commonly available, some investigators of research and scale-up cohorts in sub-Saharan Africa, Barbados, Brazil, China, Thailand, and Cambodia have reported effects of ART on clinical and immunologic outcomes that were comparable to those observed in resource-rich settings. However the majority of the studies in developing countries have had follow up times of 1 to 2 years, Thus while it has been shown in developed countries, the degree which CD4 responses can be maintained for longer periods of time after ART initiation in developing countries has not been demonstrated.

The study was conducted to describe the rate of long term immune recovery (CD4 response) of adult HIV-1 infected patients in ALERT hospital, Addis Ababa Ethiopia within 5 years after initiation of ART and it will have a great input ‘when to start’ HAART in Ethiopian context based on recommendation recently proposed by WHO.

2. Literature review

Highly Active Antiretroviral Therapy (HAART) has dramatically modified the morbidity and mortality of HIV disease. Virologic response to HAART, specifically the suppression of plasma HIV RNA to below the level of quantification, typically results in a substantial rise in CD4 cell counts. This rise, sometimes termed the immunologic response to HAART, is central to restoration of integrity of the immune system. A summary analysis of 23 clinical trials of triple combination therapy in HAART-naive subjects found that the mean CD4 cell increase 12 months after HAART initiation was 160 cells/mm3. However, immunological and virologic response to HAART is neither universal nor homogeneous. Moreover, discordant immunologic and virologic responses, where either CD4 counts rise without complete viral suppression or HIV RNA levels are suppressed to below detection but CD4 counts do not rise, have been documented. "Complete" responses, where patients experience both virologic and immunologic responses, are associated with improved clinical outcomes when compared with patients with discordant responses or compared with patients who experience neither a virologic nor immunologic response [36, 37].

After the initiation of antiretroviral therapy, peripheral CD4 cell count starts rising, continuing for at least 3–5 years [38]. The initial increase in CD4 cell count is very rapid and is usually observed in the first 3–6 months [39]. This initial increase relies on a reduction in T-cell activation and primarily consists of a release of memory CD4 cells trapped in the lymphoid tissue [40]. A second phase of slower increase follows; approaching stable CD4 cell counts at 4–6 years [41]. During this second phase, naïve CD4 T-lymphocytes from the thymus, as well as memory CD4 T-lymphocytes,

contribute to the reconstitution of the immune system. Achieving a CD4 cell count over specific thresholds (e.g., 200 cells per μL) depends on baseline CD4 cell count and may take substantially longer in patients who initiate antiretroviral therapy at lower values [42, 43].

The factors that determine CD4 cell responses are only partly known and depend on both the host and the virus. Considerable individual variation in the reconstitution of CD4 T-lymphocytes has been noted. In HIV-1-infected patients with excellent virological responses and continuous plasma HIV-1 RNA levels below 1000 copies per μL, higher age, a longer duration of HIV-1 infection and lower CD4 cell count at baseline represent important risk factors for maintaining lower CD4 cell counts [44]. In a recent study of the Swiss HIV Cohort, 36% of patients receiving antiretroviral therapy did not reach CD4 cell counts above 500 cells per μL after 5 years despite continuous suppression of plasma HIV-1 RNA to levels below 1000 copies per μL, and almost half of these patients reached a plateau in CD4 cell count. Hence, the number of patients with CD4 cell counts in the normal range after of 4–5 years of antiretroviral therapy is smaller than expected [45].

Theoretical factors that may impede a complete recovery of CD4 cells include increased viral pathogenicity or certain host factors such as insufficient thymic supply of T lymphocytes [46]. In addition, virus induced cell death and higher rates of T-cell apoptosis may occur in patients with well suppressed plasma HIV-1 RNA [47]. It is much debated whether some co infections e.g., HIV/hepatitis C virus may limit CD4 cell recovery, whereas other co infections e.g., HIV/GB virus C appear to enhance increases in CD4 cell count [48-52]. In general, it can be postulated that a lower CD4 cell count at initiation

of antiretroviral therapy requires longer treatment periods to reach the desired target level. CD4 cell recovery may level off in a proportion of treated patients before the ideal physiological range is reached. It remains to be seen whether in the long term those individuals with CD4 cell counts above the critical level of 200 cells per μL but below 500 cells per μL have a higher risk for opportunistic infections and HIV-1-associated malignancies such as lymphomas [53].

A total of 101 HIV-1 infected patients from outpatient unit who started HAART before February, 1997, and received treatment for at least the first 3 months were involved on the study which was conducted in Swiss. All the patients had extensive exposure to anti-retroviral and had moderate-to-advanced immune-suppression (mean CD4- cell count $162x10^6$/L) and a high rate (66%) of failure of viral suppression on treatment regimens. At 48 weeks, an increase in CD4-cell count was seen in 91 (93%) of 98 assessed participants. Of 82 taking HAART continuously, the mean CD4 count increase for those with persistent (n=28) or transient (n=21) undetectable viraemia was 138 (98–178) and 130 (77–182) $x10^6$/L, respectively. An increase of 105 (79–132) $x10^6$/L CD4 cells was also seen among participants who took HAART continuously (n=33), despite persistent detectable viraemia [54].

In Italy a long term evaluation of T- cell subsets and T-cell function after the initiation of HAART was done in patients who had an advanced HIV 1 disease and it was found that an increased body weight and reduction of opportunistic infection. All patients showed an initial increase in the CD4 memory subset, whereas naive CD4 cells consistently increased only after 1 year. The magnitude of immune recovery was stronger in patients showing a significant reduction in viral load. However seven out of 21 patients who did

not reach a sustained suppression of viral load showed also an increase in T-cell subsets. [55].

A three years follow up of first-line antiretroviral therapy in Cambodia was made to assess the impact of prior ART exposure. Among the 256 patients included in the analysis, 148 (58%) were ART-naïve while 50 (20%) had previously received two NRTI and 58 (22%) three drugs. At entry to the program, all the patients received two NRTIs and one non-nucleoside reverse transcriptase inhibitor (NNRTI). At evaluation, 46 patients (18%) were switched to a protease inhibitor-based regimen (9%, 32% and 29% of naive, 2-NRTI and 3-drug groups); $p<0.0001$). The median CD4 cell count increase was 180/μL overall (IQR: 96- 276) and was higher in ART-naïve than ART-experienced patients [56].

In another study, 4570 patients were followed for at least 1 year to assess the effectiveness of antiretroviral treatment in South Africa. Among the study participant 53.2% died. Eighty-seven percent of patients who died had not received HAART. HAART was associated with lower mortality and with the presence of tuberculosis after adjusting for age, sex, weight, clinic, district, CD4 cell count, cotrimoxazole therapy, tuberculosis at baseline, and previous antiretroviral therapy. Cotrimoxazole therapy was associated with lower mortality (hazard ratio, 0.37; 95% CI, 0.32 0.42). Each month of HAART was associated with an increase in CD4 cell count of 15.1 cells/μL and with an increase in body weight of 602 g [57].

In a study which was conducted in Mbarara, Uganda, a total of 23 HIV-1-positive volunteers were randomly recruited from a prospective observational cohort study of 250 people receiving care at the Mbarara Hospital Immune Suppression Syndrome Clinic

(ISS). All patients were ART naive and initiated ART within 2 weeks of enrollment. Demographic information, CD4+T cell count, and HIV viral load were obtained at the time of enrollment, 3 and 6 months after initiation of ART. Samples from 32 healthy, HIV-negative Ugandans served as controls to establish immune activation baselines. The mean age of the study population was 33 years and 16 were women. Twenty-one out of 23 patients received stavudine/lamivudine/nevirapine. One individual received zidovudine/lamivudine/nevirapine and another individual received stavudine /lamivudine/efavirenz. According to the revealed result potent antiretroviral treatment led to viral suppression and increased CD4 cell count within 3 months of therapy initiation in Ugandan population. The median CD4+T cell counts at baseline were 106 cells/mm3 (range: 7–490) with a significant increase at 6 months to 237 cells/mm3 (range: 54–478) but the greatest gain in CD4+T cell count was achieved in the first 3 months [58].

