Predictors of CD4 Count Changes after Initiation of Antiretroviral Treatment in University of Gondar Hospital, Gondar in Ethiopia

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Abstract

**Background:** The effort for preventing HIV/AIDS (Human Immune Deficiency Syndrome/Acquired Immune Deficiency Syndrome) ranges from behavioral intervention to introduction of antiretroviral Treatment (ART) program. ART has dramatically improved the livelihood of people living with HIV/AIDS. World Health Organization (WHO) recommends the optimum time for initiating ART should be guided by CD4 (Cluster Differentiation 4) count and clinical staging. Predictors of the change of CD4 count after initiation of ART are important for patient monitoring and AIDS prognosis prediction. This study aimed to investigate predictors of CD4 count change among patients on ART in University of Gondar Hospital, North West Ethiopia.

**Methods:** A cross sectional study was conducted among HIV/AIDS patients taking ART. A total of 2935 adults having at least two CD4 count values were included in the study. The study used both the ART data base and reviewed patient charts. The primary outcome measure was CD4 count change. Correlation and multiple linear regression analysis were used to identify predictors of CD4 count change.

**Result and discussion:** The median CD4 count has increased from 139 cells/ul at the initiation of ART to 356 cells/ul at the most recent visit. A median CD4 count change of 208 (IQR 224) cells/microliter was observed after 194.4 (IQR 148.6) weeks on ART. The median rate of CD4+ T cell increase was 1.06 cells/week on ART. Age (β = 97.59, p=0.000), Baseline CD4 count (β = 0.222, p=0.000), hemoglobin level (β = 4.029, p=0.000) were significant predictors of CD4 count change. Patient’s functional status when commencing ART, WHO clinical stage, ART adherence status, cotrimoxasole adherence status, educational status, marital status were also found to be significant predictors of CD4 count change.

**Conclusion:** Age when starting ART, educational status, marital status, WHO clinical staging, baseline hemoglobin level, baseline CD4 count, ART adherence status, cotrimoxasole adherence status, functional status, and recent follow up CD4 are significant predictors of CD4 count change. Clinicians need to closely monitor patients who initiated ART at a lower baseline hemoglobin level, and/or CD4 count level.

ABBREVIATIONS

AIDS: Acquired Immune Deficiency Syndrome; ART: Anti-Retroviral Treatment; CD4: Cluster Differentiation 4; CD8: Cluster Differentiation 8; CDC: Center for Disease Control; HAART: Highly Active Anti-Retroviral Treatment; HIV: Human Immunodeficiency Virus; i-Tech: International Training and Education and Center on HIV/AIDS; WHO: World Health Organization

INTRODUCTION

International and national guidelines advocate the use of CD4 count for treatment decisions, as a predictor of disease progression, a criterion for treatment initiation, and as a marker of treatment outcome in both adults and children. Therefore it is recommended at multiple instants in the course of patient care. After tested for HIV, positive results will go to CD4 count for staging the disease and assessing eligibility for ART. Most guidelines say an adult patient is eligible for ART, if his/her CD4 count is less than 200 or 350 cells per micro liter. After starting ART it is recommended to have CD4 counts every 3-6 months, but if a patient is not initially eligible, it is recommended to have every 6-12 months. As ART program is expanding the need for CD4 count becomes very high [1].

The effort for preventing HIV/AIDS ranges from behavioral intervention to introduction of ART program [2]. Once ART is started, it is needed to take the treatment throughout lifetime, and because of the associated side effects long term continuation is found to be a major challenge. Because of this problem, WHO recommends the optimum time for initiating ART should be
The change in CD4 count is the difference between baseline CD4 and most recent follow up CD4 counts. The baseline CD4 count is the initial CD4 count measured when a patient is ever enrolled on ART. There will be follow up CD4 counts measured after being enrolled on ART to assess immune system reconstitution. Therefore there will definitely be a change in CD4 counts, either negative, zero or positive. CD4 count change is affected by numerous predictors so this study will investigate different factors associated with CD4 count change after initiation of ART.

