CD4 Count Progression of Adult HIV Patients Under Art Follow Up At Mekelle General Hospital, Tigray Region: A Longitudinal Data Analysis Approach

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DOI: ***10.2427/13234***
Accepted on December 06, 2019

ABSTRACT

Background: The number of people receiving antiretroviral therapy (ART) in low- and middle-income countries continues to show encouraging growth, indicating that the global effort to scale up HIV treatment has exceeded 15 million people by the end of 2015.
Methods: A retrospective cohort study, comprising of the quantitative method of data collection was conducted among randomly selected 210 adult ART users enrolled in the first 6 months of 2011 and followed up to mid-2016 which is a five year follow up. Data were analyzed using a linear mixed model to identify the determinant factors, which importantly incorporates the effect of factors over time.
Results: Ninety-five (45%) were males and 115 (55%) were females. Composition of patients' WHO clinical stage were; stage I (25 (11.8%)), stage II (30 (14.2%)), stage III (102 (48.8%)), and stage IV (52 (24.6%)). The mean CD4+ count at baseline was 218 cells. The progression of CD4+ count for males is lower than that of female over time (coef. =-0.0779, p-value=0.0062). There was a direct relationship between time in month and CD4+ count progression i.e., the CD4+ count progression of the adult HIV patients was increasing during the subsequent number of times measured or followed up under the ART clinic (coef. = 0.0435, p-value=0.0000). Patients with WHO stage II (coef. = -0.0982, p-value=0.0109) , stage III (coef. = -0.0884, p-value = 0.0010) and stage IV (coef. = 0.0859, p-value = 0.0095) had lower CD4+ count than the reference category WHO clinical stage I over time.
Conclusion: In conclusion, we found that the WHO clinical stage, Time, Weight, Gender and the Interaction effects of Weight with Time were significantly associated with the progression of CD4+ counts over time.

Key words: Linear mixed model, Mekelle General Hospital, CD4+ counts, HIV/AIDS

BACKGROUND

Without treatment, HIV infection leads to AIDS and death. The predominant mode of HIV transmission is through sexual contact. Other modes of transmission are mother-to-child transmission (in which the mother passes HIV to her child during pregnancy, delivery, or breastfeeding), use of contaminated blood supplies for transfusions, and injections using contaminated needles or syringes [1].

Acquired Immuno Deficiency (AIDS) is one of the most serious public health and development challenges in sub-Saharan Africa.

The number of people receiving antiretroviral therapy (ART) in low- and middle-income countries continues to show encouraging growth, indicating that the global effort to scale up HIV treatment has exceeded 15 million people by the end of 2015 [2]. As of the end of 2015, the number of people receiving ART had reached 15.9 million in low- and middle-income countries, indicating a stable annual growth rate of 1.8 million per year since 2012 [2].

HIV-related deaths declined by 24% between 2000 and 2014, with HIV claiming an estimated 1.2 million (980 000–1.6 million) lives globally in 2014, compared to 1.6 million (1.3–2.1 million) in 2000, however, the mortality rate has fallen by 42% since the 2004 peak in HIV related deaths [3].

Early treatment initiation is associated with clinical and HIV prevention benefits, improving survival and reducing the incidence of HIV infection at the community level.2013

Guidelines Development Group recommends that national HIV programs provide ART to all people with a confirmed HIV diagnosis with a CD4 count of 500cells/mm3 or less, giving priority to initiating ART among those with severe/advanced HIV disease or a CD4 count of 350 cells/mm3 or less [4]. It is also recommended to initiate ART in people with active tuberculosis (TB) disease and hepatitis B virus (HBV) co-infection with severe liver disease, all pregnant and breastfeeding women with HIV, all children younger than five years living with HIV and all individuals with HIV in serodiscordant relationships, regardless of CD4 cell count [4].

HIV infection causes the patients to decrease their CD4+ T lymphocytes from lymphoid tissues and peripheral blood. Therefore the assessment of peripheral blood CD4 count is found to be the standard whether to initiate ART or not as well as monitoring response to ART [5].

It is obvious that HIV/AIDS is one of the foremost public health challenges in Sub-Saharan Africa, and Ethiopia, as one of these countries has been affected by the epidemic with a prevalence of 1.5 % [1]. Currently, the only available treatment for HIV/AIDS is antiretroviral therapy (ART). Because HIV affects the CD4+ T-cell (CD4) counts in the human body it can be employed to make appropriate decisions for the initiation of ART and proper management of the progression of the infection [6, 7]. Access to antiretroviral treatment (ART) has improved substantially in Sub-Saharan Africa over the past decade, but high rates of mortality are still reported, especially in the first few months of treatment [7].

Objectives of the Study

To evaluate the progression of CD4+ cell counts and to find determinant factors for the change in CD4+ cell counts of adult HIV positive patients in ART using a linear mixed model.

