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MODELING THE LONGTIUDNAL CHANGE OF VIRAL LOAD OF HIV POSITIVE PATIENTS ON ANTIRETROVIRAL THERAPY

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ABSTRACT

HIV/AIDS continues to be a major public health concern and cause of death in the world. Even-though WHO recommended viral load testing as the preferred monitoring approach to diagnose and confirm ARV treatment failure, but in most cases, factors influencing the trend of viral load were not well identified. The main objective of this study was to modeling the change of viral load and identifying its associated factors among HIV positive patients. In this retrospective longitudinal data analysis, data was collected from 287 HIV positive patients registered for ART between January 2017 and June 2019 in Zewditu hospital and unstructured covariance structure was parsimonious for the data. linear mixed model with different random effect were applied to the data. Linear mixed model with random intercept and slope were selected as a best model to fit the data based on different model selection criteria. The findings of the study revealed that there was a decrement over time in the log VL of patients with HIV on ART. Furthermore, time, baseline CD4 count, WHO clinical stage, functional status of the patient, adherence, smoking status, initial ART Regimen and Time interaction with adherence and WHO stage were found to be significant predictors of log VL evolution. Therefore, Patients should take ART regimens with good adherence to decrease their viral load over time.

Keywords: *HIV/AIDS, Viral load, unstructured covariance structure, Linear mixed model*

Background

Human immunodeficiency virus is a virus spread through certain body fluids that attacks the body's immune system, specifically the CD4 cells, often called Tcells. Over time, HIV causes Acquired Immune-Deficiency Syndrome, a condition in which the immune system begins to decline, exposing infected individuals to life-threatening opportunistic infections(Callaway et al., 1999).Viral load which isthe number of HIV viral particles per milliliter of blood, determinations are important prognostic marker of disease progression than CD4 count and, when used appropriately, provide a valuable tool for the management of individual patient (Saag et al., 1996).

According to UNAIDS report globally around 74.9 million [58.3 million–98.1 million] people have been infected with HIV and 32.0 million [23.6 million–43.8 million] people have died of AIDS related illnesses sinceits emergence in 1981.By the end of 2018, about 37.9 million people globally were living with HIV of which 36.2 million are adults. Sub-Saharan Africa remains among the hardest hit regions by the pandemic, with nearly one in every 25 adults (4.2%) living with HIV, accounting for nearly two-thirds of the global total HIV cases(UNAIDS, 2019).

In Ethiopia, 2018, about 690,000 people were living with HIV and 23,000 people were newly infected with HIV. The Ethiopian demographic and health survey (EDHS) 2016 report showGambella region (4.8%) and Addis Ababa (3.4%) to have the highest HIV prevalence rates while Somali (< 0.1%), and Southern Nations, Nationalities and peoples (SNNP, 0.4%) regional states have the lowest. The adult HIV prevalence in Ethiopia is 0.9%, with varying burden by sex, age, and other demographic characteristics, across sub-regions and population groups. The urban HIV prevalence (2.9%) is seven times higher than the prevalence in rural settings (0.4%), women (1.2%) having twice higher HIV prevalence than men (0.6%)(CSA, 2016).

In Ethiopia there are no studies to the best of our knowledge that documented on the area of longitudinal change of viral load among HIV infected patient even in Africa few numbers of studies like(Chendi et al., 2019) with their massive limitations attempted to determine the trend of viral load and its associated factors after patients startedART.Lack of appropriately applied statistical modeling which accurately describes the trend of viral load on each individual patient and inability of incorporating sufficient number of predictors that can affect the trend of viral load are the main problem of those few studies. Therefore, the aim of this study wasmodeling the

change of viral load and identifying its associated factors among HIV-positive patients following ART by applying advanced models that can account correlation within a patient over time since classical statistical models are not appropriate for longitudinal viral load data.

Material and Methods

Study Design and Period

A retrospective follow-up study was conducted in patients who are 15 and above years of age (adult) from January 2017 to June 2019.

Study Population

Study populations were all adult HIV/AIDS patients on ART at Zewditu Hospital from January 2017 to June 2019.

Inclusion Criteria

All enrolled adult HIV patients during the study period with at least two measurements of viral load were included.

Sample size and Sampling procedure

As with cross-sectional studies, investigators conducting longitudinal studies need to know in advance the number of subjects approximately required to achieve a specified statistical power.(Diggle et al., 2002)suggested that the required number of subjects can becomputed by:

$$N = \frac{4\left(\frac{Z\alpha}{2} + Z\beta\right)^2 \sigma^2(1 + (m-1)\rho)}{md^2}$$

$N$is the total sample size, d is effect size sample, m is number of times points repeated measurement was taken, ρ is correlation between repeated measurements and σ^2 is variance of outcome variable.

Assuming significance level of 0.05, power of 0.8, ρ = 0.475 and effect size of 0.5 from study conducted by(Brien et al., 1996), we have m = 6, σ^2=4.06, Using $\frac{Z\alpha}{2}$= 1.96, $Z\beta$=0.842 and inserting all quantities in the formula:

$$N = \frac{4(1.96+0.842)^2 4.06(1+(6-1)0.475)}{6*0.5*0.5} = 287$$

Therefore, the final sample size for this study was287. Sampling frame was prepared by collecting the identification number of RVI patients from the registration book. After identifying the patients who fulfill inclusion criteria, study subjects were selected by simple random sampling technique.

Variables in the Study

Dependent Variable

In this study viral load in copies/ml is used as dependent variable.

Independent variable

The independent variables are Age, Sex, Marital status, Adherence, Hemoglobin at Baseline, Functional status of patients, WHO RVI clinical stage, Educational level, Occupation, Baseline CD4 counts, Alcohol use, Cigarette smoking, Initial ART Regimen, History of TB, Opportunistic infection, and Body mass index.

Exploratory Data Analysis

Exploratory Data Analysis is a way to visualize the patterns of data relative to research interests. Since exploratory data analysis can serve to discover as much of the information regarding raw data as possible, plotting individual curves to carefully examine the data should be performed first before any formal model fitting is carried out. Thus, this study was assessed the nature of the data by exploring individual profiles, the average evolution, and the correlation structure.

Linear Mixed-effects Model

Linear Mixed-effects Model is an extension of a linear regression model to model longitudinal data for the continuous response. It contains fixed effects and random effects model.In the context of longitudinal data, there are three special cases of LMMs. The first one is the random intercept model, which is the simplest type of linear mixed model, the second one is random slope model and the last one is random intercept and slope model.

Linear Mixed-effects Model with AC Errors

To allow for various forms of autocorrelated errors, we will instead assume that the errors are distributed asε_i~N $(0,\sigma^2\Omega_i)$, replacing the identity matrix I_i with the autocorrelation matrix Ω_i.This phenomenon is called linear mixed-effects model with AC errors(Hedeker and Gibbons, 2006).whereΩ_i depends on q autocorrelation parameters, with q varying depending on the type of autocorrelated error structure being considered.