**OBJECTIVES**

The objective of this study is to identify predictors of CD4 count changes in University of Gondar Hospital, North West of Ethiopia, 2013.

**METHODS**

**Ethical considerations**

Ethical approval was obtained from the institute of Public Health and college of Medicine and Health Science, University of Gondar and support letter was obtained from University of Gondar Hospital. This research was done using secondary data and all study subjects within the ART data base are anonymized for the purpose of de-identification and maintaining confidentiality. The data set is kept confidential. It is protected by using password to protect it from unwanted manipulations and unethical usage.

**Study setting**

This study was conducted in University of Gondar Hospital in 2013. University of Gondar Hospital started free ART service since March 2005. In December 2012, the university hospital reported a total of 6444 patients have ever enrolled for ART, among these 3888 (1561 male, 2327 female) were reported actively taking ART. 77 (36 male, 41 females) of the 3888 are taking ART in University of Gondar Hospital. The hospital’s ART Clinic is constructed by 2 physicians, 1 Master of Public Health professional (RH), 2 Health Officers, 6 Nurses (2 Degree Nurse, 4 Diploma Nurses), 2 data clerks, 2 data base cleaners. The facility is linked with laboratory department which has CD4 counting machines (1 Celldyn and 1 FACS Calibyur CD4 counting machines).

**Study design**

Cross sectional study was conducted. Variables were taken both from the ART data base and patients’ cards. The primary outcome measure was CD4 count change which can be calculated by subtracting baseline CD4 count from most recent follow up CD4 count. Socio-demographic variables, baseline and follow up clinical as well as laboratory variables were included as independent variables.

**Data collection**

Data was collected both from ART data base and from the review of patient charts. The ART electronic data base was an...
MS access database composed of many tables and relationships including the baseline and the follow up tables. Around 20 variables were taken from the MS Access database and changed into the excel spreadsheet format.

As discussed earlier, part of the data was collected from the ART database, and it was collected by the ART database administrator, and the manually entered data was collected by one ART Nurse working in the ART clinic and Card Clerk working together. The data base which is collected for their own purpose does not include all the required variables important for predicting CD4 count changes. That is why there was a need to include excel manual extraction format prepared by the investigator for the purpose of this research. One card clerk and one computer literate ART nurse entered the manual extraction format from the patient card folders by taking out the patient card folders from the archive department. At the same time, this manually extracted document is appended with the electronic data sets by using the patient identifier code called Medical Record Number. With this number, the data base and the data collected from the manual patient card folder are connected together to produce one excel spreadsheet data format with the required variables. From this excel sheet, some variables like CD4 count change, time gap in week, time gap in month, and the number of days added.

The formula comes from the idea that there are 7 days in a week, there are 30/4 weeks in a month and there are around 52.14 weeks in a year. Using this concept, the following equation was derived. Day, month, and year were written in the Ethiopian calendar in separate columns in excel sheet. The Ethiopian calendar has 13 months in one year and 30 days in each month but the 13th month has only 5.25 days. Therefore the formula doesn’t work for the 13th month, Pagume. To avoid this problem, while entering the data, if we have any date in Pagume, we need to subtract the number of days from the date (MM/DD/YYYY) when most up-to-date CD4 count is measured. It can be simplified by adding the days in pagume, and the follow up date will also slide to another new date by considering the number of days added.

Duration of ART = ((Follow up day – ART start day)/7 + (Follow up month – ART start month)*30/4 + (Follow up Year – ART start year)*52.14)

Assume that, ART start day as “d1”, follow up day “d2”, ART start month “m1”, follow up month “m2”, ART start year “y1” and follow up year “y2”, it can be simplified by the following equation.

Duration of ART = ((d2-d1)/7 + (m2-m1)*30/4 + (y2-y1)*52.14)

Data quality

The principal investigator gave training how to fill the manual extraction formats and supervises the overall quality of data collection process and also the investigator was together with the data collectors in almost all the time during the data collection.

While filling the excel manual extraction format part of the data was checked by crosschecking the electronic based data sets with the paper based documents and check out for matches and also many of the manually filled documents were cross checked for similarity and consistency with the electronic data sets.

