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A Joint Model for Longitudinal CD4 Count and Body Weight of Adult HIV patients after ART initiation, Asella Hospital

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A Joint Model for Longitudinal CD4 Count and Body Weight of Adult HIV patients after ART initiation, Asella Hospital

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As thesis research advisors, we herby certify that we have read the thesis prepared by **Asiya Naser** under our guidance, which is entitled "**A Joint Model for Longitudinal CD4 Count and Body Weight of Adult HIV patients after ART initiation, Asella Hospital**.", in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including tables and figures are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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As the members of the board of examiners of M.Sc. thesis open defense examination, we certify that we have read and evaluated the thesis and examined the candidate. Hence, we recommend that the thesis be accepted as it fulfills the requirements for the degree of Master of Science in Biostatistics.

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List of Acronyms

AIDS	Acquired Immune Deficiency Syndrome.
HIV	Human Immunodeficiency Virus.
UNAIDS	Joint United Nations program on HIV/AIDS.
FMOH	Federal Ministry of Health.
ART	Antiretroviral Therapy.
HAART	Highly Active Antiretroviral Therapy.
CDC	Centers for Disease Control and Prevention.
TB	Tuberculosis.
FHAPCO	Federal HIV/AIDS Prevention and Control Office.
MOH	Ministry of Health.
NGO	Nongovernmental organization.
WHO	World Health Organization.
LMM	Linear Mixed Model.
GLM	Generalized Linear Model.
GLMM	Generalized Linear Mixed Model.

ABSTRACT

BACKGROUND: The clinical symptoms of HIV infection were evolved on the depletion of CD4 cells and the replication of HIV RNA. Therefore, the HIV cocktail therapy is a combination reagent which focuses on inhibits the replication of HIV RNA at different stages of HIV life-cycle. Wasting syndrome is included in the current case definition of acquired immunodeficiency syndrome (AIDS), as specified in 1993 by the Centers for Disease Control and Prevention (CDC). The CDC defines wasting syndrome as unexplained weight loss of more than 10 percent, accompany by fever or diarrhea. The development of wasting syndrome is considered an indicator condition for AIDS. Therefore this study focuses on the association of the two outcomes since both are an indicator of the progression of the disease.

OBJECTIVE: The main objective of this study is to investigate the joint evolution of CD4 cell counts and body weight of HIV patients after ART initiation and identify factors affecting the two end points.

METHODS: In this study secondary data were used from Asella referral hospital in HIV adult patients. The study population consists of 300 HIV patients, measured repeatedly at least three times on each patient who is 18 years old or older those treated with antiretroviral therapy from September 2000 to August 2005. Joint and separate model are considered to study the joint evolution and identify the potential risk factors affecting the two end points.

RESULT: First the two outcomes are analyzed separately for purpose of identifying associated risk factors for the progress of CD4 count and weight separately. Include on the joint analysis those factors for to investigate the joint evolution and association of CD4 count & weight, and associated risk factors for the progress of the two end points by considering a joint linear mixed effects model. To capture the association of the two outcomes we use a common parameter which is the correlation of the evolution of the two outcomes 0.5054 implied the two outcomes have positive (direct) correlation. Analyzing the two outcomes separately and jointly the covariates are the same that is covariates associated with CD4 count separate analysis was also significant in the joint analysis the same for the weight outcome. The covariates sex, time, WHO

stage, functional status, TB status and quadratic time were have significant association with CD4 count. Sex, time, functional status, TB status and sex by time interaction term were significantly associated with weight. Among all covariates, sex and time were negatively associated with weight that means when the time increases after sometime the weight decreases.

CONCLUSION: The results of the separate and joint model analysis are consistent. When the joint model is compared with the separate model, the joint model fitted the data better than the separate model. The result from the joint model suggested a significant statistical association between the evolutions CD4 count and weight.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Acquired Immune Deficiency Syndrome (AIDS) is a disease caused by a retrovirus known as human immunodeficiency virus (HIV). Although important progress has been achieved in preventing new HIV infections and in lowering the annual number of AIDS related deaths, the number of people living with HIV continues to increase (UNAIDS 2008). Since its detection in 1981, HIV/AIDS has become one of the most challenging problems of our age (Merso 2008). According to the UNAIDS 2013 report, globally, an estimated 35.3 million people were living with HIV in 2012.The virus attacks the immune system and weakens the body's natural defense system to fight against infection.