For our case the linear mixed-effects model with AC errors can be written as:

$$\text{LogVL} = \beta_0 + \beta_1(\text{time}) + \beta_2(\text{sex}) + \beta_3(\text{age}) + \ldots\ldots\ldots\ldots + \beta_j(j^{th}\ covariate) + b_{0i} + b_{1i}(\text{time}) + \varepsilon_{ij} \quad (8)$$

Where, b_{0i} and b_{1i} are the intercept and slope deviation for subject i respectively

Estimation of Parameters in Linear Mixed Models

The most often used methods of estimation in Gaussian mixed models are maximum likelihood and restricted maximum likelihood. The REML procedure is most popular when it comes to the estimation of variance components in mixed models assuming Gaussian random terms. REML maximizes the joint likelihood of all error contrasts rather than of all contrasts as in ordinary maximum likelihood (Gilmour et al., 1995).

The distinction between ML and REML becomes important only when the number of fixed effects is relatively large. In that case, the comparisons unequivocally favor REML. First, REML copes much more effectively with strong correlations among the responses for the subjects than does ML. Second, REML estimates do not have the downward bias that ML estimates have because REML estimators take into account the degrees of freedom from the fixed effects in the model. Finally, REML estimators are less sensitive to outliers in the data than ML estimators (Lee, 2017).

Model and Variable Selection

To select the important variables, first the main effect and main effect by time interaction were incorporated to the model and, then the highly non- significant interaction effects were removed and the model were refitted again and so on.i.e. Backward Elimination technique was employed to select significant factors to be included in the final model. Finally, the best model that can fit the data was selected depending on different information criteria like AIC and BIC. The model with smallest values of information criteria was selected as the best model to fit the data well.

Result and Discussion

Description of the Data

A data of 287 patients who were on ART between January 2017 and June 2019 in Zewditu hospital were collected. As we can see from Table 2, the mean baseline age of patients was 36.93 years with a standard deviation of 12.04 years whereas, the mean baseline CD4 count was 296.51

cells/$mm^3$with a standard deviation of 158.79 cells/mm^3.Also patient's average hemoglobin level at the baseline was 13.67g/dl with a standard deviation of 6.43 g/dl.

Table1: Descriptive Statistics of Continuous Covariates at baseline for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019

	Baseline CD4 count	**Body mass index**	**Baseline Hemoglobin**	**Baseline age**
Mean	296.51	22.02	13.67	36.93
Std. Deviation	158.79	3.9	6.43	12.04

The socio-demographic characteristics of patients are displayed in Table 3. For example, looking at the sex distribution, more than half 56.4% of them were females. About 9.4% of patients had no formal education, 28.9% had primary education, 40.4% had secondary education and 21.3% had tertiary education level. Considering the marital status of the patients, 30.3% were single, 48.1% were married while smaller number 2.4% were separated. Also, 33.4% of the patients were alcohol users and 16.4% had smoking status.

Table 2: Frequencies and percentages of the socio-demographic characteristics of HIV/AIDS patients on ART in Zewditu Hospital, Jan 2017 - Jun 2019

Variables	**Category**	**Frequency**	**Percent (%)**
Sex	Male	125	43.6
	Female	162	56.4
occupation	Government employed	45	15.7
	Private	93	32.4
	Merchant	34	11.8
	Driver	19	6.6
	Student	42	14.6
	House wife	54	18.8
Marital status	Single	87	30.3
	Married	138	48.1
	Divorced	27	9.4
	Widowed	28	9.8
	Separated	7	2.4
Educational Status	Primary	83	28.9
	Secondary	116	40.4
	Tertiary	61	21.3

Alcohol	No	191	66.6
	Yes	96	33.4
Smoking Status	No	240	83.6
	Yes	47	16.4

According to Table 3, 53.3% of the patients were at clinical stage 15.7% were at clinical stage 2, 20.6% were at clinical stage 3 and the rest 10.5% were at clinical Stage 3 at the time of starting the ART treatment. On the other hand, predominant ART regimen prescribed for patients at baseline were a combination of (TDF-3TC-EFV). Also, with regard to adherence, there were 65.9% patients who had good adherence status.

Table 3: Frequencies and percentages of the clinical characteristics of HIV/AIDS patients onART in Zewditu Hospital, Jan 2017 - Jun 2019

Variables	Category	Frequency	Percent (%)
WHO clinical stage	Stage 1	153	53.3
	Stage 2	45	15.7
	Stage 3	59	20.6
	Stage 4	30	10.5
Initial ART regimen	AZT-3TC-NVP	69	24.0
	AZT-3TC-EFV	31	10.8
	TDF-3TC-EFV	142	49.5
	TDF+3TC+NVP	45	15.7
Functional status	Working	234	81.5
	Ambulatory/bedridden	53	18.5
Adherence	Good	189	65.9
	Fair	56	19.5
	Poor	42	14.6
History of opportunistic infection	No	229	79.8

	Yes	58	20.2
History of TB	No	240	83.6
	Yes	47	16.4

The descriptions (number of observations, mean and standard deviation) of the longitudinal response variable are presented in Table 4. Here, instead of the actual observations of the response variable, the descriptive of the transformed (log VL) were calculated. The main reason behind the transformation of viral load is due to it has a non-normal distribution.

Table 4: Number of observations, mean and standard deviation of log VL at each time point

Time	N	Mean	Standard Deviation
Time 1	287	3.9235	0.7019
Time 2	287	3.1186	0.8079
Time 3	273	2.7854	0.8121
Time 4	225	2.6391	0.6822
Time 5	153	2.4985	0.4833
Time 6	77	2.4549	0.3886

All the 287 patients were observed at baseline time and at the second visit. Then, 95.72% patients were observed at the third visit and only 26.82% patients stayed at the last visit. It is not possible to say the number of patients is decreasing in successive time points as the patients entered into study at different times.

Individual Profile Plot

From the transformed viral load, there is evidence of between subject's variability as well as within subject variability. Subjects have different viral load values at the start and also possibly different evolutions over time; this suggests that perhaps linear mixed models with random intercepts and slopes could be plausible starting point. The evidence also supported by a high within individual variability over time from both females and males profile plot.