HIV-infected female sex workers from a longitudinal cohort, with at least 1 year of pre-ART and 6 months of post-ART follow-up (n = 79), were enrolled in Kenya. The median pre-ART follow-up was 4,040 days. CD4 counts were measured biannually and viral loads where available. The median CD4 count at ART initiation was 180 cells/ul, which increased to 339 cells/ul at the most recent study visit. The rate of CD4+ T cell increase on ART was 7.91 cells/month. LTNP status prior to ART initiation did not associate with the rate of CD4 recovery on ART. In univariate analyses, associations were observed for CD4 recovery rate and duration of pre-ART immunosuppression ($r = 20.326$, $p = 0.004$) and CD4 nadir ($r = 0.284$, $p = 0.012$). In multivariate analysis including age, CD4 nadir, duration of HIV infection, duration of pre-ART immunosuppression, and baseline viral

load, only CD4 nadir (p = 0.007) and not duration of immunosuppression (p = 0.87) remained significantly associated with the rate of CD4 recovery[59].

There are limited published studies on CD4 recovery rate in patients taking HAART in Ethiopia. A total of 368 HIV positive patients, with median age 30 years were enrolled in the study which were conducted in Felegehiwot Hospital, North west Ethiopia. Of these, 207 were uneducated and 233 had monthly income ≤ 250 birr. Three hundred fifteen started ART within 6 months of HIV diagnosis. The mean (95% CI) CD4 cell count at baseline was 153 (139-167); 156 (137-175) for females and 122 cells/µl (105-139) for males (p<0.01). At baseline, 280 (76.3%) and 134 (36.4%) patients had CD4 cell count <200 and ≤ 100 cells/µl, respectively. Six months follow-up CD4 counts were enumerated for 225 (61%) patients and their mean CD4 cells increased from 143 to 261 cells/µl (p < 0.05) with a mean cell gain of 117 cells/µl. Of the 166 follow-up patients with CD4 count <200 cells/µl at baseline, 130 (78%) attained a higher CD4 cells count after treatment compared to 50 (85.6%) of the 59 with CD4 cell >200 cells/µl (p=0.21) [60].

A historical retrospective cohort study was conducted at eight randomly selected public hospitals in South Nations, Nationalities and Peoples Region, Ethiopia to assess the outcome of antiretroviral treatment. The median age was 30 years and 73.6% were in the age group 25-40 while the higher HIV risk age group 14-24 covered only 12.8%. The proportion of females was 56.3%. The hazard of death was higher in male and those who had a baseline CD4 cell count < 50 cells /ml compared to these with a count of above 200. Patients with WHO stage IV at baseline had a higher risk of death compared to these with a WHO stage I [61]

3. Hypothesis

- ✓ Early initiation (CD4 ≥ 200 cells/µl) of HAART has positive impact on the recovery of CD4 number than late initiation (CD4 ≤ 200 cells/µl) of HAART for HIV infected patients

4. Objective of the study

4.1 General objective: To determine immune recovery of HIV-infected adult patients taking HAART as measured by CD4 + T cell counts in ALERT hospital, Addis Ababa Ethiopia.

4.2 Specific objectives

- To determine immune recovery of HIV-infected adults as measured by CD4 + T cell counts at 6, 12,18, 24, 36, 48 and 60 months of follow up periods within different age groups.
- To determine the frequency of different opportunistic infections before and after initiation of HAART in the study participants
- To determine the mean gain of weight in the study participants during their follow up periods
- To determine the mean CD4 recovery of the study participants at different CD4 categories during their follow up periods

5. Material and methods

5.1 Study Setting and period

The study was conducted in ALERT hospital Addis Ababa, Ethiopia from Aug 2011- Oct 2012. ALERT is a referral Hospital for four Health Centers in Kolfe Kranyo and Nafas-Silik Subcities in Addis Ababa. It is one of the hospitals which are providing ART care in the country and provides comprehensive care for HIV infected patients. It serves as HIV treatment catchment referral hospital for four health centers, Kolfe HC, Woreda 24 HC, Woreda 23 Health Center, and Woreda 19 Health Center. The Hospital has enrolled more than 11,000 patients into the chronic care and treatment and more than 5000 of them have been started with ART. The retention rate of patients on ART is estimated as 70%. The hospital started provision of HAART in 2004 with the provision of free HAART for those who need it.

The ART clinics are standard in national context having 4 rooms which include doctor's room; ART nurse's room, ART pharmacist and data clerk's room. All patients having HIV positive HIV test result from different service outlets like Voluntary counseling and testing (VCT) clinic, ANC clinic, outpatient department, TB clinic, inpatient department or referred from elsewhere are able to get the service. The clinical services are provided by a team comprising of a doctor and a nurse with good continuity of care. The initial clinical evaluation and clinical staging is done by ART trained nurse. Subsequently patients will be evaluated ART physician for decision of eligibility of antiretroviral treatment and further screening for opportunistic infection. This activity may sometimes be replaced by nurse during the absence of physicians.

All the initial patient information will be documented on standard national forms called "ART intake forms" and "follow up" forms which are prepared by the MoH. The completed information on intake and follow up forms will be transferred to National ART/Pre ART registers every day by data clerks. Initial evaluation of patients includes medical history, physical examination and CD4 cell count. Follow-up appointment will be given one or two weeks later.

The patients will be clinically staged based on WHO criteria and CD4 count. The initiation of treatment is determined by clinical and immunological assessment. In such settings, hospitals, as CD4 tests are available treatment initiation is mainly guided by both WHO staging and CD4 criteria but ART may also be initiated with clinical parameter alone in times when CD4 test is not available. In addition to the medical eligibility, thorough assessment of patient adherence preparation and commitment is mandatory. Eligible patients will be put on cotrimoxazole prophylaxis therapy prophylaxis for minimum of 2 week with adherence counseling unless contraindicated and later re-evaluated for their preparation. The medical eligibility criterion of ART in Ethiopian context is presented in Table 2.

Once the decision to start HAART has been made, the next question will be which regimens to start. In our context we have few options of drugs so that the initial selection should be judged well according to the national guideline. ZDV3/3TC/NVP or EFV, D4T/3TC/NVP or EFV, TDF/3TC/NVP or EFV and ABC/3TC/NVP or EFV are the recommended 1st line regimen for adults and adolescents in Ethiopia.

Table 2 Showing eligibility criteria for ART in Adults and adolescents adapted from Ethiopian national ART guideline (6)

CD4 count not available	**CD4 count available**
WHO clinical stage IV and III irrespective of Total Lymphocyte Count (TLC)	WHO clinical stage IV, irrespective of CD4 count
WHO Clinical stage II if TLC <1200/ mm^3	WHO clinical stage III, if CD4 cell counts ≤ 350/mm^3
Do not treat WHO clinical stage I, in absence of CD4 count	All WHO clinical stages, if CD4 cell counts < 200/ mm^3
TLC is only useful in deciding when to initiate ART in symptomatic patients with WHO clinical stage II disease. The use of CD4 cell count to guide treatment decision is advisable. For example, pulmonary TB may occur at any CD4 level	

After ART is initiated based on the national guideline, patients will be advised to come after 2 weeks to determine toxicity/tolerance or adherence problems and managed accordingly. The following appointment time depends on the condition of patient but usually medication for one month will be given and advised to come after 28 days if are in good condition. Patients who missed appointments will be checked every day and tried to be contacted by data clerks through telephone within days but accessing those who don't have telephone is still a problem. A new patient tracing mechanism has been started

a year back by involvement of peer educators who often do planned home visit in patients lost from care or treatment.

After the 12th week of initiation of antiretroviral therapy, patients will be scheduled to return every eight weeks. At each visit antiretroviral drugs and CPT for two months are given and counseling of positive living, safe sexual practice, adherence assessment and support are done. Laboratory tests including liver enzyme tests are requested when indicated. CD4 test is repeated every 6 months and patients having signs of treatment failure or poor clinical response will be referred to tertiary care for viral load test and initiation of second line regimen.

5.2 Study design*:*

The study design was retrospective document analysis (i.e. Historical cohort) of HIV infected who started HAART in ALERT hospital.

5.3 Population:

5.3.1 Source population: were all HIV infected patients attending ART clinic of ALERT hospital.

5.3.2 Study participants: were all HIV positive patients at ALERT hospital who started first line HAART and who have been on ART for more than or equal to 6 months.

5.4 Sample size: Clinical documents of all patients who started HAART and who had a minimum of one follow up period were recruited.