There were 2.3 (1.9–2.7) million new HIV infections globally, showing a 33% decline in the number of new infections from 3.4 (3.1–3.7) million in2001. At the same time the number of AIDS deaths is also declining with 1.6 (1.4–1.9) million AIDS deaths in 2012, down from 2.3 (2.1–2.6) million in 2005. Although important progress has been achieved in preventing new HIV infections and in lowering the annual number of AIDS related deaths, the total number of people living with HIV is still rising and it is the worst public health crisis worldwide. Particularly in sub Saharan Africa the HIV pandemic created unprecedented burden on the economies and health care systems since the prevalence is highest. The countries of sub-Saharan Africa are home to approximately 22.4 million people living with HIV/AIDS (UNAIDS 2009).

The clinical symptoms of HIV infection were evolved on the depletion of CD4 cells and the replication of HIV RNA. Therefore, the HIV cocktail therapy is a combination reagent which focuses on inhibiting the replication of HIV RNA at different stages of HIV life-cycle. Highly active antiretroviral therapy (HAART) and a single or combination of several drugs, have high activity to inhibit HIV RNA replication (Zhang 2007). Antiretroviral therapy not only prevents AIDS-related illness and death: it also has the potential to significantly reduce the risk of HIV transmission and the spread of tuberculosis. Having this in mind, the world is within reach of

providing antiretroviral therapy to 15 million people by 2015. In 2012, 9.7 million people in low- and middle-income countries received antiretroviral therapy, representing 61% of all who were eligible under the 2010 World Health Organization (WHO), HIV treatment guidelines. (UNAIDS 2013). However, under the 2013 WHO guidelines, the HIV treatment coverage in low- and middle-income countries represented only 34% (32-37%) of the 28.6 million people eligible in 2013. Since 1995, antiretroviral therapy has saved 14 million life years in low- and middle-income countries, including nine million in sub-Saharan Africa. Fewer deaths from AIDS-related illnesses have transformed societies: more people, regaining their health, are returning to work and taking care of their families. But despite historic gains in expanding treatment services, efforts to reach universal treatment access face considerable challenges. In addition to persistent low treatment coverage for children, men are notably less likely than women worldwide to receive antiretroviral therapy, and key populations often experience major barriers in obtaining treatment and care services. In addition, the gap between people who can access treatment and people in need is still very large, nearly 46%, and as the demand for treatment as prevention continues to rise and increasingly outstrips availability, this treatment gap is set to grow (UNAIDS 2012).

Ethiopia is one of the countries that are affected severely by HIV/AIDS. In 2008 alone, the adult prevalence of HIV is 10.5% in urban areas and 1.9% in rural areas with average prevalence around 4%. The first antiretroviral guideline was revised in 2005 to facilitate a rapid scale up of ART. Within two years patients on treatment at 117 hospitals and 108 health centers increased from 900 to 62,221 (FMOH 2007) and in 2008 a total of 400 health care facilities, 277 public health centers and 123 public and private hospitals, are rendering ART services in the country. The number of people who were able to access ART has substantially increased from 900 in 2003 to 180,447 in 2008 and in 2010 as part of the global issue the government of Ethiopia is working to provide universal access to HIV/AIDS treatment. The use of highly active antiretroviral therapy (HAART) has resulted in a significant reduction in AIDS-related deaths and complications among adults and adolescents. However, the medical management of HIV-infected children remains challenging. Access to HIV treatment is limited and early treatment initiation can cause serious complications. Since there is currently no cure for the disease HIV, a balance between treating the disease and maintaining quality of life must be weighed carefully.

An evaluation to determine an appropriate time to initiate HAART is necessary to improve both the quality of life and survival of HIV infected adults.