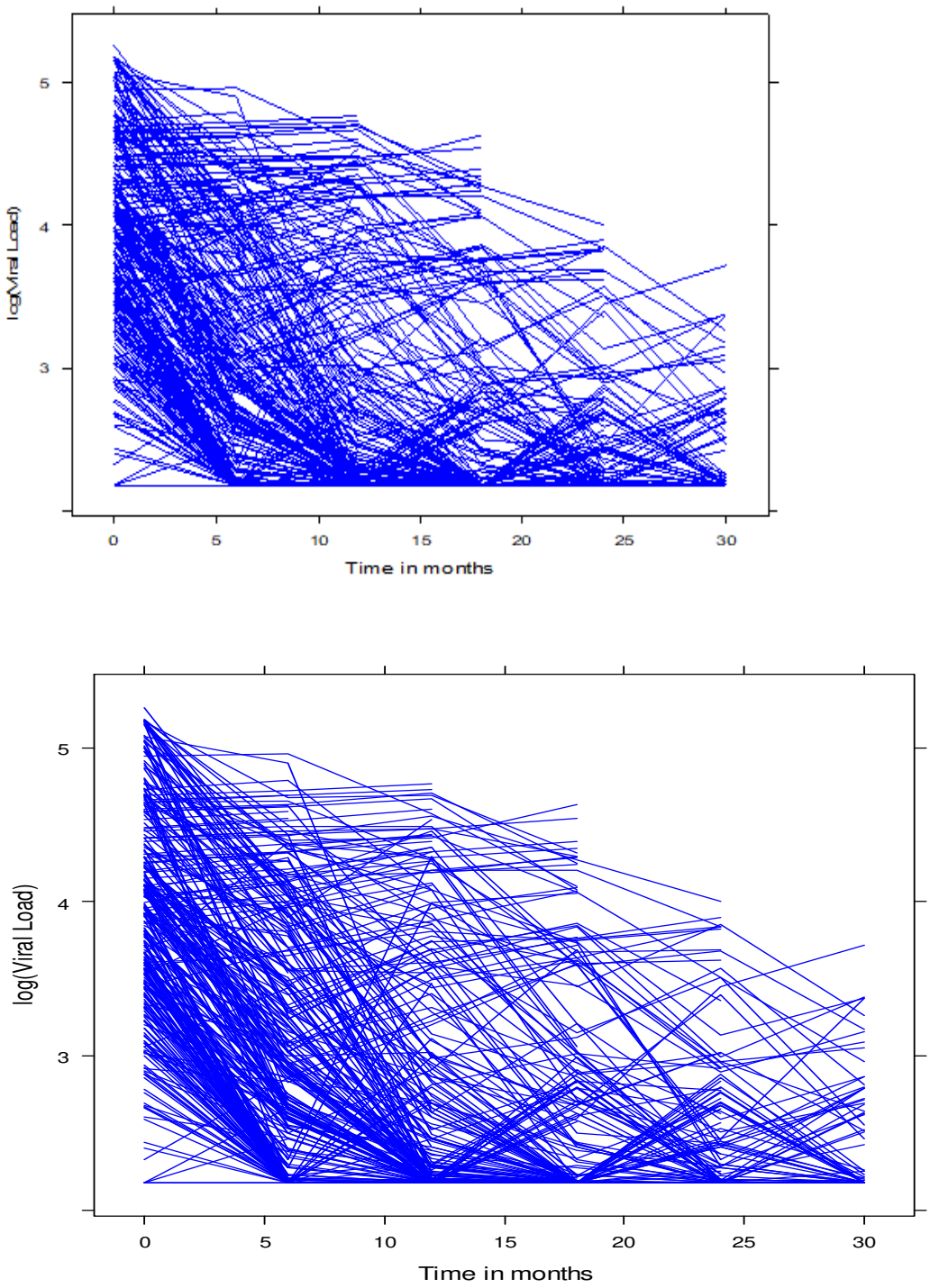

Figure1: Individual profile plot for log (viral load) for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019

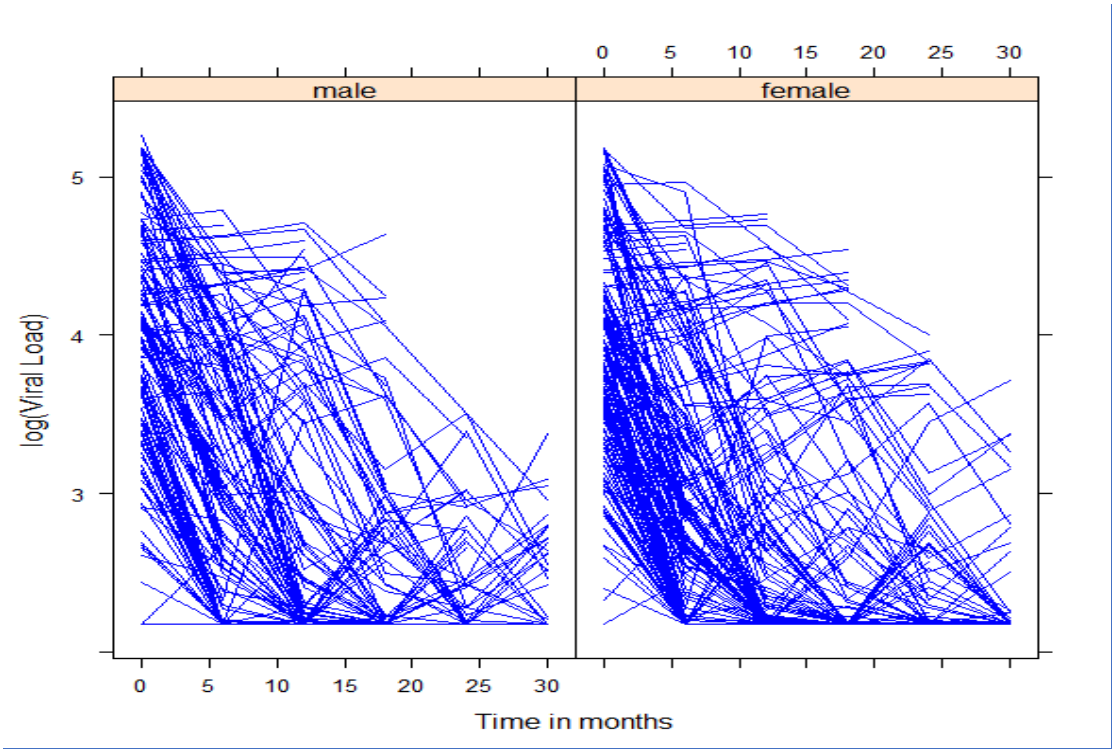

Figure 2: Individual profile plot by sex for log (viral load) for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019

Exploring the Mean Structure

The overall mean profile plot of transformed viral load shows a decreasing pattern of viral load over time (figure 3). The mean log VL decreases in a high rate from baseline till the second visit (12 months) and then it starts to decrease slowly from this point of time to the last.