5.5 Sampling procedures: General information about patients in the hospital enrolled during the study period was evaluated and those who did not fulfill the inclusion criteria or those who fulfilled the exclusion criteria were excluded. Using ART unique

identification number from ART register in ALERT hospital, documents of all patients enrolled for ART care between Dec 2004 and Dec 2010 were evaluated.

5.6 Inclusion Criteria

- ✓ All HIV positive adult patients above or equal to 15 years of age who started HAART between Dec 2004 and Dec 2010.

- ✓ HIV positive patients on HAART, who had at least been treated for 6 month, had a baseline CD4 determination and at least one follow-up CD4 determinations at or after 6 month.

5. 7 Exclusion Criteria

- ✓ HIV positive patients on HAART with a follow up period of less than six month.

5.8 Data collection procedures:

Data was extracted from medical records using the available standard national registers which have been adopted by the MoH. The first register was the Pre ART register in which confirmed HIV positive clients are registered at their first presentation. All patients who started ART were transferred to ART register at the date of treatment initiation which was another source of data. The third source of data was patient's follow-up form which was being completed for every patient during each follow up visit and information regarding progressive weight change, clinical stage, drug toxicity, adherence, new diagnosed OI and laboratory test results were documented. The data was collected by the principal investigator and 12 trained nurse from the hospital.

5.9 Measurement

5.9.1 Outcome Variables

- ✓ Change in immune recovery (i.e. CD4+ T cell number) between initiation of HAART and the most recent available follow-up times.

5.9.2 Independent variables

- ✓ Socio-demographic characteristics; Age, sex, marital status, educational status, employment, religion.
- ✓ Behavioral factors; Substance abuse(addiction) , adherence to treatment
- ✓ Baseline clinical information; Weight, WHO stage, functional status, past history of TB treatment, baseline regimens; substitution of regimen from baseline, OI at baseline.

5.10 Data Management and quality control:

Training material was developed and a two day intensive training was provided for the data collectors, supervisors and data entry personnel. Data quality was ensured through use of standardized data collection materials, pretesting and intensive supervision by the principal investigator. The collected data was checked for completeness and records with incomplete basic information were excluded from entry. Data was entered using Microsoft Access database with double entry and cleaned by trained data clerk and the overall process was supervised by the principal investigator.

5.11 Data Analysis procedures:

The entered data was checked for completeness and 4,419 records were used for analysis. After data was cleaned and checked for coherence appropriate analysis was done using SPSS version 20, STATA version 11 and Graph Pad Prism 5. Frequencies and

proportions were used to describe the characteristics of study participants in relation to relevant variables. p values less than 0.05 was considered as statistically significant.

5.12 Dissemination of results:

The survey report would be submitted to the department of Medical Laboratory Sciences, School of Medicine, College of Health Sciences, Addis Ababa University and Armaeur Hansen Research Institute (AHRI). The principal investigator would submit the study abstract to local associations like EMA, EPHA and EMLA to present the results of the survey during continuous medical education events organized through these associations. The summary of thesis would be submitted to international or national peer reviewed journal for possible publication.

5.13 Ethical consideration: After getting ethical clearance from the department of Medical Laboratory Sciences, School of Medicine, College of Health Sciences, Addis Ababa University and AHRI, the study proposal was submitted to ALERT hospital and permission was obtained to undergo the study. Any patient identifiers were removed. Trained nurses from the hospital collected patient data from the registers and records. All the collected patients' information was stored anonymous and data kept confidential. The results of the study would be communicated for the responsible stakeholders for ultimate service improvement and patient beneficence.

Result

Socio-demographic characteristics

Medical records of 4419 HIV infected individuals who have been taking highly active antiretroviral therapy between December 2004 and August 2010 at ALERT center were reviewed. Of these study participants, 61.6% were females, 42.9% were married, 57.0% were unemployed, 35.8% had secondary education, 40.6% belonged to the age group 31 to 40 years, and their median was 35 years (IQR: 29 - 40) (Table 3).

Table 3. Baseline socio-demographic characteristics of adult HIV-infected population at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

Characteristics	Number	Percent
Sex (n= 4,419)		
Male	1,696	38.4
Female	2,723	61.6
Age (n= 4404, Median=35, IQR[29-40])*		
15-24	280	6.4
25-34	1839	41.8
35-44	1528	34.7
≥ 45	757	17.2
Educational status (n= 4,369)*		
No Education	890	20.4
Primary	1,548	35.4
Secondary	1,566	35.8
Tertiary	365	8.4
Marital Status (n= 4,381)*		
Never Married	941	21.5
Married	1,880	42.9
Divorced	523	11.9
Widowed	898	20.5
Separated	139	3.2
Employment (n=4,267)*		
Working full time	1718	40.3
Working part time	56	1.3
Ill health	62	1.5
Unemployed	2431	57.0

*There were missing data

Baseline clinical information

Baseline clinical characteristics of the study participants are summarized in Table 2. The mean weight was 53.0 (IQR, 4559) kilograms. The mean weight was 57.7 Kg (SD=10.4) for males and 50 Kg (SD=9.5) for females. An independent samples t-test was conducted to compare the baseline weight for males and females. There was a statistical significance difference in weight for males (Mean=57.7, SD=10.4) and females [Mean=50.0, SD=9.5); t (4313) =24.9, *p*=0.000]. The magnitude of the difference in the means was large (eta=0.13).

The median CD4 count of the study participants at a baseline was 135 cells/ µl (IQR, 72-201) (Table 4). The median CD4 count was 120 cells/µl (IQR, 62-186) for males and 144 cells/µl (IQR, 79-209) for females. The difference was statistically significant (z =-7.232, *p*-value-0.0000, Mann-Whitney U test).

The proportions of patients with CD4 counts ≤50, 51-200, and ≥201 cells/ µl were 16.0%, 59.0% and 25.0%, respectively (Figure 1). In addition, 64.2% of the study participants had symptomatic disease with 52.0% WHO stages III diseases while 12.2% of them having WHO stages IV diseases. The vast majority (79.6%) of the study participants had a working functional status. Out of 4,323 study participants, 951(22.0%) had a record of past history of tuberculosis treatment. Moreover, 488(11.2%) of them had experience of taking antiretroviral treatment before they enrolled at ALERT center ART clinic.

Among the total 4419 reviewed medical records, 468 addiction habits were identified of which the frequent ones were alcohol (62.0%) and tobacco (20.0%). A habit of single addiction was identified in 73.5% while 16.5% had double habit of addiction.

Table 4. Baseline Clinical information of adult HIV 1/2 infected population in ALERT hospital Addis Ababa, Ethiopia, December 2004 to August 2010.

Characteristics	Number	Percent
CD4 cells/µl at baseline (n=4,419, Median=135 cells/ µl (IQR, 72-201)		
Weight (n= 4,315, Median=52 , IQR[45-59])*		
WHO Stage (n= 4,362)*		
I	430	9.9
II	1131	25.9
III	2269	52.0
IV	532	12.2
Functional Status (n= 3,999)*		
Working	3182	79.6
Ambulatory	690	17.3
Bedridden	127	3.2
Past history of TB treatment (n= 4,323)*		
Yes	951	22.0
No	3372	78.0
Past History of ART treatment (n= 4355)*		
Yes	488	11.2
No	3867	88.8

*There were missing data

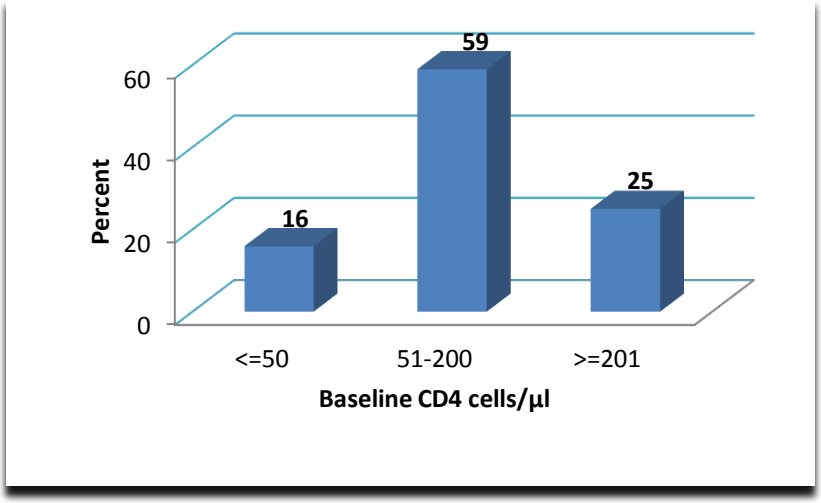

Figure 1. Distribution of baseline CD4+ T cells/µl during the initition of antiretroviral treatment at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010. ***Per cent values are shown on the bar graph.***

Reviewing of medical records of the patients also revealed that 69% had common opportunistic infections and co-morbidities during the initiation of antiretroviral treatment. The majority 1636(37%) had single opportunistic infections and co-morbidities followed by 21.0% with dual opportunistic infections and co-morbidities. As depicted in Figure 2, among the total opportunistic infections and co-morbidities, the common ones were Herpes zoster (1042, 20.7%) followed by skin disease (865, 17.1%), Candidiasis (775, 15.4%) and Pulmonary tuberculosis (637, 12.6%).

