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# LONGITUDINAL ANALYSIS OF CHANGE IN CD4+ CELL COUNTS OF HIV-1 PATIENTS ON ANTIRETROVIRAL THERAPY (ART) IN THE BUILSA DISTRICT HOSPITAL

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**Abstract**

The monitoring of CD4+ cell counts are a basis for assessing the effectiveness of most HIV treatments. Understanding the way CD4+ cells change over time could provide insight into the way Patients respond to treatment and how effective treatment is with time. In this study, we obtained secondary data from the HIV/AIDS Monitoring Program at the Builsa District hospital, in which patients were enrolled and their CD4+ cell counts were regularly monitored and thus generating repeated measures of their CD4+ cell counts. The purpose of the study was to investigate some plausible determinants of change in CD4+ cell count. Mixed effects modelling approach was used for modelling the CD4+ cell counts of the patients. The results showed that, the correlation between CD4+ cell counts at different times had a first order autoregressive moving average variance-covariance structure. The Initial CD4+ cell count of a patient, the duration of treatment and the drug type used in the treatment, were the factors that significantly determined a patient's current CD4+ cell count.

**Keywords:** CD4+ cell count, Longitudinal Analysis, HIV virus, Therapy, Patients

## 1.0. Introduction

Human Immunodeficiency Virus (HIV) is a lentivirus that causes Acquired Immunodeficiency Syndrome (AIDS) by reducing a person's ability to fight infection. HIV attacks an immune cell called the CD4+ cell which is responsible for the body's immune response to infectious agents. An uninfected individual has around 1100 CD4 cells per millilitre of blood. This $CD4^+$ cells, decrease in number with time from the HIV virus, so that an

infected person's $CD4^+$ cell count can be used to monitor the progression of the disease (Diggle *et al* 1994).

Since the beginning of the disease, somewhere between 1884 and 1924 in modern day Kinshasa, in the West Central African Republic (Louise, 2011), about 70 million people have contracted HIV and over 30 million people worldwide have died of it or related causes (UNAIDS, 2012).

Antiretroviral Therapy (ART) services have been available to HIV patients and the Guidelines from the National AIDS Control Program (NACP) recommend that patients should initiate treatment when their CD4+ cell count is less than 350 cells/$\mu l$ or when they become symptomatic with HIV infection as in WHO stages I to IV. Once a patient enrols on the ART treatment, the CD4+ cell count of the patients is examined from time to time to check whether there is an increase in its count to a relatively normal level (> 500 cells per $\mu l$) or otherwise (NACP, 2001).

Many researchers have carried out studies on the change in $CD4^+$ cell counts of HIV patients. Several of these studies have look at change from the cross sectional point of view without considering the pattern of change over the period of study or the obvious possible correlation among successive CD4+ counts. For example, Kulkarni *et al* (2011) and Xiuhong *et al* (2011) both used linear regression models to study some predictors of CD4+ Cell Count recovery in HIV-1 positive subjects receiving Antiretroviral Therapy and CD4+ T-Cell Counts and Plasma HIV-1 RNA Levels beyond 5 Years of Highly Active Antiretroviral Therapy respectively. These studies largely assumed that the change in $CD4^+$ cells is linear. Luguterah and Adams (2013) however used a longitudinal approach and suggested that the pattern of growth in $CD4^+$ cell was not Linear.

This study therefore took into consideration, the changes in the CD4+ cells count over the period of study. By considering changes over time, the longitudinal approach has the added advantage of observing changes more accurately, by increasing the power and validity of measuring the change in CD4+ cell counts.

## 2.0 Materials and Method

### Data

We obtained retrospective data from the HIV/AIDS Monitoring Program at the Builsa District hospital in the Upper East Region of Ghana, in which patients were enrolled and their $CD4^+$ cell counts initially taken on enrolment into the ART program and thereafter, counted every six months. This program included individuals who were diagnosed of HIV/AIDS, and were enrolled in the centre's HIV/AIDS Drug Treatment Program between the period 2008 and 2012: Our data was restricted to all individuals who had

their therapy between January 1, 2008 and December 31, 2012. Patients who were 15 years or older at the time of diagnosis, and had enrolled in the ART program and had their $CD4^+$ cells counted at least thrice, were considered in our study.