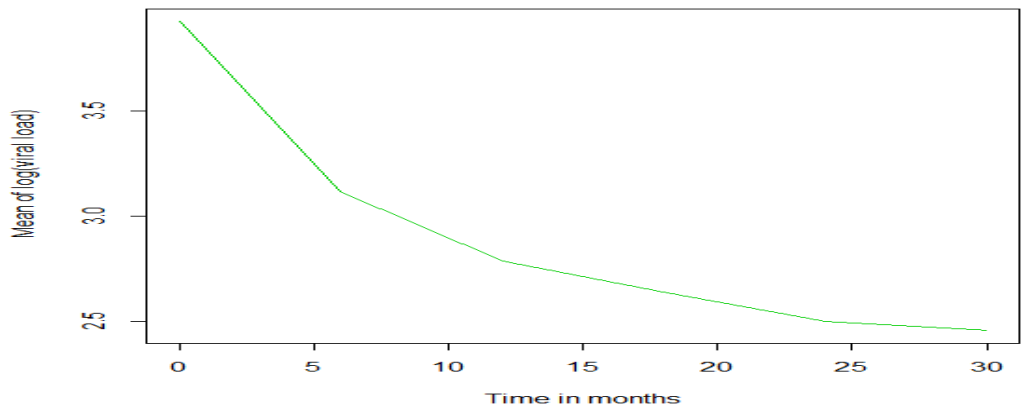

Figure 3: Mean profile plot of log (viral load) for HIV/AIDS patients under ART in ZewdituHospital, Jan 2017 - Jun 2019

As we have seen from figure 4, the mean log VL profile of the treatment TDF-3TC-EFV is lower than the other treatment combinations. Moreover, the plot also shows decreasing pattern on treatment combination AZT+3TC+EFV at some time points and a mild increase at another time points.

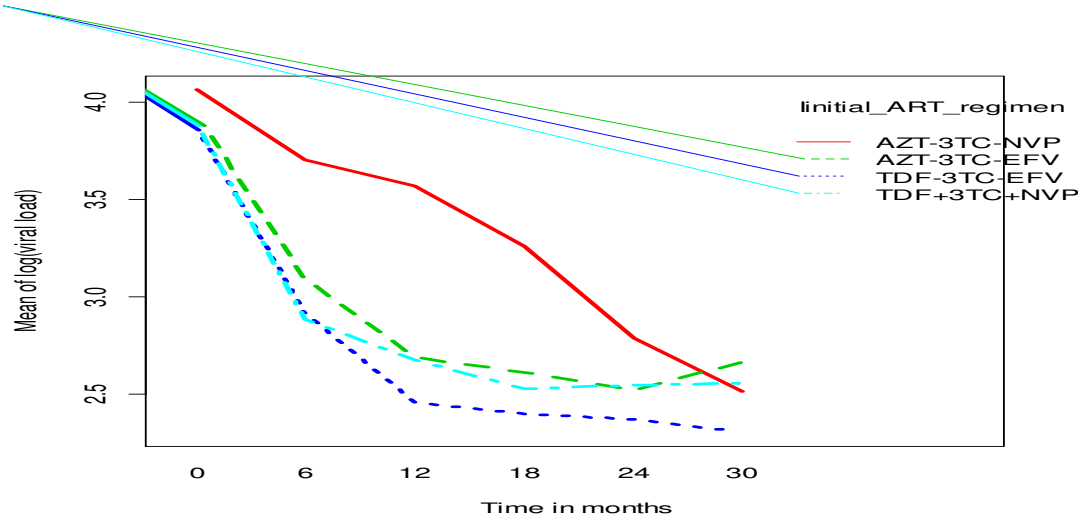

Figure 4: Mean profile plot of log (viral load) by treatment for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019

Exploring Correlation Structure

From Table 5 the correlation structure looks like irregular overtime. That means the off diagonal correlation has no known pattern over time, thus unstructured correlation might be appropriate.

Table 5: Correlation matrix of log VL over time

	Time 1	Time 2	Time 3	Time 4	Time 5	Time6
Time 1	1.0000					
Time 2	0.7086	1.0000				
Time 3	0.4216	0.5687	1.0000			
Time 4	0.4085	0.4654	0.4167	1.0000		
Time 5	-0.3651	0.5655	0.3053	0.4649	1.0000	
Time 6	-0.0139	0.4481	0.3498	0.5583	0.5610	1.0000

Random Effect Selection

As clearly shown in Table 6, the random intercept and slope model is a much better fit because it has a much lower AIC (2485.1)as compared with the other models. It is in line with the individual profile plot.

Table 6: Selection of appropriate random effect for modeling log viral load data

Model	AIC	logLik	Likelihood-ratio test	p-value
Mixed model with Random intercept only	3249.9	-1621.9		
Mixed model with Random slope only	2822.3	-1406.1	431.6	<.0001
Mixed model with Random intercept and slope	2485.1	-1236.5	339.2	<.0001

Determining Time Effect

Table 7 showed that we have evidence to incorporate cubic time effect. It is in agreement with the mean profile of log VL

Table 7: Comparison of linear mixed effect model with different time effect

Time Effect	AIC	logLik	Likelihood-ratio test	p-value
Linear	2485.1	-1236.5		
Quadratic	2063.9	-1021.9	429.2	<.0001
Cubic	1957.1	-963.5	116.8	<.0001

Correlation Structure Selection

We refitted the linear mixed effects model of log VL under all possible and proposed correlation structures. So, in fitting the model by REML method of estimation, the selected correlation structure was unstructured. Therefore, the final model to fit the data is mixed effect model with unstructured correlation structure.

Table 8: Correlation structure selection for linear mixed model

Correlation Structure	AIC	BIC
Independent	1990.7	2068.3
Exchangeable	1991.7	2074.4
AR (1)	1979.5	2062.2
Toeplitz	1984.2	2066.3
ARMA (1,1)	1976.1	2064.1
Unstructured	1933.4	2048.5

Final Result of Linear Mixed Effects Model with AC Errors

To explore the relationship between log VL and each of the covariates, bivariable analysis for each independent variable was assessed and those found to be significant ($p<0.25$) were selected for the multivariable analysis. Based on the results obtained from the linear mixed effects model; time, baseline_CD4, WHO_ clinical stage, adherence, functional status, smoking status, initial ART Regimen, interaction of time with adherence and interaction of time with WHO_ clinical stage were found to be significant factors of log VL of a patient.

The estimated model for log VL is stated as follows:

$$LogVL = 4.1476 - 0.1773(Time) + 0.0078(Time^2) - 0.0001(Time^3) - 0.0011(baseline\_CD4) + 0.1908\ (WHO\ Stage\ 3) + 0.3434\ (WHOStage\ 4) + 0.3736\ (Fair\ Adherence) + 0.6595(Poor\ Adherence) + 0.2299(Ambulatory/bedridden) + 0.1679(Smoking/yes) - 0.1855(TDF\text{-}3TC\text{-}EFV) - 0.1298(TDF+3TC+NVP) + 0.0106(Fair\ x\ Time) + 0.0185(Poor\ x\ Time) - 0.0042(Stage\ 3\ x\ Time) + b_{0i} + b_{1i}(11)$$

Where, b_{0i} is the individual deviation from average intercept and b_{1i} represent the individual deviation from the average linear trend component.

Because of too small deviation from their respective average trend components, $b_{2i}\ and\ b_{3i}$ are discarded from the final model. Beside to this, they also had no any contribution tothe total variation in log VL.

From multivariable linear mixed model (Table 9), it was found that the average log VL of patients at stage 1, good adherence, working status, had not smoking status, taking (AZT-3TC-NVP) drugwas4.1476. keeping all other variables constant, for a unit increase in baseline CD4 count, the average log VL of a participant would decrease by 0.0011 copies/ml.