Source population

All HIV positive patients dataset is present in the ART clinic.

Study population

All adult ART clients dataset which is registered in the ART data base and has baseline and follow up CD4 counts was included in the study.

Inclusion criteria: All adult (age greater than 14) ART clients who started ART and have baseline and follow up CD4 count after starting ART.

Exclusion Criteria: Clients who started ART and their information is incomplete, unreadable or their manual record is lost, and also clients who have not at least one follow up CD4 count measure.

Sample size and sampling procedures

From all the 3888 adults who ever started ART and actively taking the drug during the time this investigation is undertaken, a total of 2935(75.5%) were included in this study. The remaining ART users were under the exclusion criteria and were not included.

Operational definitions

∆CD4: it is the difference between baseline CD4 and most recent follow up CD4 counts

Time gap: the difference between the date (MM/DD/YYYY) when 1st CD4 count is measured at the start of ART and the date (MM/DD/YYYY) when most up-to-date CD4 count is measured. It is calculated by the following formula entered in excel functions.

Duration of ART in weeks = ((d2-d1)/7 + (m2-m1)*30/4 + (y2-y1)*52.14)

Good ART Adherence: People living with HIV/AIDS on antiretroviral therapy registered to have taken 95% or higher of their prescribed ART medication or missed <= 3 doses as to their agreement with health care provider.

Poor ART Adherence: level of Adherence below 95% of their prescribed ART medication or missed >3 doses as to their agreement with health care provider.

Good Cotrimoxazole adherence: Patients who are on ART and are registered to have taken 95% or higher of the prescribed cotrimoxazole medications or missed <= 3 doses as to their agreement with health care provider.

Poor Cotrimoxazole adherence: Registered level of Adherence below 95% of their prescribed Cotrimoxazole medication or missed >3 doses of cotrimoxazol as to their agreement with health care provider.

Data processing and analysis

The data identified for data analysis was collected,
preprocessed, assessed, consolidated, cleaned, recoded, transformed and changed to appropriate format to be ready for analysis.

The MS excel 2013 spreadsheet format is transported in to SPSS version 20, variables recoded and analyzed. Descriptive statistics were used to describe the socio-demographic characteristics of study participants. Pearson correlation and point biseries correlation statistics were computed to investigate the correlation between the independent variables and dependent variable. Bivariate and multivariate linear regression analyses were used to identify associated predictors. Model fit was examined using Omnibus comparison test. Tolerance or Variance inflation factor, Eigen values and condition index were used as collinarity diagnostic tools. Regression coefficients of the final model and their 95% confidence intervals were used as measures of association between the predictors and dependent variable. A p value of less than .05 was considered to be statistically significant.

RESULT
Baseline characteristics
Of the total of 2935 patients who were included in this study, 60.9% (1785) were females and 48.6% (1426) of them were married. The mean age of the clients is about 33.5 years (SD 8.63), nearly half of them (48.6%) were married (Table 1).

The mean baseline hemoglobin level was 13.2 mg/dl (SD 2.27). The mean weight and CD4/CD8 ratio of patients when started the ART was 49kg (SD 9.232) and 0.19(SD 0.15) respectively. The median CD4 count, CD8 cell count and platelet count of patients was 139 cells/μl, 853cells/μl, 259,000 cells/μl respectively. Of the total patients who started ART, 79.3% of patients were with functional status of working and 60.7% of patients were on WHO stage III at the initiation of ART. About 37% of patients were taking AZT-3TC-NVP ART regimen. And for 33.4% of patients who were on ART, their original regimen was changed to other combination during their follow up period (Table 2).

The median CD4 count has increased from 139 cells/ul, at the initiation of ART to 356 cells/ul, at the most recent visit. A median CD4 count change of 208 (IQR 224) cells/μl was observed after 194.4(IQR 148.6) weeks on ART (Figure 1). The median rate of CD4+ T cell increase was 1.06 cells/week on ART.