Different drug regimens are usually given to the patients based on their medical history: Drugs combinations that were prescribed to the patients in this study are; Combivir / Nevirapine (CBV+NVP), Tenofovir / Lamivudine / Nevirapine (TDF+3TC+NVP), Combivir/ Efavirenz (CBV+EFV) and Combivir / Lopinavir / Ritonavir (CBV+LPV+R) Tenofovir/Lamivudine/Efavirenz (TDF+3TC+EFV).

In all, a total of 139 patients' formed the sample and were used in this study. Other factors of the patient such as age, gender, level of education, marital status, religious affiliation and drug regimen issued were also obtained and used in this study.

**Modelling Approach**

We explored the data using basic descriptive statistics and a profile plot of the mean CD4+ cell count over the period of our study. Linear mixed effects model was used to model the change in $CD4^+$ cell count over time. This modelling approach was used because mixed models take into account both the within and between sources of variation, are flexible enough to account for the natural heterogeneity in the population, and can handle any degree of missing and drop-out data in the data. The general structure for the model is

$$\boldsymbol{y_i = X_i\beta + Z_i b_i + \varepsilon_i}, \qquad \boldsymbol{i = 1, \ldots, m}$$

Where

$\mathbf{y_i} = (\mathbf{y_{i1}}, \mathbf{y_{i2}}, \ldots \mathbf{y_{ini}})^{\mathbf{T}}$, $\quad \mathbf{b_i} \sim \mathbf{N_q}(0, \psi) \quad \boldsymbol{\varepsilon_i} \sim \mathbf{N_{ni}}(0, \sigma^2 I)$

$\beta =$ fixed effects, $\mathbf{b_i} =$ Random effect for unit $i$

$\psi =$Between-unit covariance matrix

$\sigma^2\mathbf{I} =$Within-unit covariance matrix

$\mathbf{X_i}$ is an $n_i \times j$ matrix with $jth$ columns, matrix $Z_i$ is an $n_i \times k$ matrix $jth$ columns. Both matrices $\mathbf{X_i}$ *and* $Z_i$ depend on $i$ through $t_i$. Averaging over the distribution of the latent random effects $b_i$, the marginal (population-average) distribution of $y_i$ is

$y_i \sim N(X_i\beta, \Sigma_i)$, *where*

$\Sigma_i = Z_i \psi Z_i^T / \sigma^2 I$

The resulting estimated ***b***, the fixed-effect parameter for each predictor in this model, represents the average change in $CD4^+$ cell count for a unit increase in that predictor. The age of the patient and the Initial $CD4^+$

cell count were considered continuous variables. The type of correlation between successive CD4+ cell counts is reflected in the covariance structure. This was explored using the "smaller is better" criteria of the Akaike Information Criteria (AIC) and Bayesian Information criteria (BIC). The selected covariance structure, as well as the pattern of change of the CD4+cell count, was used to fit a No-intercept model for the data: Both a full and a reduced model were fit to the data.

**Results**

The descriptive statistics of the data, showing the distribution of the patients across the various levels of the factors considered, as well as their minimum and maximum CD4+ cell counts, are shown in Table 1. The pattern of Change of the time plot, as shown in figure 1, suggests a logarithmic trend of the change in CD4+ cell count. The Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) of the variance covariance structures model (shown in table 2), suggest that the correlation between the CD4+ cell counts at various times followed the first order autoregressive moving average (BIC = 4920.6). Thus, even though a patient's CD4+ cell count depended on his/her past CD4+ cell counts, the strength of the relationship was stronger with his/her immediate past CD4+ cell count, and weakened with increasing time difference between counts.

We incorporated the logarithmic trend into the linear mixed effect model to obtain the parameter estimates, and their significance, of the model as shown in Table 3. The table shows that the initial CD4+ cell count, age, gender and duration of treatment (in months) are significant determinants of the CD4+ cell counts of a patients on ART. The rate of change in CD4+cell count, expressed as a logarithmic function (ln(t)) is 105.01 counts per unit increase in logarithmic time. This suggests that the rate of increase in CD4+ cell count decreases with time.