Time had a significant effect on log VL of patients, keeping the effect of other covariates constant over time the average log VL of an individual decreased by a cubic time effect. But specifically, individuals who have fair and poor adherence have additional 0.0106 copies/ml and0.0185copies/ml increment respectively, in their log VL for a one-unit increment in the duration of treatment. Conversely, individuals who are at WHO stage 3 have a significant decrement in their log VL by 0.0042 copies/ml for a unit increment in time.

Regarding on individual's functional status, Patients who are either ambulatory or bedridden in their functional status have an average0.2299 copies/ml exceedance in their log VL, as compared with those who have working functional status. In addition, patients who have smoking habit have an average0.1679 copies/ml exceedance in their log VL, as compared with those who have no smoking habit with keeping the effect of other covariates constant.

The importance of treatment was strongly reveled from this study. keeping the effect of other covariates constant, taking a combination of (TDF-3TC-EFV) drug lowers patients log VL by 0.1855 copies/ml on average as compared to those who take a combination of (AZT-3TC-NVP) drug. Furthermore, patients who take a combination of (TDF+3TC+NVP) drug also have a lower log VL as compared to those who take a combination of (AZT-3TC-NVP) drug. Even though a combination of (AZT-3TC-EFV) drug lowers patientslog VL by 0.1728 copies/ml on average, but it does not show a statistical significance.

Table 9: Parameter estimates for full linear mixed-effects model

Fixed effects	Estimate	95 % CI		p-value
		Lower	Upper	
Intercept	4.1476	3.9682	4.3269	0.0000
Time	-0.1773	-0.1943	-0.1604	0.0000
$Time^2$	0.0078	0.0065	0.0092	0.0000
$Time^3$	-0.0001	-0.0002	-0.00009	0.0000
baseline_CD4	-0.0011	-0.0013	-0.0008	0.0000
WHO Clinical Stage				
Stage 1	Ref.			
Stage 2	0.0148	-0.1672	0.1968	0.8734

Stage 3	0.1908	0.0191	0.3625	0.0295
Stage 4	0.3434	0.0953	0.5915	0.0067
Adherence				
Good	Ref.			
Fair	0.3736	0.2047	0.5425	0.0000
Poor	0.6595	0.4431	0.8759	0.0000
Functional Status				
Working	Ref.			
Ambulatory/bedridden	0.2299	0.1089	0.3509	0.0002
Smoking Status				
No	Ref.			
Yes	0.1679	0.0491	0.2868	0.0058
ART Regimen				
AZT-3TC-NVP	Ref.			
AZT-3TC-EFV	-0.1728	-0.3357	0.0099	0.0949
TDF-3TC-EFV	-0.1855	-0.3128	-0.0582	0.0043
TDF+3TC+NVP	-0.1298	-0.2821	-0.0226	0.0376
Adherence × Time				
Good x Time	Ref.			
Fair x Time	0.0106	0.0016	0.0196	0.0206
Poor x Time	0.0185	0.0067	0.0302	0.0021
WHO Clinical Stage × Time				
Stage 1 x Time	Ref.			
Stage 2 x Time	-0.0016	-0.0124	0.0093	0.7791
Stage 3 x Time	-0.0042	-0.0138	-0.0015	0.0391
Stage 4 x Time	-0.0021	-0.0152	0.0111	0.7572

Ref. =reference category

Table 10: Estimates of random components

Random components	Estimate
$\boldsymbol{\sigma_{boi}}$	0.4864
$\boldsymbol{\sigma_{b1i}}$	0.0163
$\boldsymbol{\rho_{boi\ b1i}}$	-0.925
$\boldsymbol{\sigma_{\varepsilon}}$	0.4115
Intraclass Correlation (ICC)	0.5499

From Table 10, there are two estimated variance components; these are the random effects standard deviation and the residual standard deviation. The residual standard deviation is$\sigma_{\varepsilon ij}$ =0.4115and for the random effects, σ_{b0i} = 0.4864 andσ_{b1i}=0.0163. The total variability between individuals is estimated as σ_{b0i}+ σ_{b1i}= 0.5027 whereas the total variability within individual is 0.4115. However, the total variation in log VL is estimated to be 0.5027 + 0.4115= 0.9142. The proportion of total variability that is attributed to within person variation is given by 0.4115/0.9142 is 45.01% while the proportion of total variability attributed to between individual variations in their general level of log VL is 0.5027 /0.9142 is 54.99%. Therefore, more than half of the variation is explained by random effects. Finally, the correlation between the intercept and linear trend is negative; it equals -0.925, which is very strong in size. This suggests that patients who have initially high viral load (i.e., greater intercepts) improve at a greater rate.

Discussion

Duration on treatment have a positive effect on HIV positive patients. For a one-unit increase in treatment duration the log VL of patients decreased; this finding is in line with studies in Cameroon (Chendi et al., 2019). This means patients with longer time on treatment have a lower viral load than those of patients with short duration on the treatment.

When other effects held constant in the model for a unit increase in baseline CD4 count, log VL significantly decreased by 0.0011. Implying that there is a negative association between CD4 count and viral load. This finding is supported by other studies like a study conducted in Botswana (Farahani et al., 2016) and in Vietnam (Rangarajan et al., 2016).

When compared with patients at a fair and poor adherence status, those at good adherence have a decreased log VL. This is because low level of antiretroviral in the body owing to the non-adherence is not sufficient to suppress viral replication, hence leads to detection of higher viral load in the blood. This indicates that adherence play an important role in maintaining successful decrement in viral load of patients. This finding is in agreement with the studies of (Bayu et al., 2017) and (Hailu et al., 2018).

This study was concordant with a study done by Teshome and Yalew, they showed that starting of ART at later stages of WHO (third and fourth) had a risk of poor outcomes as compared as initiation of ART at early stages(Teshome and Yalew, 2015).

There was a significant decrease in the viral load in combination of (TDF-3TC-EFV) and (TDF+3TC+NVP) drugs. This result is in consistent with the study done by(Abu-Raddad and Awad, 2014) and (Edwards et al., 2015).

We found an association between cigarette smoking and viral load. Smokers had exceedance in their log VL by 0.1679 copies/ml than non-smokers. This association is supported by evidence from a study conducted inCameroon(Ande et al., 2015) and in Vietnam(Pollack et al., 2017).

This study also revealed that baseline functional status of an HIV/AIDS patients is an important predictor of viral load evolution. Being either ambulatory or bedridden increases log VL by 0.2299 when compared with those who are working. This might be due to the fact that those worker patients may have better income that in turn creates opportunity to get better care and support. This finding is in disagreement with the studies of (Sultan et al., 2019). The main reason behind the disagreement might be the follow up time of their study was relatively shorter as compared to our study.

Conclusion

Linear mixed model with random intercept and slope wereselected to fit the data based on different information criteria.cubic time effect was incorporated only in the fixed effect part. The main reason behind cubic time effect was not included in the random effect part was it had no any contribution to the total variation in log VL.