During the initiation of antiretroviral treatment, the most prescribed first line combination of antiretroviral treatment were 1a (30) D4T (30]/ 3TC/NVP accounting for 28.9% (1,278), 1c AZT/3TC/NVP accounting for 19.1% (844) and 1d AZT/3TC/EFV accounting for 18.4% (813).

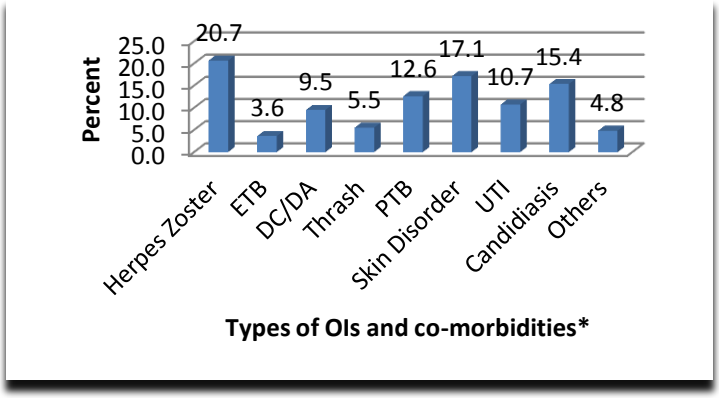

Figure 2. Distribution of OIs during the initiation of antiretroviral treatment for the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010. ***ETB=extra-pulmonary tuberculosis, DC/DA= Diarrhea chronic/ Diarrhea acute PTB=pulmonary tuberculosis, UTI= Urinary tract infection. Per cent values are shown on the bar graph.*

Follow up periods

The CD4 count at the first six months of follow up has shown dramatic increment across the age strata. Particularly patients with age group 15-24 years had the highest rate (21.8 cells per µl per month) of CD4 cells increment. Whereas age group above 45 years had relatively low rate of CD4 cells increment with a rate of 16.7 cells per µl per month during the six month follow up period (Table 5). The overall rate of increment during the first six month was 18.7 cells per µl per month.

The differences between the slopes during the 6 years follow up periods across each age group were highly significant. The slopes for the age groups 15-24, 25-34, 35-44, ≥ 45 were 6.5 (95% CI, 4.78-8.3), 3.4 (95% CI, 2.4-4.3), 3.3 (95% CI, 2.4-4.2), 2.6 (95% CI, 1.8-3.5), respectively (Figure 3A and Table 5). The overall slope during the 72 month follow up period was 3.3 (95% CI, 2.4 to 4.1).

As depicted on Figure 3B, the median CD4 counts during different follow up periods was determined for males and females and there was improvement overtime for both. Although there was no noticeable difference in the rate of change of CD4 cell count between the two sexes (F (1) =1.98, p =0.174), the difference observed at baseline was maintained for the follow up time.

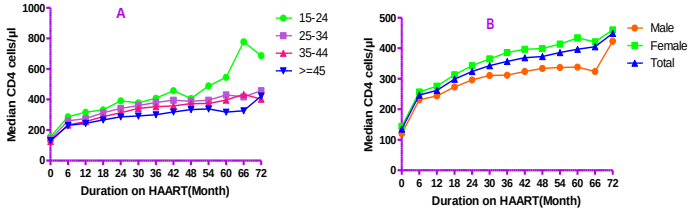

Figure 3. Distribution of the Median CD4 cells/µl at baseline and follow up periods stratified by age (A) and sex (B) for the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010. ***The respective number of study participants for 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 months of follow up periods were 4419, 4419, 2477, 2299, 2140, 2045, 1817, 1555, 1119, 642, 258, 97, and 26, respectively.***

Table 5 Median rates of CD4 cell increases (cells/ µl/month) at different months stratified by age of the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

Age Category	Time(Month)												Slope	95% CI
	6	12	18	24	30	36	42	48	54	60	66	72		
15-24	21.8	13.4	9.8	9.8	7.4	7.0	7.2	5.2	6.2	6.5	9.4	7.4	**6.5**	**4.78 to 8.3**
25-34	19.7	11.1	9.5	8.3	7.3	6.6	6.0	5.1	4.7	4.8	4.2	4.4	**3.4**	**2.42 to 4.3**
35-44	18.0	10.8	9.0	7.8	7.2	6.3	5.5	5.1	4.6	4.5	4.7	3.8	**3.3**	**2.4 to 4.2**
>=45	16.7	9.4	7.5	6.5	5.4	4.7	4.5	4.2	3.9	3.1	3.0	4.1	**2.6**	**1.8 to 3.5**
Total	18.7	10.6	9.1	7.9	6.9	6.2	5.6	5.0	4.6	4.4	4.1	4.4	**3.3**	**2.4 to 4.1**

Number in the body shows an increase in the median CD4 from baseline to the given follow up time divided by the length of that follow-up time

Separate analysis was performed to check changes in CD4 count overtime for the different baseline clinical characteristics. As shown in Figure 4, there was no noticeable difference in the rate of change of CD4 count between different patients defined by their baseline WHO stage, functional status, past history of ART treatment and past history of TB treatment. Moreover, the differences between the slopes were not statistically significant for all study variables listed above.

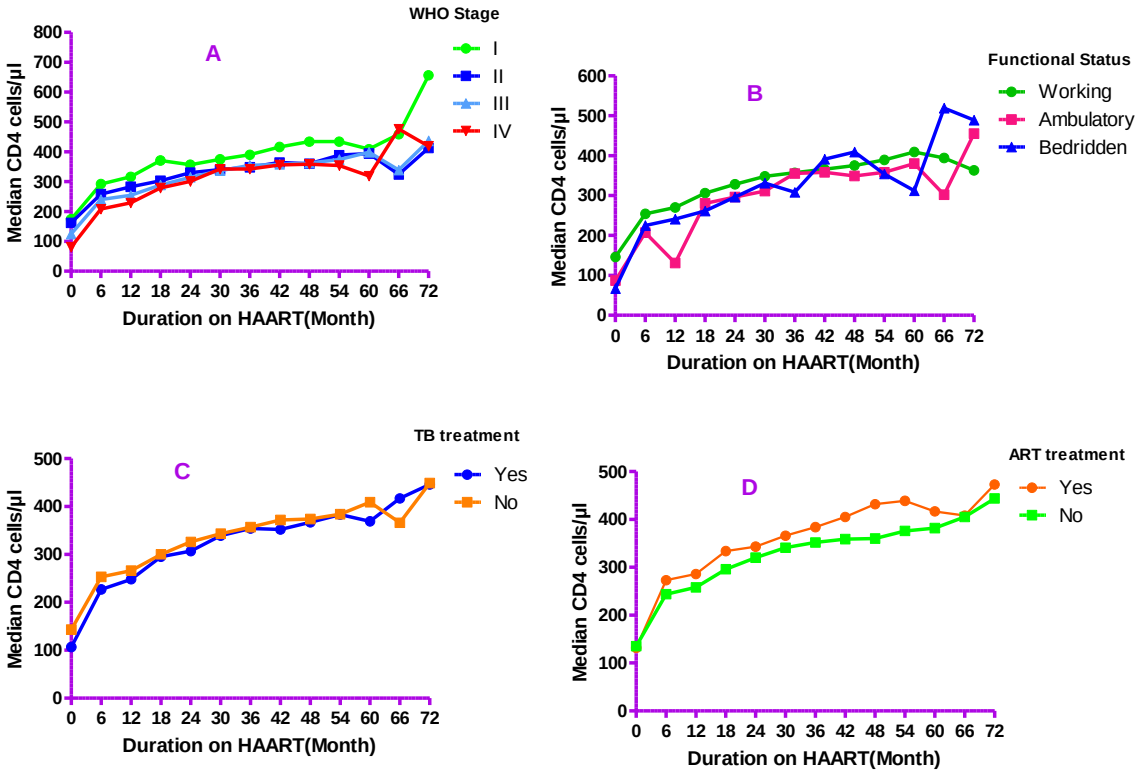

Figure 4. Distribution of the Median CD4 cells/µl at baseline and follow up periods stratified by WHO stage (A), Functional status(B), Past history of TB treatment (C) and Past history of ART treatment (D) for the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