METHOD

A retrospective cohort study contained only; the quantitative method of data collection was conducted for randomly selected patients from January 1-15, 2017 enrolled in the first 6 months of 2011 and followed up to mid-2016 that was five year follow up in Mekelle General Hospital.. Mekele town is located in the northern part of Ethiopia, at a distance of 783 km from Addis Ababa. Its *astronomical location is 13°32'' **North latitude and 39°28' East longitude.*** Mekelle General Hospital contains ART, Mother, and Child Health, Dental, Dermatology, Multiple Drug Resistant, Inpatient clinics with laboratory and pharmacy services. In 2017 the number of outpatient services provided was 238,000. The numbers of ever enrolled ART patients in the hospital were 5200. The manpower of the ART clinic is 23, of which 1 is a Medical Doctor, 1 Health Officer, 15 Clinical Nurses, and 6 Case Managers including, Data Clerk, Health Information Technology Unit, and Medical Data Clerk. Patients with age less than 15 years old, an incomplete record of CD4 counts, less than 3 number of CD4 count records (i.e., a patient with visits to ART clinic less than three times) were excluded from the sampling procedure from the electronic medical record software. The sample size was determined according to Diggle [8] for comparing two groups across time with a confidence of level of 95%, 5% degree of precision, 80% power of test, ratio of male to female 1:1, effect size 0.3, the assumed correlation of the repeated measure 0.4, the number of repeated measures 3. The estimated sample size was 210 adult HIV patients from the General Hospital.

A stratified sampling technique was used to select the study participants. First, the study population was clustered into gender, Male and Female, according to the sample size determination formula. A simple random sampling technique has been applied to select from both groups, male and female.

Data were extracted from ART electronic medical records, where only a few and important variables were recorded, CD4+ count and age. Thus, we used this for sampling purpose i.e., to identify adult patients and patients with at least three times CD4+ count records. Then we collected other socio-demographic characteristics and clinical variables from the review of patient charts. It was collected by three nurses who work at the ART clinic of Mekelle General Hospital under the supervision of the principal investigator. The training was given to the data collectors for two consecutive days on the purpose of the study, the contents of the extraction sheet, prepared based on the available variables and particularly on issues related to the confidentiality of the responses and the rights

of respondents.

Data were entered, cleaned and analyzed using R software for windows (R version 3.2.3 (2015-12-10)). A descriptive analysis was computed. To identify predictors of CD4 Count Progression, a linear mixed model was employed. The linear mixed model is statistical model applied for a continuous outcome (response) variables where the residuals are expected to follow the Gaussian or normal distribution but may not be independent or may have not constant variance [9]. The model includes both **fixed-effect parameters** associated with one or more continuous or categorical covariates and **random effects** associated with one or more random factors [10]. The mix of fixed and random effects gives the *linear mixed model as* its name. According to [10], the fixed effect parameters measure the relationships of the covariates to the dependent variable for the whole population, whereas the random effects are specific to subjects (some other clustering variable(s)) within the population under study. As a result, random effects are directly used in modeling the random variation in the response variable at different levels of the data as classified above. The general matrix specification of LMM according to [10-13] for given subject i, for individual observations by t into vectors and matrices as below;

$$\boldsymbol{Y_i} = \underbrace{\boldsymbol{X_i\beta}}_{\boldsymbol{fixed}} + \underbrace{\boldsymbol{Z_i u_i + \varepsilon_i}}_{\boldsymbol{random}}$$

Where, $\mathbf{u}i \sim N(0, D)$

$$\varepsilon i = (0, Ri)$$

In this equation, Yi represents a vector of continuous response CD4 count for the i-th subject (adult HIV patient) in our case.

The vector notation can be represented as below:

$$\boldsymbol{Y}\mathrm{i} = \begin{pmatrix} \mathrm{Y1}i \\ \mathrm{Y2}i \\ \dots \\ \dots \\ \mathrm{Y}nii \end{pmatrix}$$

In the case of vector Yi, the number of repeated measures, n_i, may vary from one subject to another subject.

X_i in the equation is an n_ix p design matrix, which represents the known values of p covariates, $X^{(1)}$. . ., $X^{(p)}$, for each of the n_i observations collected on the i-th subjects:

$$\mathbf{X}i = \begin{pmatrix} X_{1i}^{(1)} & X_{1i}^{2} \dots & X_{1i}^{p} \\ X_{2i}^{(1)} & X_{2i}^{(2)} \dots & X_{2i}^{(p)} \\ \vdots & \vdots & \vdots \\ X_{nii}^{(1)} & X_{nii}^{(2)} \dots & X_{nii}^{(p)} \end{pmatrix}$$

In this case, the X_i matrices are assumed to be of full rank; that is none of the columns or rows is a linear combination of the remaining ones. In general, the Xi matrices may not be of full rank which may lead to parameter identifiability problems for fixed effects stored in the vector β.

$$\boldsymbol{\beta} = \begin{pmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_p \end{pmatrix}$$

The symbol β in the equation is a vector of p unknown regression coefficients or the fixed-effect parameters) associated with the p covariates used in constructing the X_i matrix:

Zi is an n_ix p in the equation above and is a design matrix that represents the known values of the covariates, $Z^{(1)}$, …, $Z^{(q)}$, for the i-th subject. This matrix is very much like $\mathbf{X}_i$ matrix in that it represents the observed values of covariates; however, it usually has fewer columns than the $\mathbf{X}_i$ matrix: The columns in the Zi matrix represent observed values for the q predictor variables for the i^{th} subject, which have effect on the continuous response variable that vary randomly across subjects. In many cases, predictors with effects that vary randomly across subjects are represented in both the Xi matrix and the Zi matrix. In an LMM in which only the intercepts are assumed to vary randomly from subject to subject, the Zi matrix would simply be a column of 1's.