A patient's initial CD4+cell count significantly determined his/her current CD4+ cell count at the 5% significance level ( *p*-value < 0.0001). A patient has a 0.4624 CD4+ cell count disadvantage, for every count lower in initial CD4, when compared to his/her counterpart. The duration of treatment is also shown to significantly determine a Patients CD4+ cell count ( $\beta = 39.685,\ p = < 0.0001$) as shown in table 4.4.3, a patients' CD4+ cell count increased by about 40 cells/$\mu L$ every 6 months. This suggested that there is strong positive association between CD4+ count and duration of treatment (time). The effect of age on change in CD4+ cell count was also statistically significant at the 5% significance level. A patient has an average of 2.551 count disadvantage for every year older he /she is at the time of diagnosis.

There was also significant gender differential among patients that were on treatment (*p*-values < 0.0001): The average CD4+ cell count for males is about 29 counts higher than that of their female counterparts. While there were no educational differentials in this study, Marital and Religious differentials were observed in this study. Only married patients significantly differed from the widowed. They had significantly lower CD4+ cell count ($\beta$ = -83.58 and *p*-value = 0.0084) when compared to the widowed. Patients who professed Islam or No Religion, had significantly lower change in CD4+ count ($\beta$ = -149.24, *p*-value = 0.0079 and $\beta$ = -127.79, *p*-value = 0.0433 respectively) when compared to those who belonged to the traditional religion.

The general model for patients $CD4^{+}$ count is given by

$$\begin{aligned} CD4\ count = {} & 0.4626PreCD4 - 2.4551Age + 105.04Time + 410.49Female \\ & + 440.16Male + 7.121No\ education - 87.0608Primary + 53.5336JHS \\ & + 4.5824SHS - 10.4645\text{Divorce} - 83.5799Married - 33.6269Seperated \\ & - 93.4106\text{Single} - 13.344Christianity - 149.24\text{Islam} \\ & - 127.39\text{No Religion} + 45.2674(\text{CBV} + \text{EFV}) + 16.0026(\text{CBV} + \text{LPV} + \text{L}) \\ & + 86.3384(\text{CBV} + \text{NVP}) + 3.8756(\text{TDF} + 3\text{TC} + \text{EFV}) \end{aligned}$$

With a reduced model for prediction given by

$$\begin{aligned} CD4\ count = {} & 0.49511PreCD4 + 113.6098Time + 289.4341(\text{CBV} + \text{EFV}) \\ & + 249.045(\text{CBV} + \text{LPV} + \text{R}) + 324.552(\text{CBV} + \text{NVP}) \\ & + 248.552(\text{TDF} + 3\text{TC} + \text{EFV}) + 241.186(\text{TDF} + 3\text{TC} + \text{NVP}) \end{aligned}$$

Where Combivir/Nevirapine = CBV+NVP, Tenofovir / Lamivudine / Nevirapine = TDF+3TC+NVP, Combivir / Efavirenz = CBV+EFV, Combivir / Lopinavir / Ritonavir = CBV+LPV+R and Tenofovir / Lamivudine / Efavirenz = TDF+3TC+EFV

**Discussions**

We compared the Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the known covariance structures and chose the autoregressive moving average model as the best fit for the covariance structure of the CD4+ cell counts based on the information criteria. This means that there is a correlation between CD4+ cell counts and that the correlation weakened with distance between counts. Our mixed model results showed that the duration of treatment is a significant determinant of a Patients CD4+ cell count (as expected of all treatments). This study is in contrast with the study by Viviane *et al* (2009), who showed a decline in CD4+ cell count over time among untreated HIV/AIDS patients; the net effect of these studies, together with the significance of duration of treatment shown in our study, confirms the well accepted fact of the effectiveness of the ART.

The significant gender differentials shown in this study, contradicts results from earlier studies by Mair *et al* (2008) that males were associated with significantly lower CD4+ cell counts when compared to females. Our results showed that male patients responded to treatment better than females which could be a result of socio cultural practises and norms associated with gender.

Consistent with findings by Xiuhong *et al* (2011), that older men had 60 fewer $CD4^+$ cells per microliter on average when compared with the younger men, our study showed that treatment was less effective for elderly patients: This is expected, because it is well known from literature that immune function declines with age.

The importance of early treatment was shown in this study: The Initial CD4+ cell count was shown to significantly determine a Patient's CD4+ count. A higher initial CD4+ cell count would result in a better rate of recovery of patients on ART: This agrees with findings of Viviane *et al* (2009) and Kulkarni *et al* (2011).