In this study, we have found an overtime decrement in the log VL of patients with HIV on ART. There was a significant variation in log VL of patients at baseline and through ART treatment time. The effects of several factors on the evolution of log VL were identified. Among these time, baseline CD4 count, WHO clinical stage, functional status of the patient, adherence, smoking status, initial ART Regimen and Time interaction with adherence and WHO stage were found to be significant predictors of log VL evolution. Specifically, patients with higher duration on treatment, who have high baseline CD4 count,with earlier WHO clinical stage (stage 1 and stage 2), with working status, with good adherence, had no smoking status, used acombination of (TDF-3TC-EFV) and (TDF+3TC+NVP) drugs were significantly reduce in their log VL.

Ethical Consideration

Ethical clearance and Letter of cooperation for selected Hospital was obtained from the Institutional Review Board of the University of Gondar. Waiver letters was obtained from the medical director of Zewditu Hospitalin order to access the medical records of patients. Confidentiality during all phases of research activities was kept and data was held on a secured password-protected system.

List of abbreviations

AC: Autocorrelated, AIC: Akaike's Information Criterion, AIDS: Acquired Immune Deficiency Syndrome, ART: Anti-Retroviral Therapy, ARV: Anti-Retro Viral, BIC: Bayesian Information Criterion, EDA: Exploratory Data Analysis, HIV: Human Immunodeficiency Virus, LMM: Linear Mixed Model, MI: Multiple Imputation, ML: Maximum Likelihood, MAR Missing at Random, MCAR: Missing Completely at Random, MNAR: Missing not at Random, NASBA: Nucleic Acid Sequence-based Amplification, PCR: Polymerase Chain Reaction, RVI: Retroviral Infection, REML: Restricted Maximum Likelihood, VL: Viral Load

Ethical Consideration

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Consent for publication

Not applicable.

Availability of data and materials

Authors have Considered HIV/AIDS datasets from Zewditu Hospital patient history card and now, attached as supplementary materials of the submission system.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' Contributions

Dawit Getachew conceptualized the proposal identified and reviewed all papers, and analysis, and interpretation of data. Dr. Aragaw Eshtieand and Dessie Melese participated in the conception of the study, provided methodological guidance, supervised the analysis, and prepared the manuscript. All authors reviewed the manuscript critically for content and approved the final version to be submitted.

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REFERENCE

1. ANDE, A., MCARTHUR, C., AYUK, L., AWASOM, C., ACHU, P. N., NJINDA, A., SINHA, N., RAO, P. S. S., AGUDELO, M., NOOKALA, A. R., SIMON, S., KUMAR, A. & KUMAR, S. 2015. Effect of Mild-to-Moderate Smoking on Viral Load, Cytokines, Oxidative Stress, and Cytochrome P450 Enzymes in HIV-Infected Individuals. *PLOS ONE*.
2. BAYU, B., TARIKU, A., BULTI, A. B., HABITU, A. Y., DERSO, T. & TESHOME, D. F. 2017. Determinants of virological failure among ptients on highly active antiretroviral therapy inUniversity of Gondar Referral Hospital, Northwest Ethiopia: a case–control study. HIV/AIDS - Research and Palliative Care.
3. CALLAWAY, D. S., RIBEIRO, R. M. & NOWAK, M. A. 1999. Virus phenotype switching and disease progression in HIV-1 infection. Proceedings of the Royal Society of London. Series B: Biological Sciences, 266**,** 2523-2530.
4. CHENDI, B. H., ASSOUMOU, M. C. O., JACOBS, G. B., YEKWA, E. L., LYONGA, E., MESEMBE, M., EYOH, A. & IKOMEY, G. M. 2019. Rate of viral load change and adherence of HIV adult patients treated with Efavirenz or Nevirapine antiretroviral regimens at 24 and 48 weeks in Yaoundé, Cameroon: a longitudinal cohort study. BMC infectious diseases, 19**,** 194.

5. CSA*, 2016,* Addis Ababa, Ethiopia and Rockville, Maryland, USA:, ICF International and central Statistical Agency.
6. EDWARDS, J. K., COLE, S. R., MARTIN, J. N., MOORE, R., MATHEWS, W. C., KITAHATA, M., ERON, J. J., SAAG, M. & MUGAVERO, M. J. 2015. Dynamic visual display of treatment response in HIV-infected adults. *Clinical Infectious Diseases,* 61**,** e1-e4.
7. FARAHANI, M., NOVITSKY, V., WANG, R., BUSSMANN, H., MOYO, S., MUSONDA, R. M., MOETI, T., MAKHEMA, J. M., ESSEX, M. & MARLINK, R. 2016. Prognostic Value of HIV-1 RNA on CD4 Trajectories and Disease Progression Among Antiretroviral-Naive HIV-Infected Adults in Botswana: A Joint Modeling Analysis. AIDS RESEARCH AND HUMAN RETROVIRUSES.
8. GILMOUR , A. R., THOMPSON, R. & CULLIS, B. R. 1995. Average information REML: an efficient algorithm for variance parameter estimation in linear mixed modelsBiometrics, (pp. 1440–1450).
9. HAILU, G. G., HAGOS, D. G., HAGOS, A., WASIHUN, A. G. & DEJENE, T. 2018. Virological and immunological failure of HAARTandassociatedriskfactorsamong adultsandadolescentsintheTigrayregionof NorthernEthiopia. *PLOS ONE*.
10. HEDEKER, D. & GIBBONS, R. D. 2006. *LONGITUDINAL DATA ANALYSIS* New Jersey, John Wiley & Sons, Inc.
11. LEE, S.-C. 2017. The restricted maximum likelihood estimation of a censored regression model. The Korean Statistical Society, and Korean International Statistical Society., 24, No. 3, 291–301.
12. POLLACK, T., DUONG, H., PHAM, T., DO, C. & COLBY, D. 2017. Cigarette smoking is associated with high HIV viral load among adults presenting for antiretroviral therapy in Vietnam. PLoS ONE.
13. RANGARAJAN, S., COLBY, D. J., LE TRUONG GIANG, D. D. B., NGUYEN, H. H., TOU, P. B., DANH, T. T., TRAN, N. B. C., NGUYEN, D. A., NGUYEN, B. T. H. & DOAN, V. T. N. 2016. Factors associated with HIV viral load suppression on antiretroviral therapy in Vietnam. Journal of virus eradication, 2**,** 94.
14. SAAG, M. S., HOLODNIY, M., KURITZKES, D., O'BRIEN, W., COOMBS, R., POSCHER, M., JACOBSEN, D., SHAW, G., RICHMAN, D. & VOLBERDING, P. 1996. HIV viral load markers in clinical practice. Nature medicine, 2**,** 625.