Figure 5 shows the frequency distribution of CD4 counts of the study participants at the baseline and during follow up periods. Overtime there was a marked shift from baseline over the follow up periods of treatment, clearly demonstrating the effect of HAART. Moreover, While the proportion of individuals with CD4 count <=50 cell/ µl was 16% at baseline, this proportion decreased to 1% by the 12 months of treatment. Similarly, the proportion of individuals with a CD4 count ≥201 cells/ µl was 25% at baseline, but this proportion increased to 70% by 12 month and to 88% by 48 months of treatment (Figure 6).

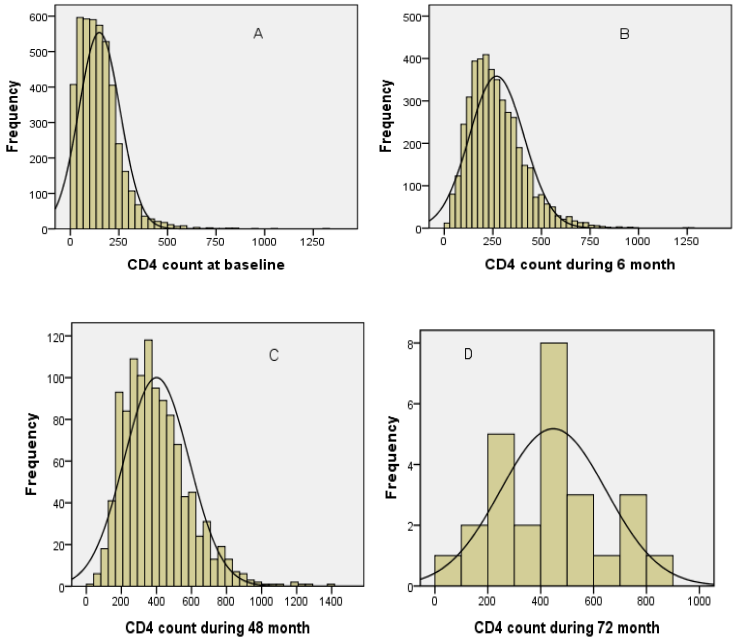

Figure 5. Frequency distribution of absolute CD4 counts (A) at baseline (N=4,419), (B) after 6 months (N=1,119), (C) after 48 months and (D) after 72 months of antiretroviral treatment at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

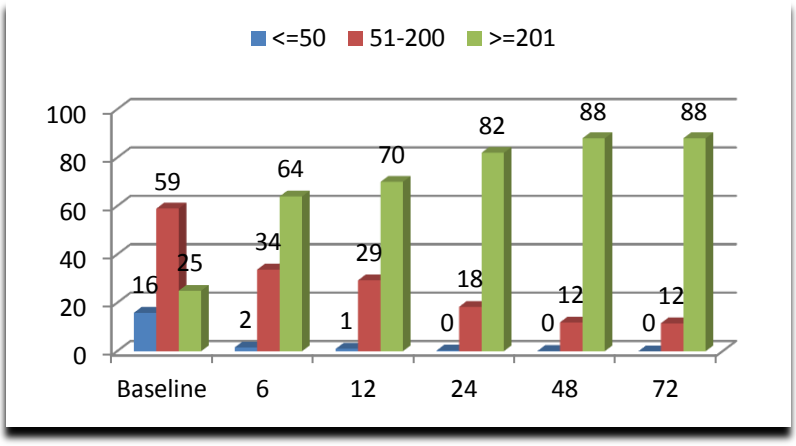

Figure 6. Distribution of CD4 cells/µl during the follow up periods of treatment at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

Analysis of the rate of CD4 change in the respective CD4 categories revealed that regardless of the baseline CD4 category, there was improvement in median CD4 count overtime. Of note, the immunologic response to HAART among those with a CD4 count <=50 cells/µl was excellent during the first six months of follow up period with a rate of 21.2 cells/ µl/month (Figure 6). The median CD4 cells/ µl change during the follow up periods across each baseline CD4 category had no a statistically significant difference. The slopes for the respective CD4 categories were 2.6 (95% CI, 1.2-4.1), 3.5 (95% CI, 2.7-4.3), 2.8 (95% CI, 1.8-3.8) (Table 6).

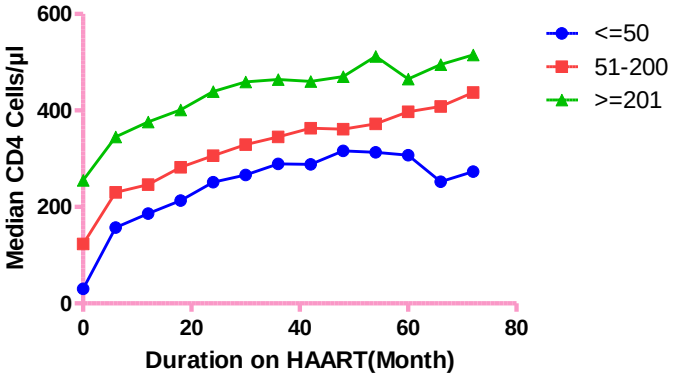

Figure 7. Distribution of the median CD4 cells/µl at baseline and during follow up periods stratified by baseline CD4 count of the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

Table 6 Median rates of CD4 cell increases (cells/ µl/month) at different months stratified by baseline CD4 counts of the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

CD4 cells/µl	Time(Month)												Slope	95% CI
	6	12	18	24	30	36	42	48	54	60	66	72		
<=50	21.2	13.0	10.2	9.2	7.9	7.2	6.1	6.0	5.2	4.6	3.4	3.4	**2.6**	**1.2 to 4.1**
51-200	17.8	10.3	8.8	7.6	6.9	6.2	5.7	5.0	4.6	4.6	4.3	4.4	**3.5**	**2.7 to 4.3**
>=201	15.0	10.3	8.5	7.7	7.1	6.1	5.0	4.7	4.6	3.5	3.4	3.4	**2.8**	**1.8 to 3.8**

Number in the body shows an increase in the median CD4 from baseline to the given follow up time divided by the length of that follow-up time

The mean weight was determined at different months of follow up periods for all study participants. Overall, at the end of the follow up period (72 months) the study participants have shown a mean weight increment of 6.2 Kg from the baseline. Males and females have gained a mean weight of 7.1 kg and 6.2 Kg at the end of 72 months of treatment,

respectively. Similarly, after 12 months of treatment 4 and 4.6 kg was the respective mean weight gain for male and female. However, there was no noticeable difference in the mean weight gain between the two sexes (F (2) =0.96, *p* =0.39), the difference observed at baseline was maintained for the follow up time (Figure 8).

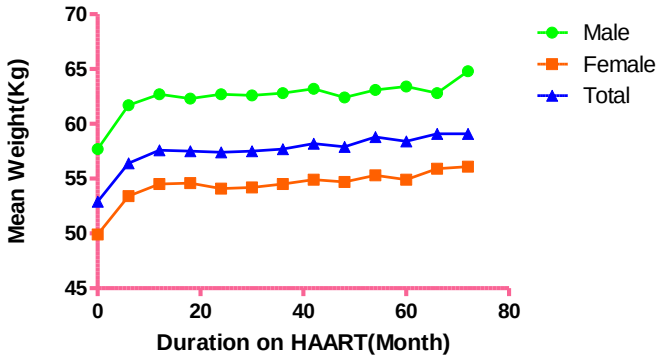

Figure 8. The trend of weight of the study participants at baseline and during follow up periods at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

After the initiation of antiretroviral treatment a total of 754 opportunistic infections and co-morbidities were recorded during the follow up months of treatment. Of these, UTI, pulmonary tuberculosis (PTB) and diarrhea acute/chronic (DC/DA) were the most prevalent opportunistic infections and co-morbidities with a proportion of 215(28.5), 185(24.5) and 77(10.2), respectively. UTI was the only opportunistic infection and co-morbidity which persist throughout the follow up months. But its frequency has shown a marked decrement at the end of 72 months of treatment (Figure 9). Generally there was a remarkable change of total OIs during the follow up periods.