Wasting syndrome is included in the current case definition of acquired immunodeficiency syndrome (AIDS), as specified in 1993 by the Centers for Disease Control and Prevention (CDC). The CDC defines wasting syndrome as unexplained weight loss of more than 10 percent, accompanied by fever or diarrhea. The development of wasting syndrome is considered an indicator condition for AIDS.

Wasting syndrome in patients with human immunodeficiency virus (HIV) infection is a multi-factorial process that can be associated with a variety of infectious, neo-plastic, metabolic and nutritional abnormalities. Early identification and treatment can improve functional status and, perhaps, survival as well. Although not yet thoroughly studied with regard to HIV-related wasting, highly active antiretroviral therapy seems to have significantly reduced the prevalence of wasting syndrome among patients with HIV infection. Nonetheless, treatment failure despite intensive antiretroviral therapy is not uncommon, and HIV disease activity may place patients at risk for significant weight loss.

Profound weight loss commonly occurs late in the course of HIV disease and is associated with a poor prognosis, more rapid disease progression, significant disability and increased mortality. HIV-related wasting is a starvation state characterized by protein–energy malnutrition in which mobilization of fat is accompanied by loss of somatic and visceral proteins. Loss of more than 30 percent of ideal body weight is associated with high mortality and weight loss of as little as 5 percent of the patient's usual weight is associated with more rapid progression of disease. Weight loss, however, is a relatively insensitive indicator of early wasting syndrome, and decreases in certain body compartments, particularly body cell mass, may portend early death if this depletion is not reversed.

Loss of body cell mass can result from a variety of causes but usually is a result of inadequate intake in the context of active opportunistic disease, HIV disease (as indicated by a high viral load), or both. Certain pro-inflammatory cytokines, particularly tumor necrosis factor-α (TNF-α), have also been implicated, although their precise roles in wasting syndrome are still unclear.

This research work was undertaken against the above background and explores the factors that have strong association jointly with the CD4 count and weight of HIV-infected adult who started ART in one of the government hospitals in the regional state of Oromia at Asella Hospital.

1.2 Statement of the problem

HIV attacks the CD4 cells that play a major role in maintaining the body's immune system. Several years after initial infection with the virus, the number of CD4 cells in the blood falls to below 200 per cubic millimeter, leading to infections such as tuberculosis (TB), septicemia and pneumonia, which people without the virus can usually resist this result the so called wasting syndrome unexplained weight loss in which some of it is accompanied by fever and diarrhea. AIDS is the stage of the disease where the immune system has weakened to the extent that these opportunistic infections appear. Most opportunistic infections can be treated, but as the immune system deteriorates over several years, treatment becomes increasingly ineffective. Without access to ARVs, people with HIV often die within one or two years of the onset of AIDS.

In Ethiopia, there is no known and well-developed joint analysis longitudinal research has been conducted to know the mean evolution of CD4 counts and weight, to assess whether ART initiation change jointly the CD4 count and weight over time, and whether change in CD4 count and weight varied by covariates.

The above main reasons have been considered to address the following issues

- Is the CD4 count and weight of patients changing over time jointly after initiation of ART?
- What are the factors other than ART for the change CD4 count and weight?
- Is there a significant variation in CD4 count and weight of patients?

1.3 Objective of the study

1.3.1 General objective

The main objective of this study is to assess the joint evolution of CD4 count and weight of HIV patients after they started ART at Asella hospital in Oromia region.

1.3.2 Specific objectives

- To explore the association of the evolution of CD4 count and weight of HIV patient over time.
- To fit an appropriate joint model and identify the associated factors for CD4 count and weight other than ART.
- To compare the separate model with the joint mixed model for the two outcomes.