15. SULTAN, H., MOHAMMEDAMAN, M., BITEW, M., MANAYE, Y., MULUGETA, S., NEGUSSIE, B. & MOHAMMED, S. 2019. Predictors of Time to Viral Load Suppression of Adult PLWHIV on ART in Arba Minch General Hospital: A Follow up Study. *Ethiop J Health Sci.*

16. TESHOME, Y. Y. & YALEW, A. 2015. Magnitude and Predictors of Anti-Retroviral Treatment (ART) Failure in Private Health Facilities in Addis Ababa, Ethiopia. PLOS ONE
17. UNAIDS 2019. UNAIDS report on the global AIDS epidemic.

Figures

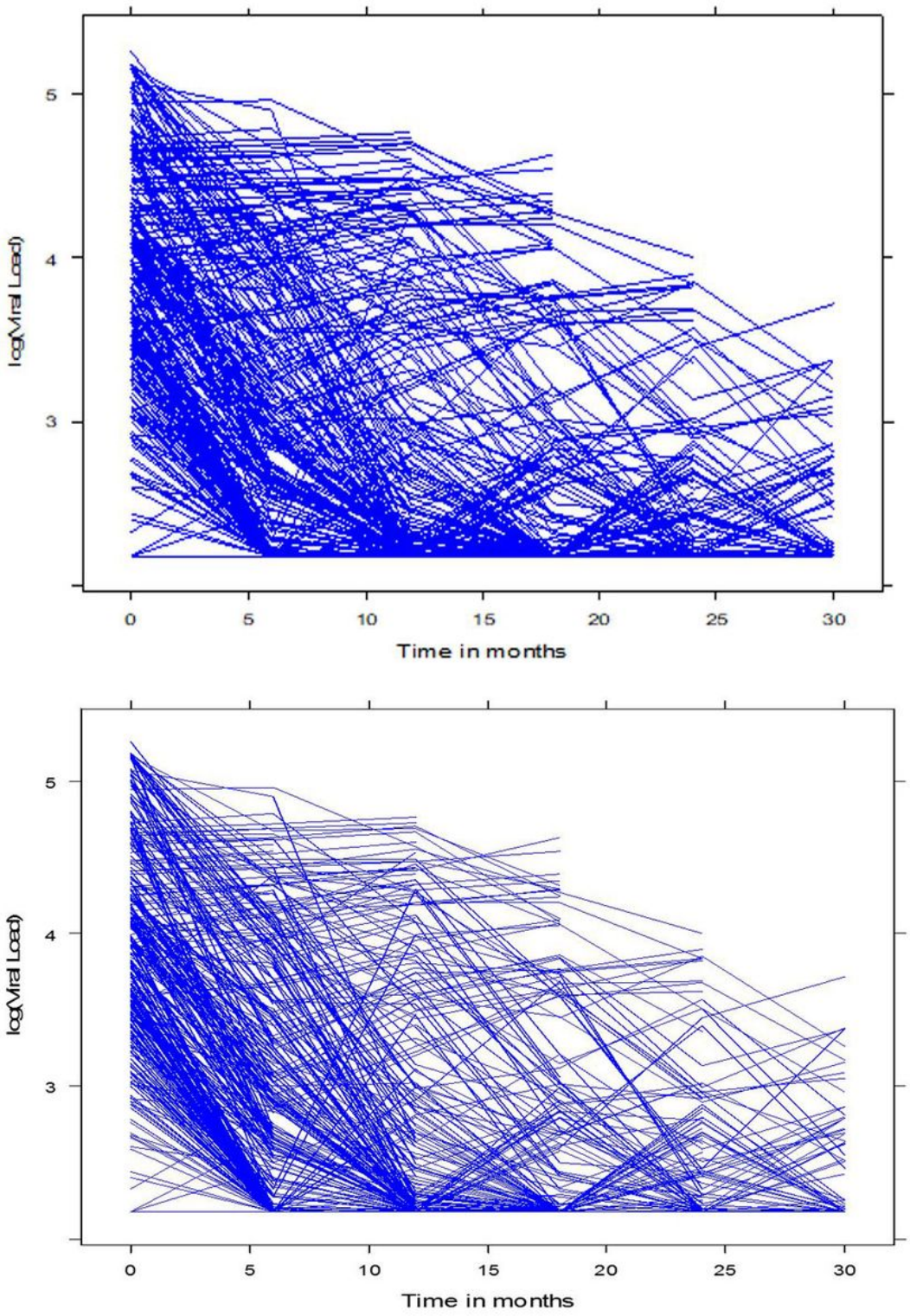

Figure 1

Individual profile plot for log (viral load) for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019.