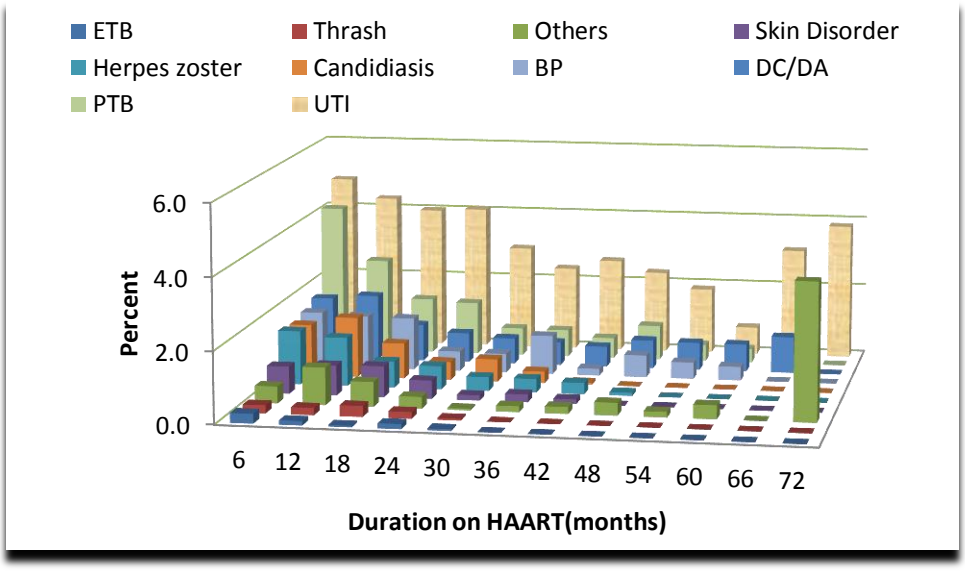

Figure 9. Distribution of opportunistic infections (OIs) after the initiation of antiretroviral treatment for the study participants at ALERT Hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

As indicated in Table 7, the presence of initial regimen modification was assessed and 1350 (30.5 %) of the study participants changed their regimen. The main reasons which contributed for their regimen modification were lipoatropy in (56.0%) cases, followed by toxicity in (22.6%) cases and new TB diagnosis in (7.3%) cases. Regimen change due to virologic failure immunologic failure, and clinical failure was recorder in 22, 18, and 11 cases of the study participants, respectively.

Table 7 Frequency distribution regimen change and reason for regimen change for the study participants at ALERT hospital, Addis Ababa, Ethiopia, December 2004 to August 2010.

	Value	Percent
Regimen change		
Present	1350	30.5
Absent	3069	69.5
Reason for regimen change		
Lipoatrophy	758	56.0
Toxicity	305	22.6
New TB diagnosis	98	7.3
Peripheral nephropathy	59	4.4
Anemia	37	2.7
Pregnancy	28	2.2
Others*	65	4.8

*Others include risk of pregnancy, new drug available, drug out of stock, clinical failure, immunologic failure, and Virologic failure

Discussion

The study reported herein aimed to determine the rate of long term immune recovery of adult HIV-1 infected patients taking HAART in ALERT hospital, Addis Ababa. In this study the majority of the study participants were females, which is consistent with other reports (Tsegaye and Worku 2011 and Alemu et al 2010) [61,62] and parallels with the national figure of higher proportion of HIV female population compared to males [6]. The baseline median age was 35 years (IQR, 29-40) and most of the study participants were between the ages of 25 and 34 years, a sexually more active age group and thus have a high risk of infection compared to the other age groups. This finding is consistent with other studies in Oromiyaa region of Ethiopia (Alemu et al 2010) [62] and that from the Botswana National Antiretroviral Treatment Program [63].

The observed difference in weight between males and females at baseline in the current study is comparable with a study from Felegehiwot referral hospital, Northwest Ethiopia, which found a baseline mean difference of weight between the two sexes [64].

At baseline, the median CD4 count of the study participants was 135 cells/ µl. The findings of the current study are consistent with those of Tsegaye and Worku (2011) [61] but slightly higher than the baseline median count of 103 cells/ µl reported by Alemu et al [62]. However, the result is lower than the reports from Swiss HIV cohort study, Switzerland [65]. Delayed presentation and/testing, differences in the criteria for HAART initiation, differences in educational and socio-economic levels could explain the observed differences. In our study, female HIV patients had higher median CD4 counts than males (p-value <0.0001) before HAART was initiated. This may be due to sex-related differences in the overall CD4 counts among males and females as reported by

Tsegaye et al in healthy Ethiopians; [66]. The finding was supported by studies by Abera et al from Ethiopia [60], Kippa et al from Uganda [67] and Nicastria et al from Italy [68].

Our data indicates that the majority of HIV patients started treatment with more advanced immunodeficiency status. Since the majority (75.0%) of HIV patients had AIDS as defined by their CD4 counts < 200 cells/µl. This was lower when compared to the 85% reported by Alemu et al (2010) [62] from Oromiyaa but higher when compared to the studies conducted in Vancouver, Canada which reported a lower rate (48.0%) of AIDS at the initiation of ART [69]. Similarly, the majority (64.2%) of the study participants had a symptomatic disease having WHO clinical stage of 3 and 4. This finding is in agreement with Alemu et al (2010) [62] and Tsegaye et al (2011) [61] findings which found high proportion of WHO clinical stage of 3 and 4. These findings are expected as the Ethiopian ART guideline was based on CD4<200 and WHO clinical staging depending on the patient's status to commence ART [6].

The current study found Herpes zoster was the most common OIs and co-morbidities before the initiation of HAART. However, this finding does not support the study from Botswana [63]. A possible explanation for this is that lack of adequate registration of opportunistic infections and co-morbidities at the time of patient enrollment and lack of a means for identification. Sixty nine percent of the study participants had one or more OIs and co-morbidities before the initiation of antiretroviral treatment. However, this proportion has not previously been described by Abera et al (2010) [60] and Alemu et al (2010) [62]. It seems possible that these results are due to difference with sample size. Another possible explanation for this is that all possible co-morbidities were not considered together with opportunistic infections.

This study demonstrates that the greatest increases in CD4 counts as a consequence of HAART occurred during the first 6 months of therapy for all age groups, although the increase continued with relatively lesser degree during the entire follow-up period. This observation is consistent with findings in the western populations. The largest gains in the number of CD4 cells occurred in the first 6 months after starting HAART, presumably because of redistribution of memory CD4 cells from lymphoid tissue. Thereafter, the rate of increase in CD4 cell counts gradually slowed [41].

After the initiation of antiretroviral treatment there was a median difference of CD4 count across the age group. Particularly, the younger age groups (15-24 years) had a better increment than the older age groups (45 and above years). This finding is in agreement with Kaufmann et al [44]. The younger age groups had a highest recovery rate of CD4 cells/µl (13.6 cell/ µl/month) at the first twelve months of treatment than the other age groups. It is encouraging to compare this figure with Bussmanna et al from Botswana who found the rate of 13.4 cell/ µl/month CD4 increment [63]. Another important finding was that, females had a better recovery than males although the observed slope difference between males and females in this study was not significant. This also accords with Nicastria *et al* observations [68], which showed that female participants maintained a higher CD4 count compared to males during the entire follow-up periods.

The results of this study indicate that the frequency distribution of blood CD4 counts shifted markedly from the baseline over the 48 months of follow up periods of treatment. And the proportion of individuals with CD4 cell counts <=50 cell/µl has shown much decrement over a period of time. The present findings seem to be consistent with Lawn *et al* report which revealed similar finding [70].