Our study did not show any age differentials: While is it conceivable that educational level could among other things influence knowledge of, and behavioural patterns about HIV/AIDS, and even response to treatment, as shown in several studies (McMahon *et al*., 2011; Upreti, Regmi, Pant and Simkhada, 2009; Ho and Loke, 2003) once a Person is diagnosed as having the virus and on monitored treatment, any medication taken is not expected to act differently on Patients because of their educational status.

On the marital status of Patients on ART, only married patients CD4+ cell counts significantly differed, when compared with the widowed: The significantly lower expected CD4+ cell counts for married couples is consistent with several studies and could be as a result of reinfection of each other.

The religious differentials shown in this study are not only a reflection of the deeply religious Ghanaian society, but also the high influence of Religion on the beliefs, attitude and practises on people. It is therefore imperative that in the education, treatment and other activities of HIV/AIDS in highly religious environments, not only should religious concerns be taken into account but also, the support of Religious leaders should be sought.

## Conclusion

Generally all the patients that were considered in our study between January 2008 and December 2012 had their CD4+ cell count increased at different levels after been put on treatment at a certain initial CD4+ cell count. The determinants of CD4+ cell counts, as well as the effect of the factors studied on patients CD4+ cell count were shown in this study. While

this study is useful to guide education to the Public, particularly Patients, and also guide policy and management of treatment, further studies are recommended to expand the scope of study as well as include more covariates.

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Table 1 Descriptive Statistics of CD4+ Count of Patients

| | Percentage | Min (CD4+) | Max (CD4+) |
|---|---|---|---|
| **Age** | | | |
| 20-29 | 21 | 324 | 1032 |
| 30-39 | 42 | 226 | 1779 |
| 40-49 | 18 | 160 | 1264 |
| 50-59 | 12 | 150 | 1203 |
| 60-69 | 7 | 140 | 1195 |
| **SEX** | | | |
| Male | 81 | 8 | 1779 |
| Female | 19 | 79 | 935 |
| **EDUCATION** | | | |
| Non | 59 | 51 | 1203 |
| Primary | 9 | 172 | 618 |
| JHS/middle | 14 | 39 | 1779 |
| SHS | 10 | 176 , | 1264 |
| Tertiary | 8 | 8 | 1195 |
| **Marital status** | | | |
| Divorce | 21 | 39 | 1779 |
| Married | 41 | 51 | 1203 |
| Single | 9 | 176 | 1264 |
| Widow(er) | 25 | 8 | 1195 |
| Separated | 2 | 172 | 618 |
| **DRUG** | | | |
| CBV+EFV | 27 | 244 | 1264 |
| CBV+LPV/R | 3 | 180 | 720 |
| CBV+NVP | 36 | 281 | 1779 |
| TDF+3TC+EFV | 14 | 155 | 769 |
| TDF+3TC+NVP | 20 | 238 | 1203 |
| **RELIGION** | | | |
| Christianity | 78 | 8 | 1779 |
| Islam | 11 | 150 | 1082 |
| Traditionalist | 7 | 185 | 634 |
| Non | 4 | 171 | 796 |