The ui vector for the i^{th} subject in the equation above represents a vector of q **random effects** associated with the q covariates in the Zi matrix:

$$u_i = \begin{pmatrix} u_{1i} \\ u_{2i} \\ \vdots \\ u_{qi} \end{pmatrix}$$

It is assumed that the q random effects in the $\boldsymbol{u}_i$ vector follows a multivariate normal distribution, with mean vector **0** and a variance-covariance matrix denoted by **D** i.e. $\boldsymbol{u}_i \sim N(\mathbf{0}, \mathbf{D})$.

Elements along the main diagonal of the ***D*** matrix represent the **variances** of each random effect in ui, and the off-diagonal elements represent the **covariances** between two corresponding random effects. Because there are q random effects in the model associated with the i^{th} subject, ***D*** is a q x q matrix that is symmetric and positive definite. Elements of this matrix are shown as follows:

$$\mathbf{D} = Var(\boldsymbol{u}_i) = \begin{pmatrix} Var(u_{1i}) & cov(u_{1i}, u_{2i}) & \dots & cov(u_{1i}, u_{qi}) \\ cov(u_{1i}, u_{2i}) & Var(u_{2i}) & \dots & cov(u_{2i}, u_{qi}) \\ \vdots & \vdots & \ddots & \vdots \\ cov(u_{1i}, u_{qi}) & cov(u_{2i}, u_{qi}) & \dots & Var(u_{qi}) \end{pmatrix}$$

The variances and covariance of the ***D*** matrix are defined as functions of a (usually) small set of covariance parameters stored in a vector denoted by ***D***. Note that the vector ***D*** imposes structure (or constraints) on the elements of the ***D*** matrix.

TABLE 1. Scio-demographic characteristics of Adult HIV patients (n=210), Mekelle general Hospita, 2017

		Follow up time in months									
		Baseline	Month 6	Month 12	Month 18	Month 24	Month 30	Month 36	Month 42	Month 48	Month 54
Gender	Male	95 (45%)	95 (45%)	95 (45%)	51(44.74%)	18(48.65%)	9(52.94%)	5(50%)	3(50%)	2(50%)	1(50%)
	Female	115 (55%)	115 (55%)	115 (55%)	63(55.26%)	19(51.35%)	8(47.06%)	5(50%)	3(50%)	2(50%)	1(50%)
Marital Status	Single	30 (14.2%)	30 (14.2%)	30 (14.2%)	16(14.68%)	3(9.09%)	2(13.33%)	1(12.50%)	1(20.00%)	1(13.33%)	0(0%)
	Married	120 (57.9%)	120 (57.9%)	120 (57.9%)	60(55.05%)	20(60.61%)	10(66.67%)	6(75%)	3(60%)	2(66.67%)	2(100%)
	Separated	9 (4.3)	9 (4.3)	9 (4.3)	5(4.59%)	1(3.03%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Divorced	36 (17.1%)	36 (17.1%)	36 (17.1%)	20(4.59%)	8(24.24%)	3(20%)	1(12.50%)	1(20%)	0(0%)	0(0%)
	Widowed	10 (4.7)	10 (4.7)	10 (4.7)	8(7.34%)	1(3.03%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Educational Status	No educ.	35 (16.6%)	35 (16.6%)	35 (16.6%)	23(21.30%)	3(9.09%)	1(6.67%)	0(0%)	0(0%)	0(0%)	0(0%)
	Primary	88 (41.7%)	88 (41.7%)	88 (41.7%)	47(43.52%)	16(48.48%)	7(46.670%)	3(37.50%)	2(40%)	2(66.670%)	2(100%)
	Secondary	56 (26.5%)	56 (26.5%)	56 (26.5%)	26(24.07%)	9(27.27%)	5(33.33%)	3(37.50%)	2(40%)	0(0%)	0(0%)
	Tertiary	25 (11.8%)	25 (11.8%)	25 (11.8%)	12(11.11%)	5(15.157%)	2(13.33%)	2(25.00%)	1(20.00%)	1(33.33%)	0(0%)
Residence	Urban	168 (79.6%)	168 (79.6%)	168 (79.6%)	83(73.45%)	28(75.68%)	14(82.35%)	7(70.00%)	4(66.67%)	2(50%)	1(50%)
	Rural	42 (19.9%)	42 (19.9%)	42 (19.9%)	30(26.55%)	9(24.32%)	3(17.65%)	3(30.00%)	2(33.33%)	2(50%)	1(50%)
Functional Status	Working	190 (90.5%)	190 (90.5%)	190 (90.5%)	109(95.61%)	36(97.30 %)	16(94.12%)	9(90.00%)	5(83.33%)	3(75.00%)	2(100%)
	Ambulatory	20 (9.5%)	17 (8.1)	17 (8.1)	5(4.39%)	1(2.70%)	1(5.88%)	1(10%)	1(16.67%)	1(25%)	0(0%)
	Bed driven	6 (2.8%)	3 (1.4)	3 (1.4)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
WHO Stages	Stage I	25 (11.8%)	25 (11.8%)	25 (11.8%)	61(53.51%)	24(64.86%)	8(47.06%)	5(50%)	3(50%)	2(50%)	2(100%)
	Stage II	30 (14.2%)	30 (14.2%)	30 (14.2%)	10(8.77%)	4(10.81%)	2(11.76%)	0(0%)	0(0%)	0(0%)	0(0%)
	Stage II	102 (48.8)	102 (48.8)	102 (48.8)	30(26.32%)	7(18.920%)	5(29.41%)	4(40.00%)	2(33.33%)	1(25%)	0(0%)
	Stage IV	52 (24.6%)	52 (24.6%)	52 (24.6%)	13(11.4%)	2(5.41%)	2(11.76%)	1(10.99%)	1(16.67%)	1(25.00%)	0(0%)