1.4 Significance of the Study

- Since Longitudinal studies and more than one outcome are common in health area; this research could be used as a basis for future studies on health areas to show how to apply joint mixed model.
- This study has an important role to identify the associated factors of CD4 count and weight, to evaluate the effectiveness of ART, and to give suggestions and recommendations to the respective bodies to adjust their intervention.
- This study can be the corner stone for future longitudinal studies about the CD4 count and weight in Ethiopia and the health professionals will evaluate their interventions to improve the CD4 count and weight of patients over time.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The human Immune deficiency virus (HIV) directly attacks the CD4 cells that play a major role in maintaining the body's immune system.CD4 cells are the very important types of white blood cells that protect the human body from any types of infections (Panos 2006). After several years of HIV infection the number of CD4 cells falls below normal count (800-1000) to (below 200) per cubic millimeters leading the body to become susceptible to any type of infections. This condition is identified by health professionals as the stage of AIDS. AIDS is a group of diseases manifested in the person who has HIV. (Panos 2006).The risk of HIV infection increases by number of sexual partners, intravenous drug use, any sex without condom, alcohol and other drug use, tattoos and body piercing with contaminated needles or instruments. Since AIDS was first identified, researchers and scientists have tried their best to find medicine and vaccine but the results everywhere were not successful. Consequently the lives of human beings have been observed to be threatened due to infection with the virus. On top of all, since the disease is affecting the most productive citizens, it is natural that it causes damage to national economy (Bollinger 1999; UNAIDS 2000).

2.1.2 HIV/AIDS in Ethiopia

HIV/AIDS has created an enormous challenge to mankind since it became known in1981.The first evidence of HIV epidemic in Ethiopia was detected in 1984. Since then, AIDS has claimed the lives of millions and has left behind hundreds of thousands of orphans (FHAPCO 2007). Ethiopia is one of the hardest hit Sub-Saharan Africa countries by the HIV pandemic. In 2009 a single point estimate of AIDS related deaths for 2010 was 44,751 of which 37,537 were adults. (National AIDS resource center 2009).

According to the National Factsheet, 2010 of National AIDS resource center the total number of HIV positive people in 2010 is estimated to be 1,326,329 including 137,494 new HIV infections

and excluding 28,073 AIDS related deaths during the year. According to the FHAPCO single point estimate for prevalence of HIV/AIDS in Ethiopia, the adult (15-49) HIV prevalence for 2007 is estimated at 2.1% of which 7.7% is urban and 0.9% is rural (EMOH 2007). In 2010, the FHAPCO estimates of the overall adult (15-49) HIV prevalence is 2.4%. Urban and rural HIV prevalence rates were 7.7% and 0.9%, respectively.

HIV/AIDS has been and still is the greatest challenges to the Ethiopian health system, as elsewhere in sub-Saharan African countries. It has remained among the major causes of deaths over the past two decades. Currently in 2010, more than one million people are estimated to be living with HIV in Ethiopia of who nearly 397,818 need ART care and treatment (National Factsheet 2010).

2.1.2 HIV/AIDS and Anti-Retroviral drugs in Ethiopia

Ethiopia is among the Sub Saharan African countries where a large number of people live with HIV. According to Federal HIV/AIDS Prevention and Control Office (HAPCO) single point HIV prevalence estimate, in 2007, the national adult prevalence was estimated to be 2.1% with the urban rural prevalence 7.7% and 0.9% respectively. The total number of people living with the virus in the same year was estimated to be977, 394. AIDS also killed an estimated 71,902 individuals in the same year. Until recently; the major concern on HIV/AIDS was on prevention of new infections through various approaches with little attention given to those who have been already living with the virus. Nevertheless, prevention efforts must be accompanied by treatment of those who live with HIV.

It is recognized that treatment for HIV positive individuals after diagnosis has different stages which include an emphasis on positive life, treatment of opportunistic infections, provision of antiretroviral drugs and palliative and end of life care services (Whiteside &Sunter 2000). However, in Ethiopia as in many other developing countries the availability of these chains of comprehensive care and treatment services are either nonexistent or only one or two of them are available.

The introduction of ART is indeed a breakthrough and step forward in the whole setoff HIV/AIDS response in the country, it has brought a new hope for those who are living with the virus. Without access to ARVs, people with HIV often die within one or two years of the onset of AIDS (Panos 2006). The Ethiopian Ministry of health has also anticipated that in the long run ART will have profound impact on AIDS related deaths and would increase life expectancy of the country that partly has dropped as a result of HIV and AIDS.