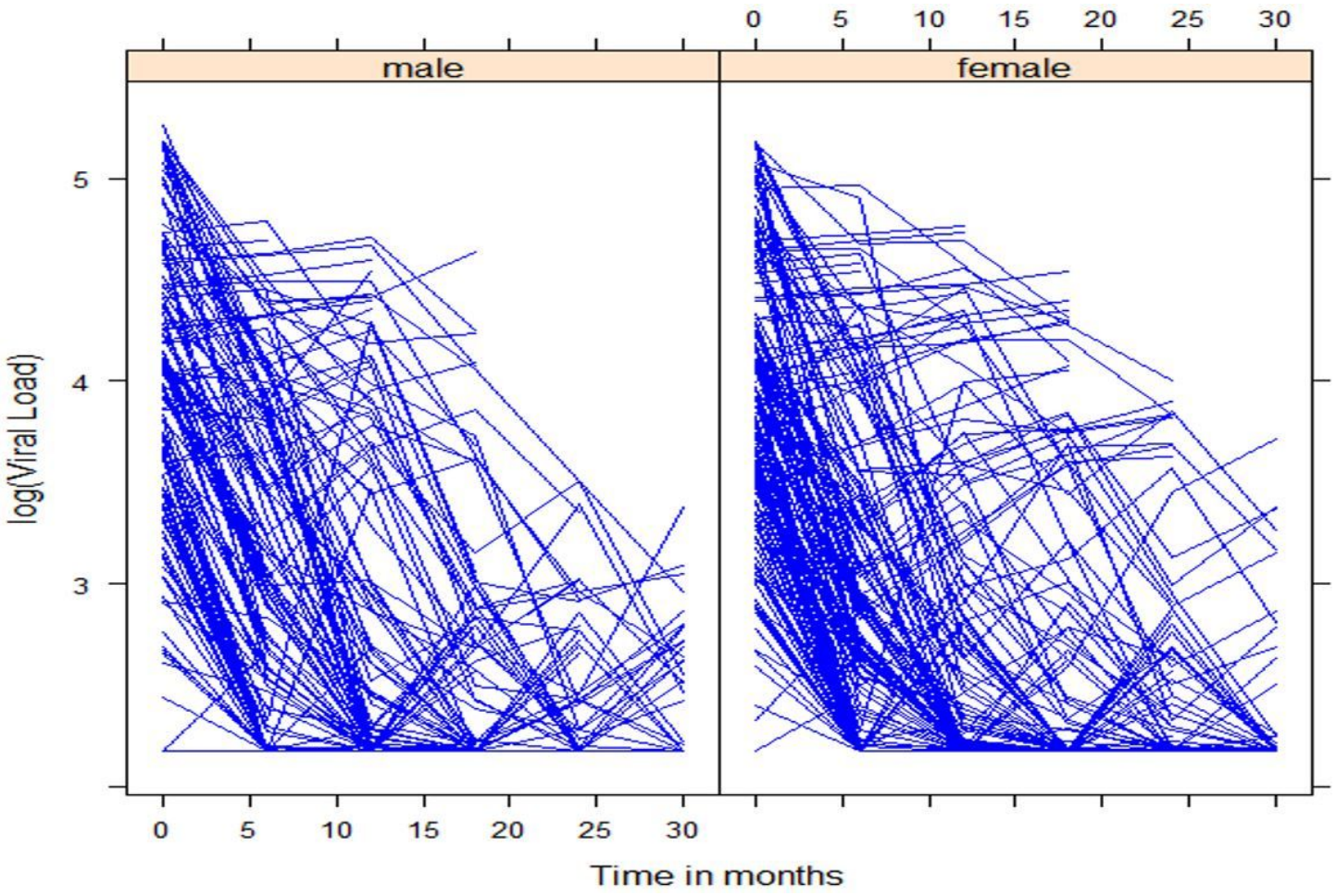

Figure 2

Individual profile plot by sex for log (viral load) for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019.

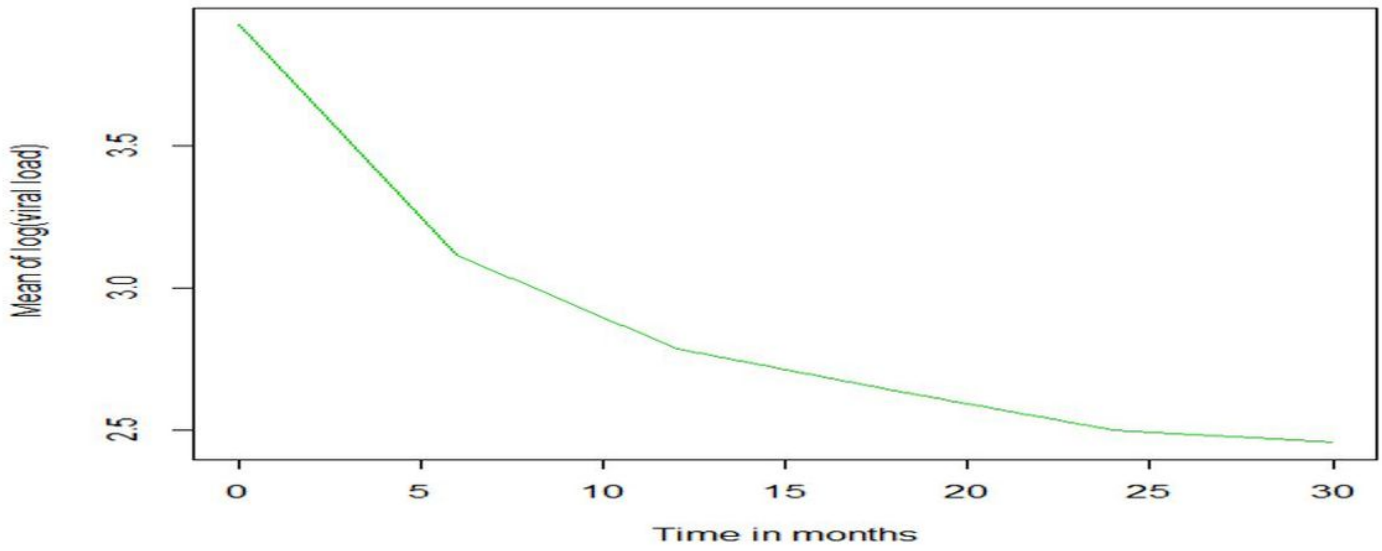

Figure 3

Mean profile plot of log (viral load) for HIV/AIDS patients under ART in ZewdituHospital, Jan 2017 - Jun 2019.

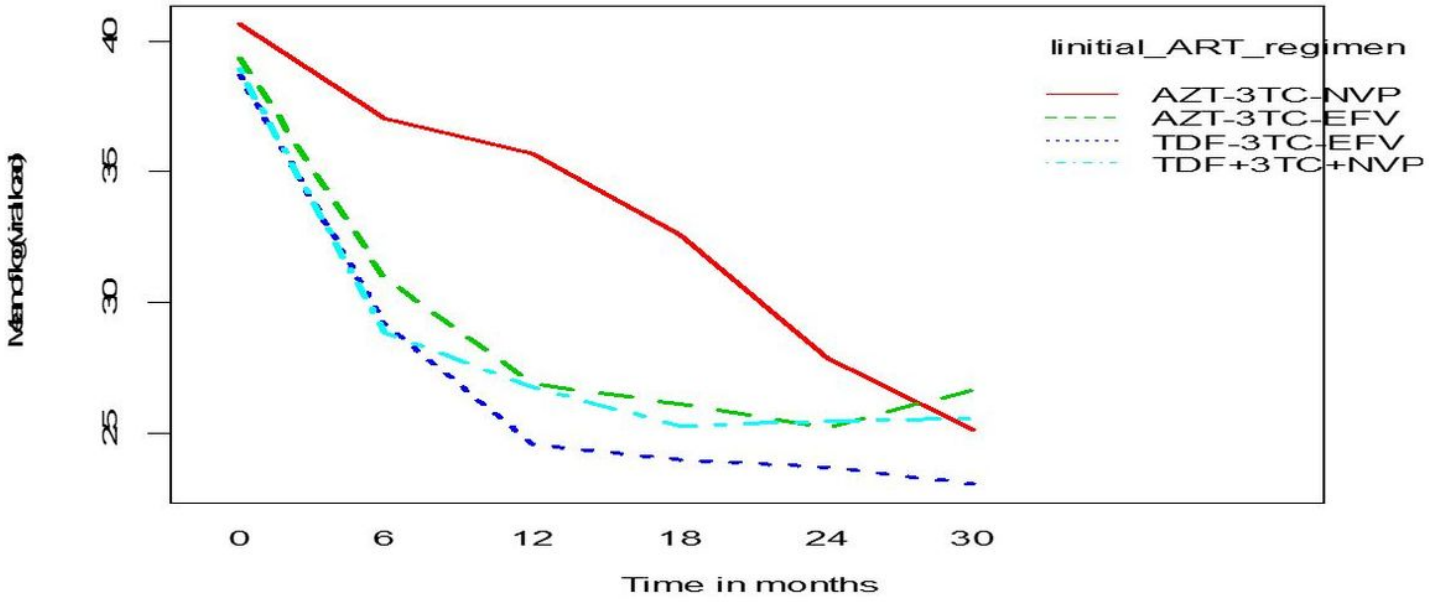

Figure 4

Mean profile plot of log (viral load) by treatment for HIV/AIDS patients under ART in Zewditu Hospital, Jan 2017 - Jun 2019.