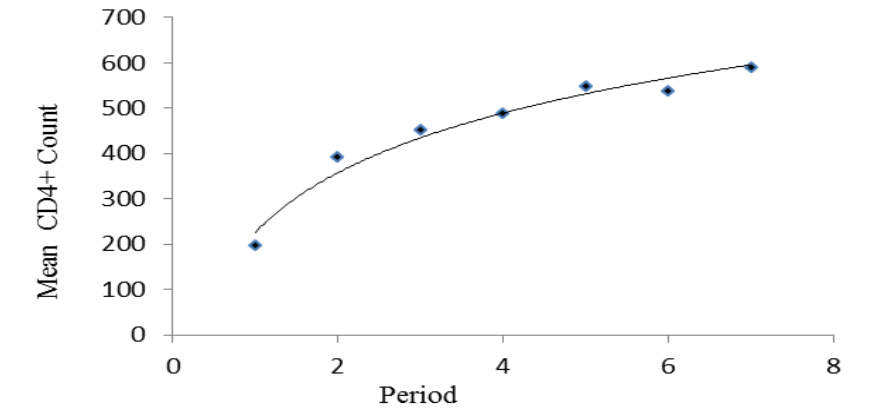


Figure 1 Profile Plot of mean CD4+ count

Table 2: Statistics for covariance Structure Models

| Structure | AIC | BIC |
|---|---|---|
| First order Autoregressive AR(1) | 4915.6 | **4920.6**** |
| Compound Symmetry | 4965.8 | 4970.8 |
| Variance Component | 5134.3 | 5136.8 |
| Toeplitz | 4917.2 | 4934.7 |
| AR (1,1) Moving Average | **4913.3**** | 4920.8 |
| Heterogeneous Toeplitz | 4960.8 | 4949.3 |
| Heterogeneous AR(1) | 4915.7 | 4935.7 |
| Heterogeneous Compound Symmetry | 4952.1 | 4972.1 |

** means Smallest

Table 3: First order Autoregressive AR (1) moving average covariance structure output

| Effect | Estimate | Standard Error | DF | t value | Pr < \|t\| |
|---|---|---|---|---|---|
| **Initial CD4+** | 0.4624 | 0.07432 | 372 | 6.22 | <.0001 |
| **Age** | -2.4551 | 1.212 | 372 | -2.03 | 0.0435 |
| **Ln(t)** | 105.01 | 17.8722 | 372 | 5.88 | <.0001 |
| **GENDER** | | | | | |
| Female | 410.49 | 105.59 | 372 | 3.89 | 0.0001 |
| Male | 440.16 | 107.76 | 372 | 4.08 | <.0001 |
| **Education (compared with tertiary)** | | | | | |
| Non | 7.1215 | 48.8301 | 372 | 0.15 | 0.8841 |
| Primary | -87.061 | 63.3908 | 372 | -1.37 | 0.1705 |
| JHS/middle | 53.5336 | 56.5644 | 372 | 0.95 | 0.3445 |
| SHS | 4.5824 | 59.2541 | 372 | 0.08 | 0.9384 |
| **Marital Status (compared with widowed)** | | | | | |
| Divorce | -10.465 | 34.0298 | 372 | -0.31 | 0.7586 |
| Married | -83.58 | 31.5647 | 372 | -2.65 | 0.0084 |
| Separated | -33.627 | -33.6269 | 372 | -0.48 | 0.6299 |
| Single | -93.411 | 49.6997 | 372 | -1.88 | 0.061 |
| **DRUG (compared with TDF/3TC/NVP)** | | | | | |
| CBV/EFV | 45.2674 | 40.222 | 372 | 1.13 | 0.2611 |
| CBV/LPV/R | 16.0026 | 72.2657 | 372 | 0.22 | 0.8249 |
| CBV/NVP | 86.3384 | 38.1768 | 372 | 2.26 | 0.0243 |
| TDF/3TC/EFV | 3.8756 | 40.9513 | 372 | 0.09 | 0.9247 |
| **RELIGION (compared with Traditionalist)** | | | | | |
| Christianity | -13.344 | 38.984 | 372 | -0.34 | 0.7323 |

| | | | | | |
|---|---|---|---|---|---|
| Islam | -149.24 | 55.8397 | 372 | -2.67 | 0.0079 |
| Non | -127.39 | 62.8247 | 372 | -2.03 | 0.0433 |

Table 4: Estimates of reduced model

| | Estimate | Standard Error | t value | Pr >\|t\| |
|---|---|---|---|---|
| Initial CD4+ | 0.49511 | 0.07045 | 7.028 | $9.61\times 10^{-12}$ |
| Ln(t) | 113.61 | 18.5267 | 6.132.15 | $2.15\times 10^{-09}$ |
| CBV/EFV | 289.434 | 29.572 | 9.787 | $<2.0\times 10^{-16}$ |
| CBV/LPV/R | 249.045 | 64.5578 | 3.858 | 0.000134 |
| CBV/NVP | 324.722 | 28.6959 | 8.881 | $2.0\times 10^{-16}$ |
| TDF/3TC/EFV | 248.552 | 30.9545 | 8.03 | $1.2\times 10^{-14}$ |
| TDF/3TC/NVP | 241.186 | 41.009 | 5.881 | $8.85\times 10^{-09}$ |