The last part of the equation above is the vector ε_{i}, which is the vector of n_i residuals, each element in ε_i denotes the residual associated with an observed response at occasion t for the i^{th} subject. This is because some subjects might have more observations collected than others (e.g. if data for one or more time points are not available when a subject or a patient in our case drops out), the ε_i vectors may have a different number of elements.

$$\boldsymbol{\varepsilon} = \begin{pmatrix} \varepsilon_{1i} \\ \varepsilon_{2i} \\ \vdots \\ n_{ii} \end{pmatrix}$$

When compared to those from the standard linear model, the residuals associated with repeated observations on the same subject in an LMM can be correlated. The n_i residuals in this vector for a given subject, **i**, are random variables that follow a multivariate normal distribution a mean vector **0** and a positive definite symmetric covariance matrix $\mathbf{R}_i$:

$\varepsilon_i \sim N(\mathbf{0}, \mathbf{R}_i)$

In this case independence is assumed, i.e. the residuals associated with different subjects are independent of each other, and additionally the vector of residuals, ε_i andrandom effects, u_i are independent of each other. The matrix representation is written as follows:

$$R_i = Var(\varepsilon_i) = \begin{pmatrix} Var(\varepsilon_{1i}) & cov(\varepsilon_{1i}, \varepsilon_{2i}) & \cdots & cov(\varepsilon_{1i}, \varepsilon_{nii}) \\ cov(\varepsilon_{1i}, \varepsilon_{2i}) & Var(\varepsilon_{2i}) & \cdots & cov(\varepsilon_{2i}, \varepsilon_{nii}) \\ \vdots & \vdots & \ddots & \vdots \\ cov(\varepsilon_{1i}, \varepsilon_{nii}) & cov(\varepsilon_{2i}, \varepsilon_{nii}) & \cdots & Var(\varepsilon_{nii}) \end{pmatrix}$$

The elements of the $\boldsymbol{R}$**i** matrix are defined as functions of another (usually) small set of covariance parameters

FIGURE 1. Mean CD4 count profile of patients by time (0=baseline, 1= month 6... and 9=month 54)

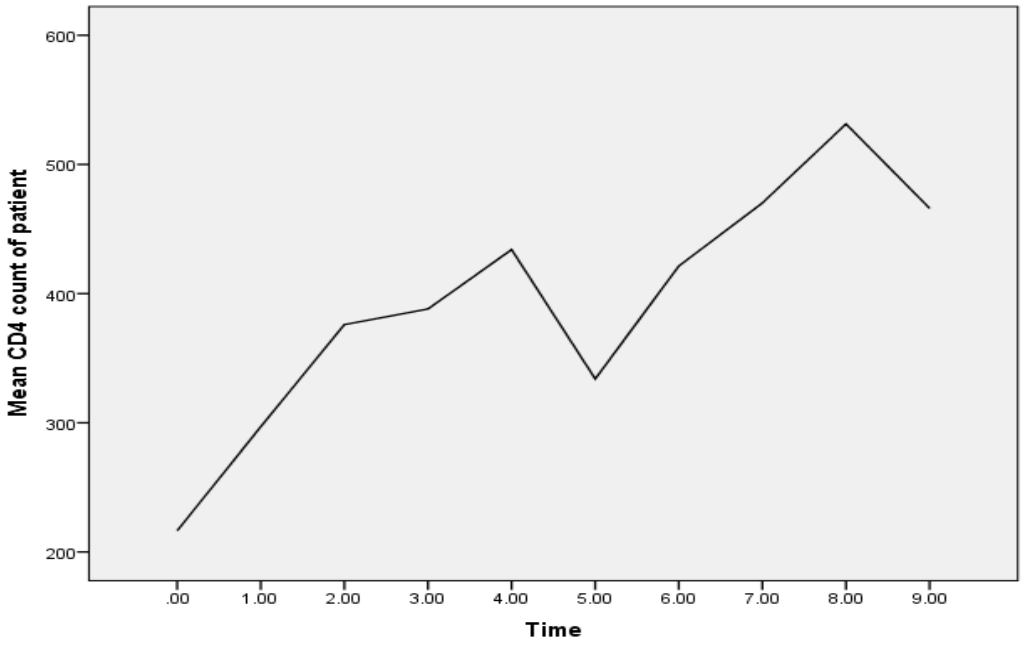
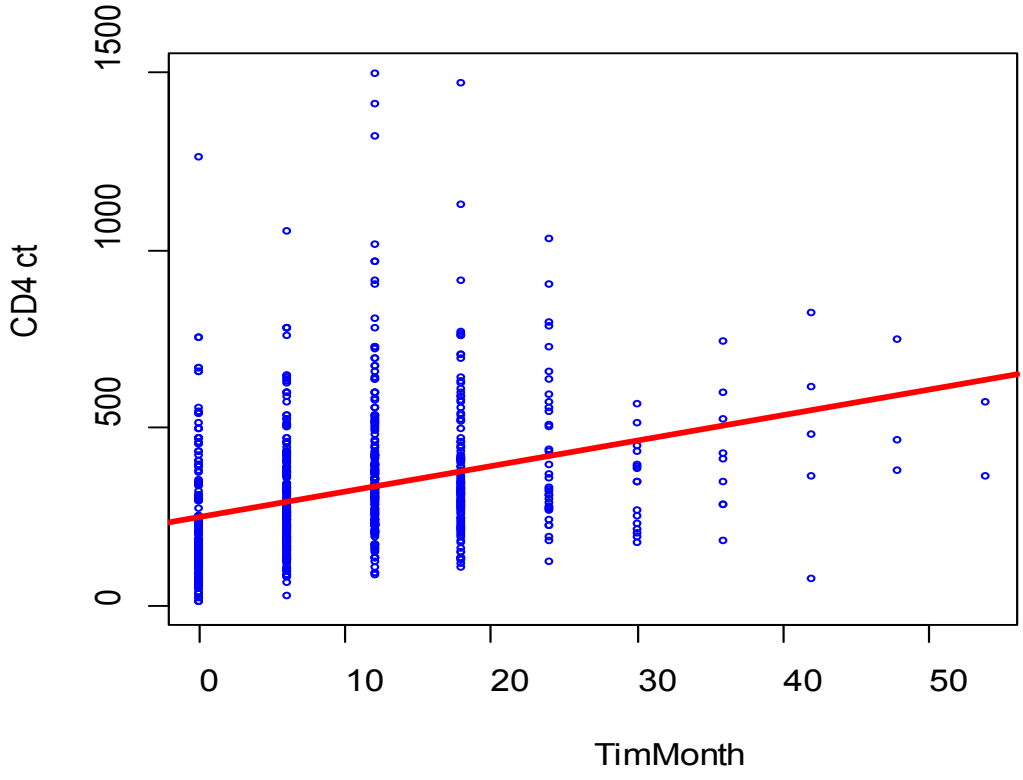