Predictors of CD4 count change
A multivariate linear regression model is built and the model fit shows 80.7% of the variability in CD4 count change is explained by the model. The mode is significant: Omnibus test (F=219.925, p<0.001). Multicollinearitv among predictors was less evident, because the variance inflation factor, Tolerance, Eigen values and the condition indices were all very good.

The factors found to predict CD4 count and their associated beta coefficients are shown in Table 3. In univariate analysis baseline CD4 count (r=0.104, p=0.000), age at the initiation of ART(r=0.289, p=0.000), duration of ART(r=0.268, p=0.000) and recent follow up CD4 count (r=0.885, p=0.000) were significantly correlated with CD4 count change. Sex (point bi-series correlation r= -0.1, p=0.000), poor ART adherence (Point bi-series correlation r=-0.197, p=0.000), poor Cotrimoxasole adherence (Point bi-series correlation r=-0.16, p=0.000) were also significantly correlated with CD4 count change.
It is known that many factors could influence CD4 count change. In a multiple linear regression analysis model that included many factors, the association of several factors with CD4 count change was investigated. Variables including age at the initiation of ART ($\beta=97.59$, $p$ value 0.000), baseline CD4 count ($\beta=0.22$, $p$ value 0.000), baseline hemoglobin level (Beta=4.029, $p$ value 0.000), poor ART adherence status ($\beta=-111.2$, $p$ value 0.000), poor Cotrimoxasole adherence status (Beta=-60.88, $p$ value 0.014), secondary Educational status ($\beta=11.2$, $p$ value 0.024), bedridden functional status of the patient at the initiation of ART($\beta=-22.13$, $p$ value 0.016), WHO clinical stage of the patient and recent follow up CD4 count were significantly associated with the CD4 count change (Table 3).

Sex, religion, employment status, CD8 cell count, CD4/CD8 ratio, platelet count, the type of ART regimen at start, condition of

| Predictor Variable | Pearson Correlation or Point-Biseries Correlation | Regression | | | |
|---|---|---|---|---|---|---|---|
| Intercept | R | P value | Coefficient | SE | 95% CI | P value |
| Baseline CD4 count | 0.104 | 0.000 | 0.222 | 0.058 | 0.108, 0.336 | .000 |
| Hemoglobin | 0.056 | 0.000 | 4.029 | 1.573 | 0.946, 7.113 | .01 |
| Education | | | | | | | |
| No formal education | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Primary | -0.030 | 0.073 | 0.66 | 4.88 | -8.9, 10.2 | .897 |
| Secondary | 0.034 | 0.051 | 11.2 | 4.98 | 1.45, 20.97 | .024 |
| Tertiary | 0.048 | 0.011 | 4.24 | 7.29 | -10.06, 18.53 | .124 |
| ART adherence status | | | | | | | |
| Good | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Poor | -0.197 | 0.000 | -111.2 | 21.98 | -154.3, -68.01 | .000 |
| Cotrimoxazole adherence status | | | | | | | |
| Good | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Poor | -0.16 | 0.000 | -60.88 | 24.79 | -109.49, -12.27 | .014 |
| Functional status | | | | | | | |
| Working | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Ambulatory | -0.065 | 0.001 | 0.58 | 4.88 | -8.98, 10.14 | .905 |
| Bedridden | -0.004 | 0.42 | -22.13 | 9.17 | -40.11, -4.14 | .016 |
| Marital Status | | | | | | | |
| Single | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Married | 0.047 | 0.013 | 11.78 | 5.47 | 1.05, 22.5 | .031 |
| Divorced | -0.009 | 0.337 | 17.4 | 6.24 | 5.17, 29.6 | .005 |
| Separated | -0.011 | 0.297 | -5.69 | 12.05 | -29.32, 17.95 | .637 |
| Widow/widower | -0.008 | 0.345 | 16.7 | 7.15 | 2.68, 30.71 | .02 |
| WHO clinical staging | | | | | | | |
| Stage I | Ref | Ref | Ref | Ref | Ref | Ref | Ref |
| Stage II | -0.061 | 0.002 | 23.01 | 7.24 | 8.8, 37.21 | .002 |
| Stage III | 0.075 | 0.000 | 27.28 | 6.3 | 14.92, 39.63 | .000 |
| Stage IV | 0.047 | 0.011 | 47.61 | 7.67 | 32.57, 62.65 | .000 |
| Recent follow up CD4 count | 0.885 | 0.000 | 0.79 | 0.009 | 0.77, 0.81 | .000 |
| Age | 0.289 | 0.000 | 97.59 | 12.9 | 72.23, 122.95 | .000 |
regimen change during treatment, current ART regimen, duration of ART, Liver function test and Renal function test results were not found to be significantly associated with CD4 count change.