In the current study, there is an overall improvement in median CD4 count overtime. This observation remained true when analysis was done separately for the three CD4 categories (<50, 50-200, >200 cells/ µl). Although there is no noticeable difference in the rate of increases between different patients in the three CD4 groups as defined by baseline CD4 count, the difference observed at baseline was maintained for the follow up periods. However, the findings of the current study do not support the previous research from San Francisco General Hospital, USA by Lawn *et al* [70]. This could be due to the difference of the living style of the study participants between the two groups.

The result of this investigation show that males had increased weight significantly throughout the follow up periods in relative to females. Males and females had a respective mean increment of 7.1 and 6.2 Kg during the 72 months of treatment, while the combined overall mean increment was 6.2 Kilograms during the entire follow up period. However, the findings of the current study do not support the South African finding [72]. Moreover, Bizuwork et al reported a mean gain of 6.0 kg in men and 5.0 kg in women from Malawi [73]. In the present study, the mean weight change in males and females for the same duration of treatment (i.e., 4kg and 3.5 kg) was much lower than the Malawi report. Similarly, in one study by Fairall *et al*, found a mean weight increment of 7.2 Kg during the 20 months of follow up periods [57]. This finding is inconsistent with the current study which shows a mean weight increment of 4.5 kg from the baseline at similar period of follow up months. These differences can be explained in part by the difference with nutritional status of the study participants between the two groups.

In our study, regiment change was recorded in 30.5% of the study participants. Lipoatrophy, toxicity and new TB diagnosis were the main reasons for regimen

modification. This proportion of regimen change is quite small as compared to the study from Southern part of the Oromiyaa region, Ethiopia by Alemu *et al* which found 88.0% of the study participants had regimen change. Moreover, this study has identified toxicity and new TB diagnosis as the main reasons for regimen modification [62]. Another study from Italy found that 5.1% of the study participants discontinued their initial regimen due to immunologic, clinical and virologic failure [74]. This almost accords with our finding which revealed 3.8% of the study participants changed their regimen due to the above three reasons. Similar findings were reported in one hospital-based study from Arbaminch, Ethiopia, where a regimen change due to drug side effects (but not due to treatment failure) was observed [75].

Taken together, our study demonstrated the effectiveness of HAART with a dramatic rate of CD4 recovery during the first 6 months post HAART as reported elsewhere, regardless of patient baseline characteristics such as CD4 category and sex. The rise continues throughout the follow-up period though with a lesser extent after the initial dramatic increment. Moreover, the frequency of opportunistic infections dramatically declines upon initiation of antiretroviral therapy with the subsequent increase in CD4 count as reported elsewhere [76-79].

Strengths and Limitations

- This study involves large number of participants making it one of the strong studies measuring HAART outcome as measured by CD4 count. On the other hand, the participants' number has continuously declined during the follow up period. The reason for the loss to follow up was not investigated.

- To our knowledge the first study to evaluate the immune status of adult HIV infected in Ethiopian.
- The observed recovery might be affected by survival bias.

Conclusion

- Despite some of the limitations, the study has shown CD4 increment which is comparable with other developed and developing countries.
- In this study, females and younger age groups relatively (15-24 years) have shown a better immune recovery.
- Herpes zoster was the most predominant observed opportunistic infection before the initiation of antiretroviral treatment.
- There was no noticeable difference in the rate of change of CD4 count between different patients defined by their baseline CD4 cells and different baseline characteristics i.e. the difference observed during the baseline was maintained throughout the follow up periods.
- During the entire follow up periods, relatively male study participants had a better mean weight gain but it is not statistically significant.
- Sharp increment of CD4 cell/µl was observed during the first six months of follow up periods.

Recommendation

- Proper screening of opportunistic infections via laboratory, including diagnosis of co-morbidities and treatment, should be part of routine care.
- The patient retention mechanisms should be strengthened to address the higher lost to follow-up rate with regard to assessment of the patient immune status (CD4 measurement).
- Steadily decline in the rate change of immunologic and clinical parameters after the first 6 months of treatment should be investigated for possible treatment failure and drug resistance.
- Even though the current ART scale-up service has included more than a hundred thousand patients, this study could contribute to our understanding of the trend of CD4 cells recovery after antiretroviral treatment.
- The utilization of routine data should be encouraged in the Ethiopian setting for the improvement of patient outcomes.

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ANNEXES

I. Training Manual for supervisors, data collectors and data entry Personnel's

Part I: Introduction on the study

1. Introduction about the study: why needed? Its purpose

With the use of combination antiretroviral therapy (HAART), HIV mortality has declined dramatically. However, early initiation of HAART has been shown to increase morbidity and mortality. In resource limited countries like Ethiopia where viral load measurements are not widely available and will remain restricted because of cost and accessibility, the CD4+ T cell count remains the strongest predictor of HIV-related complications including treatment failure in patients on HAART. Past studies revealed that immunologic response after 6 months of HAART indicates a favorable outcome regardless of virologic response. Monitoring immunologic function through periodic CD4+ T cell count in HIV positive patients on HAART is most widely used in Ethiopia. In Ethiopia where nucleoside only regimens are used as first line regimens, sufficient data on recovery of immune status is unavailable.

The critical decision on when to start HAART dictated by the rate of recovery of immune status of HIV patients, and this can be measured in three ways: clinically, by disease progression and WHO staging; immunologically, using trends in CD4+ T cell counts over time, and virologically, by measuring HIV viral loads. However, the definitions of clinical, immunological and virological failure currently used in different settings represent different biological end-points. It is not clear which criteria are optimal, as either individual measures or a mix of measures. Recent studies suggested that baseline

and serial CD4 counts better predict HIV disease progression than plasma viral load measurements.

In Ethiopian Guideline for antiretroviral treatment in adults and adolescents, immunologic failure is defined as; Fall of CD4 count to pre-therapy baseline (or below), 50% fall from the on-treatment peak value (if known) and Persistent CD4 levels below 100 cells/mm3.

Various studies in different settings revealed that factors associated with insufficient immune recovery include poor adherence, initiation of HAART at low CD4 count and high viral load at baseline, more than one missed clinic visits, older age, and HIV risk behaviors. Of these, poor adherence, more than one missed clinic visits, low CD4 at initiation of HAART have been shown to be most important predictors of immunologic failure.

As discussed above, regular CD4 cell monitoring is the widely available and best monitoring tool in Ethiopia to identify the ever growing immune status of patients on HAART. However, some important questions have to be answered. First, there is no sufficient data to show the magnitude of immunologic failure in Ethiopia. Second in Ethiopian context, behavioral and clinical factors which predict unfavorable outcome like immunologic failure in HIV patients who start HAART were not well defined. It would be best to look for ways of predicting immune recovery will help to identify those clients that are at high risk. If we are able to identify predictors of worse outcome in HAART, it will be easy for HAART providers to identify and exclude high risk patients for more aggressive follow up and therapy.

2. Title of the research project

A rate of long term immune recovery of adult HIV patients taking HAART in ALERT hospital, Addis Ababa, Ethiopia

3. Objectives of the study

To assess the immune status of adult HIV patients who have been on ART from 1-5years and identify factors influencing immune- recovery.

4. Methods of the study (Brief)

a. **Design**

The study design will be retrospective (historically cohort) follow-up study on HIV infected patients who are started on HAART in ALERT hospital.

b. Study Population

The actual study population will be HIV positive patients on HAART at ALERT hospital

c. Sample size: All clinical documents of HIV infected patients who have been taking HAART in ALERT hospital ART clinic.

d. Sampling methods

ALERT hospital is selected to have optimal follow-up period. General information about patients in the hospital enrolled during the study period will be evaluated and those who do not fulfill the inclusion criteria or those who fulfill the exclusion criteria will be excluded. Then using ART unique identification number from ART register in the hospital, a systematic sampling method will be used to draw the required sample size. If the selected sample is excluded, the next consecutive number will be included.

e. Data collection tools

Data will be collected using abstraction form ART registers and intake formats which is pretested. Existing intake formats, ART and Pre-ART registers, follow-up formats, cohort analysis formats, clinical notes, laboratory requests will be reviewed

f. Ethical issues

Ethical Clearance will be obtained from AHRI/ALERT and department of Medical Laboratory Sciences of Addis Ababa University. All data will be collected retrospectively from records and there will no contact with individual patients.