FIGURE 2. Scatter plot with smoother of CD4+ cell counts versus Time in month of all samples under study (from 0=baseline, 1=month 6, ..., 9=month 54)



stored in a vector denoted by θ_R. Many different covariance structures are possible for the R_i matrix.

RESULTS

Socio Demographic Characteristics

A total of 210 adult HIV patient cards were assessed in the study. Ninety-five (45%) of them were male and the rest 115 (55%) were female. Above half the patients 120(57.9%) were married. Of the respondents, 35(16.6) were with no education (unable to read and write), 88(41.7%) primary, 56 (26.5%) secondary, and 25 (11.8%) tertiary. Regarding residence places, 168 (79.6%) of the patients were urban and the rest 42 (19.9%) were rural. (Table 1)

Stage I: The primary HIV infection phase (or acute sero-conversion illness)

Stage II: The asymptomatic latent phase, the minor symptomatic phase

Stage III: The major symptomatic phase and opportunistic diseases

Stage IV: AIDS-defining conditions: the severe symptomatic phase

Summary Statistics for Continuous Covariates

Exploratory data analysis of the CD4+ count was conducted for randomly selected adult HIV positive patients.

The mean CD4+ count (218) of patients showed no change between baseline time until time two (the next six months), then increases [3] in time 12 (12^{th} month of the follow-up). The mean weight of patients in the baseline, month 6 and month 12 were 50.6, 52.4 and 53.5 respectively. (Table 2)

The mean profile plot indicates that the mean CD4+ count showed an increasing progress from baseline to the last time point and follows almost a linear trend. (Figure 1)

Exploring Individual profiles

The scatter plot of the sample with smoother, to observe the trends of the CD4+ cell counts over time were drawn, therefore; the number of times the patients measured was higher up to time point 18 i.e., month 18, and even there were higher average records of CD4+ count over time. In general, the overall progression of CD4 count over time, showed that the progression of CD4+ cell counts were increasing across the follow up times. (Figure 2)

The individual profiles of all patients, i.e., plotting observed profiles over time helps to identify general trends within patients, and may detect change over time that provides information about the variability at given times. Connecting the repeated measurements for each patient's CD4+ count over time shows that there is a non-constant pattern common to most patients.