**DISCUSSION**

This study aimed to investigate the predictors of CD4 count change among patients on antiretroviral treatment in University of Gondar hospital, North West Ethiopia. The findings of this study shows that baseline CD4 count (β =0.222, p=0.000), hemoglobin (β =4.029, p=0.000), age (β =97.59, p=0.000) were significant predictors of CD4 count change. Patient’s functional status when commencing ART, advanced WHO clinical stages, poor ART adherence status, poor cotrimoxasole adherence status, educational status, marital status were also found to be significant predictors of CD4 count change. Studies have shown that starting ART at higher CD4 count has better immune reconstitution and better CD4 count results [13].

A study done in Boston Massachusetts, 2005 reported that hemoglobin level and sex were significant predictors of CD4 count [14].

Patients who were bedridden when starting ART predicted a reduction in CD4 count change by 22.13 times (p value 0.016). If a patient on ART has poor antiretroviral and cotrimoxasole adherence status, there will be a reduction in CD4 count change by 111.2 (0.000) and 60.88(p value 0.014) times respectively than those who have good ART and cotrimoxasole adherence. Higher age (Beta=97.59, p=0.000), secondary education, marital status, advanced clinical stages (p=0.000) and recent CD4 count predicted improvement in CD4 count change. This finding is related with the study done in United States that has shown, patients who have self-reported poor adherence status have a loss of CD4 count [15].

The findings of this study are also consistent with research literatures that reported CD4 count change is affected by numerous predictors. CD4 count change after the initiation of ART is known to be good predictor of Health Related Quality of Life. A study done in Southern State USA reported that CD4 count change is significant predictor of Health related quality of life [16].

A retrospective cohort study by Ayalu and colleagues in Ethiopia found that duration of ART and functional status were found to be significant predictors of CD4 count change [8]. However, this study didn’t find any significant association between the duration of ART and CD4 count change. This difference might be due to the difference in study design and the sample size used for this study is large.

Similar to the findings of this study, a study done in Sub Saharan Africa in 2006 by Stephan D Lawn showed the baseline CD4 count and age were significantly associated with CD4 count change [17].

**LIMITATIONS**

Both the ART data base and ART patient’s chart are secondary sources, therefore all the problems related with using secondary data applies to this study.

This study lacks some important predictors that are known to potentially affect CD4 count change like Viral load, presence of chronic diarrhea, presence of AIDS defining illness and nutritional status of the patient need to be included if better result is to be achieved.

**CONCLUSION**

Age when starting ART, educational status, marital status, WHO clinical staging, baseline hemoglobin level, baseline CD4 count, ART adherence status, cotrimoxasole adherence status, functional status, and recent follow up CD4 are significant predictors of CD4 count change.

It looks evident that clinicians need to closely monitor patients who initiated ART at a lower baseline hemoglobin level, and/or CD4 count level. Strategies to improve ART and cotrimoxasole adherence need to be also encouraged. Understanding the multifactorial CD4 count change after the initiation of ART requires advanced study researches that include numerous predictors like Viral load values, nutritional status, presence of AIDS defining illnesses.

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**Authors’ contributions**

1) MK have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data and preparation of manuscript
2) DZ have been involved in drafting the manuscript or revising it, scholarly critics for important intellectual content, have given final approval of the version to be sent for publication
3) BZ have been involved in drafting the manuscript or revising it scholarly critics for important intellectual content, have given final approval of the version to be sent for publication

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