Part II. Training purpose and arrangements

1. The purpose of training

- Ensuring data quality is very important for many reasons including;
- Solid and reliable data supports conclusions and recommendations,
- Future policy decisions may rely on the evidence generated in the study
- Results will be publicly accessible and may be used by others and critics and opponents will look for weakness in the survey methods and results.

Past experience showed that errors during data collection and entry are common which in many occasions lead to wrong and unreliable conclusions. Data collection errors need to be verified and corrected or deleted from the results. If large segments of incorrect or unreliable data have to be excluded from the analysis or re-collected, the overall survey results will be weekend and resources will be wasted, since time and efforts have been spent on collecting data that cannot be used. There are several reasons for the data problems commonly encountered as part of a survey:

- Survey personnel may not be well acquainted with the procedures or may misunderstood it
- Supervisors, data collectors and data entry personnel may receive insufficient or poor-quality training
- The Pretest may not be conducted properly
- Work in the field may be of poor quality (insufficient supervision, no quality control for submission of completed forms, misunderstanding of instructions, etc.)
- Data were not checked at every stage of the survey process
- Data were entered only once (double entry not used)

Training survey personnel is an important element of survey preparation because it helps ensure data quality through accurate and reliable data-gathering and data entry procedures. Therefore, this training manual is prepared to train supervisors, data collectors and data entry personnel's on all the procedures that need to be followed

2. Overview of the training

Two day training will be organized and implemented before the start of the actual data collection. All personnel involved in data collection, supervision and data entry will be trained with an overall purpose of ensuring reliable and accurate data collection, completion of the data collection form and transfer of data to the database. The training fostered an appreciation among survey personnel of the importance of generating high-quality data and also will ensure a common understanding of the terms and definitions used in the study.

Overall Training Objective:

To provide supervisors, data collectors and data entry personnel with the knowledge and skills will be required to carry the study in an accurate and reliable manner.

Specific Learning Objectives;

Upon completion of the training, participants would:

- Be familiar with the key aspects of the survey and how it will be conducted;
- Understand their roles and responsibilities in the survey, including specific tasks, timelines and reporting requirements;
- Understand the critical content required to do their job effectively and possess the skills required to undertake each of their activities;
- Be aware of common issues that may arise during survey activities, and troubleshooting/problem-solving strategies to address these issues; and
- Recognize the intrinsic value of good-quality data and be motivated to ensure data quality as part of their activities.

The training will be provided by the primary investigator and AHRI Statistician group. The training venue was in AHRI meeting hall.

3. Training methods

Over the two days period the training will be addressed the following issues;

- The overall purpose and objective of the study;
- The consequences of poor-quality data;
- How to complete the Data Collection form;
- How to solve problems during data collection;
- How to enter data into the data base (double-entry feature); and

- Common data collection and data entry mistakes

The training will be provided using the following training methods

a) Lectures through presentation

The power point focused on the following area

- Introduction to the study
- Study overview; purpose, objective, methodology etc.
- Preparing for data collection
- Completing the data collection form
- Data entry
- Data quality and checking

b) Handouts

- Instructions for supervisors
- Instructions for data collectors
- Instructions for data entry personnel

c) Exercises

- A „Spot the mistakes exercise; addressed common mistakes during data collection and entry
- A data-checking exercise; participants will work on factious data developed to assist personnel in identifying common data errors.

4. Materials required

Trainer's material

- Training Manual

- Power-point slides
- Flip chart and paper
- Markers

Trainee's material

- Name card for each participant
- Training agenda for each participant
- Copy of presentations for each participant
- Copies of Data Collection forms per participant
- Note pad, pen, for each participant

5. Roles and responsibilities

Data collector

- Data collectors will be responsible for visiting patient records, registers and information and recording it on data collection checklist with a high degree of accuracy. Data collectors have to the following skills and capabilities:
- A nurse working in the ART clinic with basic understanding of ART related terminologies and familiar with clinical registers and records
- Some understanding of the principles of sample surveys, ideally with some previous experience in data collection;
- Accurately fill survey checklists and directly forward it to data entry personnel
- Notify supervisor regarding any issues that will raised during data collection
- Ensure that all collected data is placed in secure place

Data entry personnel

- Accurate data entry is vital to ensure the reliability of the results.

- Experience in using Microsoft Word Access or Epi info will be required:
- Two data clerks, one to enter the data and the other to re-enter the same data to check that the entries are correct
- Keep all study data in secure place

Supervisor

- Supervisors are responsible for overseeing all aspects of data collection. They should have a crucial role to play in ensuring data quality and consistency.
- Supervisors should have experience in data collection and entry and be familiar with the survey's terminologies.
- Supervisors are responsible for choosing data collectors and data entry personnel in collaboration with the PI.
- Responsible for coordination between data collectors and data entry personnel.

Primary Investigator

- The PI is responsible for the overall planning and coordination of the study. This includes;
- Planning the study's technical and logistical aspects,
- Recruiting and training survey personnel,
- Supervising data collection and data entry,
- Conducting data quality assurance and data analysis,
- Interpreting results and preparing a survey report.

II. Data Collection Format for long term immune recovery of adult HIV infected patients taking HAART in ALERT hospital as measured by CD4 T cells Addis Ababa, Ethiopia.

Medical Record Number_____Serial Number _____

NO	Variable	Coding category
101	Age at enrolment	_____ Year
102	Sex	1. Male 2. Female
103	Educational Status	1.No education 2.Primary 3.Secondary 4.Teritary
104	Marital Status	1. Never married 2. Married 3. Divorced 4. Widowed 5. Separated
105	Employment	1. Working full time 2. Working part-time 3. Ill health 4. Unemployed
Part B Baseline clinical information		
201	Weight	_____ Kg
202	WHO stage	1. I 2. II 3. III 4. IV
203	Functional Status	1. Working 2. Ambulatory 3. Bedridden
204	Past history of TB Rx	1. Yes 2. No
205	Past history of ART Rx	1. Yes 2. NO
206	OIs	**1**. Zoster **2**. ETB **3**. DC/DA **4**. BP **5**. Thrash **6**. PCP **7**. PTB **8**. Ulcers **9**. CM **10**. Toxoplasmosis **11**. CNS **12**. Skin Disorder **13**. UTI/URTI **14**. Candidiasis **15**.Others_____
207	CD4 + T cell number	_____ cells/mm^3
208	Addiction	1. Tobacco 2. Alcohol 3. Drugs 4. Others

	First Line	Second line
209 Regimen	1. 1a (30) D4T (30)/3TC/NVP 2. 1a (40) D4T (40)/3TC/NVP 3. 1b (30) D4T (30)/3TC/EFV 4. 1b (40) D4T (40)/3TC/EFV 5. 1c AZT/3TC/NVP 6. 1d AZT/3TC/EFV **7. TDF/3TC/EFV** **8. TDF/3TC/NVP**	9. 2a= ABC/ddl/LPv/r 10. 2b=ABC/ddl/NFV 11. 2c= TDF/ddl/LPv/R 12. 2d=TDF/ddl/NFV

C. Clinical Information during Follow up Periods

	301 weeks on ART	302 Weight	303 WHO Stage				304 Adherence			305 Functional Status			306 CD4	307 Type of OIs
			I	II	III	IV	G	P	F	W	A	B		
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														

	First Line	Second line
308 Regimen	1. 1a (30) D4T (30)/3TC/NVP 2. 1a (40) D4T (40)/3TC/NVP 3. 1b (30) D4T (30)/3TC/EFV 4. 1b (40) D4T (40)/3TC/EFV 5. 1c AZT/3TC/NVP 6. 1d AZT/3TC/EFV **7. TDF/3TC/EFV** **8. TDF/3TC/NVP**	9. 2a= ABC/ddl/LPv/r 10. 2b=ABC/ddl/NFV 11. 2c= TDF/ddl/LPv/R 12. 2d=TDF/ddl/NFV

309. Presence of drug change 1. Yes 2. No

310. If there is regimen change, what is the reason for change?

1. Toxicity
2. Pregnancy
3. Risk of pregnancy
4. Due to new TB
5. New drug available
6. Drug out of stock
7. Lipoatropy
8. Clinical failure
9. Immunologic failure
10. Virologic failure
11. Others

Collected By_____ Date of Collection _____ Signature _____

Checked By_____Signature_____