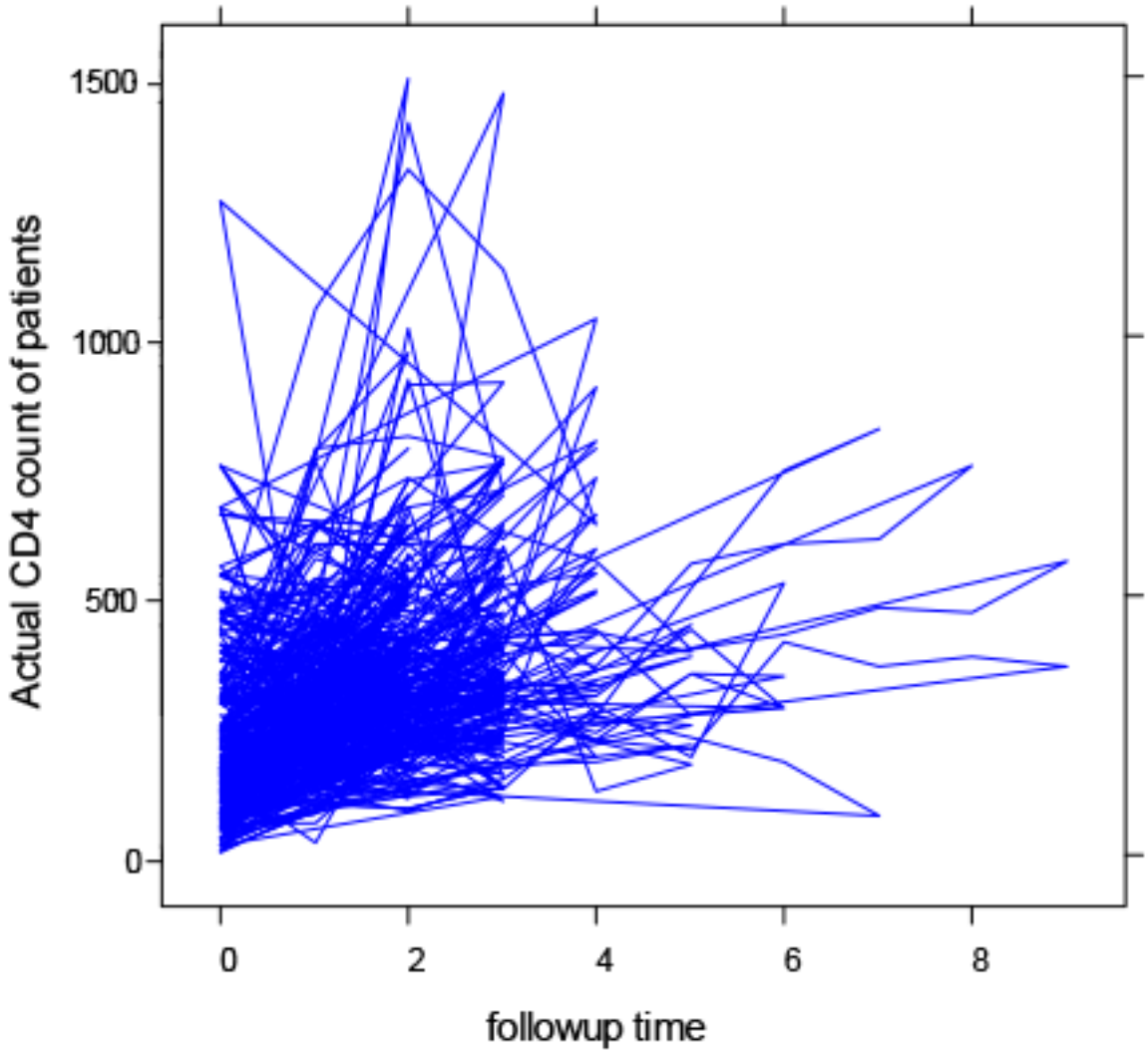
These individual profiles can also provide some information between the patient's CD4+ count variability and illustrate that there is a change among the patient's CD4+ count overtime. Similarly, it appears that there is a fluctuation in CD4+ count over time after they were initiated on ART and the variability of the patient's CD4+ count seemed larger at the beginning compared to the end of CD4+ count of patients. The variation at the beginning was higher than at the end of the follow-up periods and higher CD4 count was recorded in-between month one (month 6) and time two (month 12). (Figure 3)

Individual patient profile plot of CD4+ counts by sex, that is, the plot shows that there is an observed

TABLE 2. Descriptive Statistics of the Covariates (Continuous variables) of Mekelle General Hospital, 2017

Follow up Time	Baseline	Month 6	Month 12	Month 18	Month 24	Month 30	Month 36	Month 42	Month 48	Month 54
n (Sample)	210	210	210	210	210	210	210	210	210	210
Mean (CD4 count)	218	218	362	340	348	345	398	534	271	482
Std.Dev. (CD4 count)	166	167	217	220	203	206	345	489	76	426
Mean (Weight)	50.6	52.4	53.5	55.26	57.65	57.26	55	65.5	56	55.5
Std.Dev. (Weight)	9.8	9.5	9.2	9.71	8.53	10.14	9.14	8.89	10.03	14.85
Mean (BMI)	18.6	18.8	19.8	20.33	20.79	20.83	21.15	22.35	21.97	22.12
Std.Dev. (BMI)	3.4	3.4	3.1	3.20	2.22	2.60	2.77	2.46	2.78	3.00
Mean (Hemog. Level)	14.1	14.1	14.8	14.92	15.43	14.58	16.01	15.19	13.91	12.33
Std.Dev.(Hemg. Level)	2.7	2.8	3.3	3.15	1.99	2.51	2.41	2.73	0.90	4.48
Mean (Age)	34.5	34.5	34.5	35.89	35. 04	35.82	34.85	30.33	32	32.5
Std.Dev (Age)	9.8	9.8	9.8	9.53	8.24	10.24	10.62	3.87	3.37	1.41

FIGURE 3. Individual Profile plot of CD4+ count by Time for n=210 Adult HIV Patients of Mekelle General Hospital, 2017 (0=baseline, 1=month 6, 2= month 12, …, 8= month 48, 9=month 54)



variability in CD4+ count over time within patients and between patients. In this profile plot, there seems almost the same variability in the progression of CD4+ count of patients over time by sex group. But, it shows that there is some variability in CD4+ count progression between males and females, females CD4 count seem to be higher CD4 count than males. (Figure 4)

Factors Affecting progression of CD4+ cell counts

In the bi-variable random-effects model, we have seen that the variances for the random effects are statistically significantly different from zero, indicating that there is between patient variations. This variation does not only exist at baseline, but it exists over the follow-up time as well, implying that the random intercept and slope should be included in the linear mixed model.

The interaction term of Weight with time was found statistically significant which indicates that the weight of patients contributes towards a significant lower progression in CD4+ count over time. Gender is found to be significant which implies that the progression of CD4+ count for males was lower than that of the reference group, female over time, and this was supported by the mean profile of CD4 at the exploratory data analysis section. Time (time in month) was also found to be statistically positively significant with the Log CD4+ count; this implied that there was a direct relationship between time and CD4 count progression i.e., the CD4+ cell count progression of most of the adult HIV patients was higher as a function of the number of times measured or followed up under the

FIGURE 4. Individual Profile plot categorized by sex of CD4+ cell count of Adult HIV Patients (n=210) of Mekelle General Hospital by Time, 2017 (0=baseline, 1=month 6, 2= month 12, …, 8= month 48, 9=month 54).

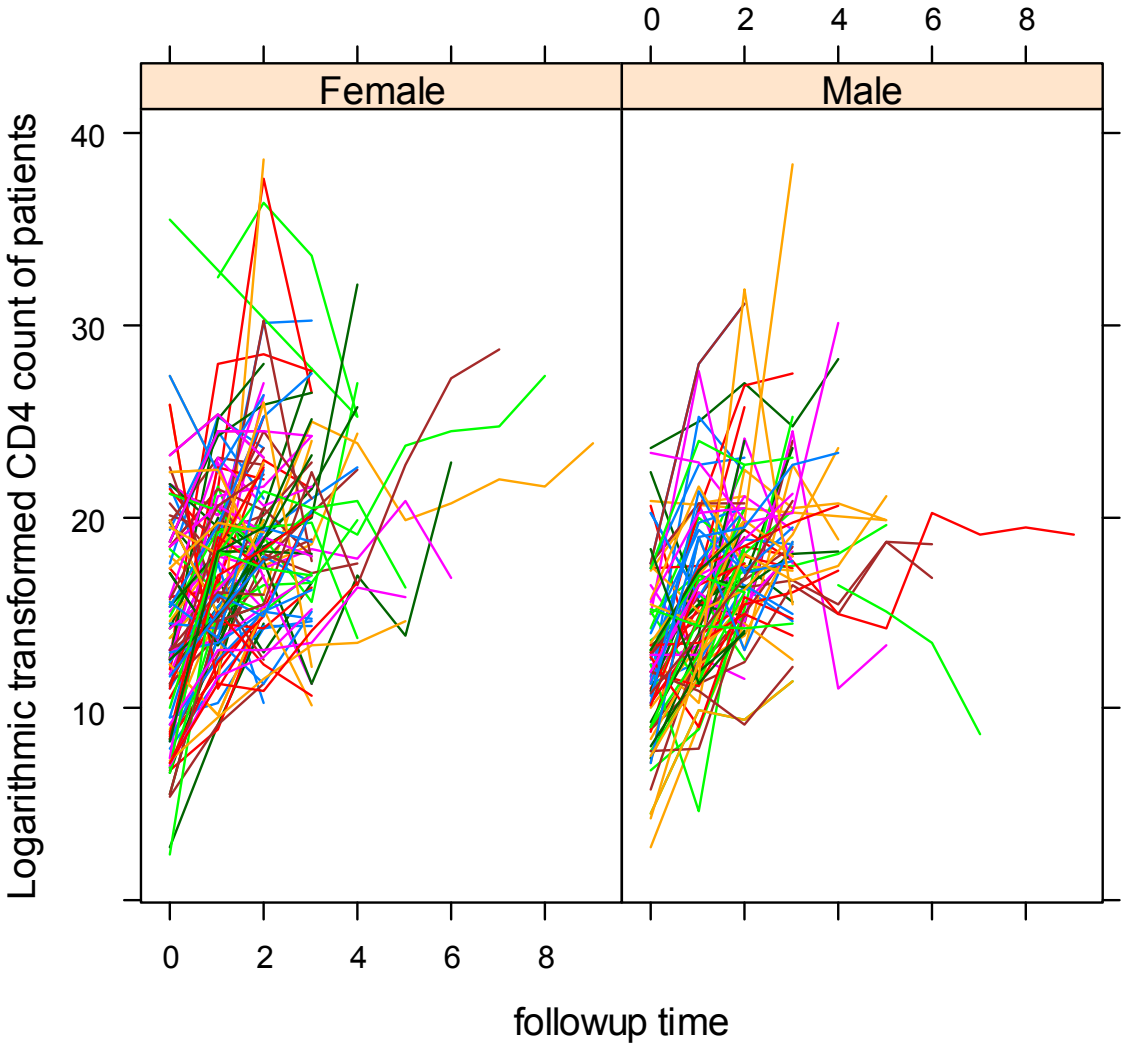


TABLE 3. Fixed Effect Parameter Estimates for Random Slope and Intercept Model (n=210) of Mekelle General Hospital, 2017.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	1.8592	0.0983	574	18.9129	0.0000
Gender (Male)	-0.0779	0.0283	574	-2.7489	0.0062
Time in month	0.0435	0.0068	574	6.4363	0.0000
Weight	0.0100	0.0018	574	5.5044	0.0000
WHO stag Stage 2	-0.0982	0.0384	574	-2.5554	0.0109
WHO stage 3	-0.0884	0.0267	574	-3.3131	0.0010
WHO stag stage 4	-0.0859	0.0330	574	-2.6034	0.0095
Weight*Time in month	-0.0005	0.0001	574	-4.2704	0.0000

ART clinic. The other variable found statistically negatively significant was that the WHO clinical stage; this result showed that patients with WHO Stage II had a lower CD4 count than the reference category WHO clinical Stage I over time. WHO clinical stage III was also statistically negatively significant, showing that the average progression of CD4+ count was lower for patients with WHO clinical stage III than that of patients with WHO clinical stage over time. Similarly, the WHO Clinical stage IV was found to be statistically negatively related to the progression of Log CD4+ count, implying that the mean progression of patients with WHO stage IV is lower than the mean progression of patients with the reference group, the WHO clinical stage I. This was supported by the mean profile plot of CD4 count by WHO stages category, which is the mean CD4 count for patients with WHO clinical stage I was higher in almost all times of follow up. (Table 3)

DISCUSSION

In this section, we will try to compare the results found in our study with other researches as described in our literature part of this research work.

Accordingly, results from exploratory data analysis show that the mean CD4 count progression increases as the time of follow up increases, this result is similar to a study conducted by Tadesse K. et al [14]. But there are contradicting results, in the interaction of gender with time, in our study this interaction was not found statistically significant, but according to the thesis work [14], the interaction was found to be significant.

According to Hunt PW et al., [15], among the actors of interest associated with increased CD4 cell count gains from the baseline measurement to the last of the follow up of adult HIV patients included, younger age and female sex. But, according to our study age was not found to be statistically significant for even the progression of mean CD4+ count. Age as a factor in this study was included in the bi-variable analysis and fulfilled the criteria for 0.25 level of significance with its similar sign (younger age is more likely associated with higher CD4 count) as was reported in [15] but removed from the final model in this study as it was not significant at 0.05 level of significance. Regarding Gender, the result of our study coincides with [15], that is, the mean progression of CD4+ count of males was lower than that of the reference category, which implies that being female is more likely to lead higher CD4+ count.

A study conducted by Tsgaye E. and Worku A. at SNNP (Southern Nations Nationalities and Peoples) region [16] showed similar result with our study i.e., there were no significant differences between patients whose age group was 25 through 30 and 31 through 40 years, but there is disparity due to the statistical method as Demidenko E. [11] utilized the Cox regression model in the survival analysis framework. The difference is the way data handling; this result may be due to an appropriate age category for the patients and survival time may not show the effect of age as time goes as already followed for about five years but the age group was categorized within five and ten years interval, in fact, it could be classified within equal interval and probably we could see a different result.

According to studies conducted in South Africa, USA and Ethiopia by Venter E. et al, Forrester J. et al, and Siyoum A. et al [17-19] respectively, there were significant correlations between the CD4 cell count and weight and between the CD4 cell count and BMI for the respective study groups as a whole. These studies mainly used the cross-sectional approach, and hence lacked the benefit of dealing with longitudinal data. They also indicated that there is growing evidence that increased BMI is associated with an increased CD4 cell count and with lower rates of the events that characterize the progression of HIV disease. According to our study, weight is positively correlated with CD4+ count (i.e., an increased weight leads to an increase in CD4+ count over time) which is a similar result with [17-19]. BMI was not found to be statistically significant, and it was removed from a bi-variable analysis step.

Other research work conducted by Siyoum A. and Temesgen Z. in Ethiopia [19] showed that both age and weight significantly affected CD4 count, and from the two-way interaction, time * level of education, time * sex, age * sex as well as age * level of education were significant for the outcome variable. The CD4+ count was higher for urban adult patients than rural areas over time, but according to our study only the results weight concurs with this study, the rest they are removed from the model in the bi-variable and full model formulation procedure.

Our study showed that among WHO clinical stages, stage II, stage III and stage IV were found to have a significant difference from the reference group stage I, implying that the mean progression of WHO clinical stages II, stage II, and stage IV is consistently lower than that of the reference group, WHO stage I. This result of our study contradicts with [14]; among WHO clinical stages, only stage 3 was found to have a significant difference from the reference group stage 4. According to Tadesse K. [14], functional status had a significant effect on CD4+ count; and this concurs with our study; that is functional status was not significantly associated with the progression of CD4+ counts over time.

LIMITATIONS

- The data were not recorded primarily for this research purpose

- The number of times the patients measured were not the same (unbalanced)
- Data were extracted by workers in the clinic and was not convenient for serious follow up of the data extraction process.

CONCLUSION

WHO clinical stage, time, Weight, Gender and the interaction effects of Weight with time are significantly associated with the progression of CD4+ counts of adult HIV patients under ART follow up over time. The average CD4+ count progression of females was higher than that of males. The mean progressionCD4+ count of patients with WHO stage II-Stage IV was lower than that of the mean CD4+ count progression of patients with WHO stage I.

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Acknowledgements

We would like to thank Mekelle University, School of public Health. Our gratitude goes to data collectors, Mekelle General Hospital ART clinic staffs and Tigray regional health bureau.

Authors' Contribution

HGM designed the study, analyzed the data using R software and drafted the manuscript and incorporated the comments from co-authors

HT and MA critically commented and reviewed starting from the design up to the final manuscript.

All authors read and approved the final manuscript.

Conflict of interest Statement

The authors declare that they have no competing interests.

Source of Funding

There was no source of fund.

Ethical Approval

The ethical review committee of Mekelle University, College of Health Sciences approved the study protocol. Any personal identifier was not encoded; identifiers of the patients were replaced with identification numbers.

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