Before 2003, some people in Ethiopia got access to ART through black market but the official ART service began in Ethiopia in July 2003 on fee bases costing ($289-$346 per month). According to MOH (2005) report at that time only 2% (3000) people living with HIV got access to the service from 12 hospitals. Clients were mainly educated and well to do men who live in urban areas. But with the "3 by 5" and concerted effort of Ethiopian Ministry of Health as well as other international donor organizations, free ART service launched in January 2005 in 49 additional sites in different regions. Currently, there are 272 ART sites all over the country.

According to the Ethiopian AIDS resource center web site, as of the end of October2007, out of the total 258,264 people who need ART in Ethiopia 109,552 are able to access it. Out of the total ART service recipients 55,888 are adult women, 48,650 are adult men and 5017 are children below 14 years old. The ministry of health has a plan to put 320,000 persons on ART by the end of 2008.Since the beginning of ART service in Ethiopia, the Ministry of Health in collaboration with other international NGOs has formulated important national documents to guide and coordinate various efforts targeting HIV and AIDS treatment.

Based on WHO public health recommendation, currently there are three class of HIV treatment drugs approved to be distributed in Ethiopia. Most patients begin from the first class regimens which are composed of three different types of pills. There are two basic conditions to switch patients to second line regimens these are: in the case of drug resistance and treatment failure. Patients will be forced to switch to second class regimens which are more complicated with serious side effects. Third class drugs are more expensive, much more complicated and the last resort in ART treatment in Ethiopian contexts. If patients reached to the third class drugs no other alternative treatment options are available (ART guideline 2003).

2.1.3 Goals of Antiretroviral Therapy (ART)

Infection with HIV causes immunologic deficiency that results in depletion of CD4 cells and suppression of cell mediated immune defenses. HIV infects CD4 cells by interacting with their CD4 receptors, which allows the virus to gain entry into the cells. The invading HIV replicates within the CD4 cells destroys them and spreads to other CD4 cells, depleting theCD4 cell population. Because CD4 cells direct and activate immune responses, many immune functions degrade as a result of HIV infection.

Individuals with weakened immune defenses are susceptible to infections caused by opportunistic pathogens that do not normally cause disease for immune competent individuals. These infections are known as opportunistic infections. Once an opportunistic infection has begun, it can rapidly spread throughout the body via the circulatory system, damages vital organs and becomes fatal (Virco 2008). The goals of therapy in treating HIV/AIDS infected individuals are, therefore, in view of the following points:

- **Clinical goal** which aims to extend life expectancy and quality of life for patients infected with HIV,
- **Viralogical goal** is to reduce the HIV viral load to the lowest level possible in order to prevent disease progression and limit development of resistance to ARV drugs,
- **Immunological goal** is to preserve and restore immunologic functioning in the normal range. This involves the quantitative component of CD4 cell count in the normal range. It also involves the qualitative goal of resisting infections by opportunistic pathogens, and
- **Epidemiological goal** is to reduce transmission of HIV to others. (Virco 2008).

The clinical symptoms of HIV infection were evolved on the depletion of CD4 cells and the replication of HIV RNA. Therefore, CD4 cell count and HIV viral load are two of the most important predictors of the clinical prognosis of HIV-infected subjects.

Highly active antiretroviral therapy (HAART) and a single or combination of several drugs, have high activity to inhibit HIV RNA replication. HIV cocktail therapy is combination reagents which inhibit the replication of HIV RNA at different stages of HIV life-cycle. The currently

available HIV inhibition reagents can be categorized into Nucleotide reverse transcriptase inhibitors, Non-Nucleotide reverse transcriptase inhibitors, protease inhibitors, and integrated zinc-finger inhibitors (Zhang 2007).

2.1.4 Importance of ART in Ethiopia

In the face of competing demands such as malaria, TB, and famine some question whether an investment on ART in Ethiopia is justifiable. Given the impact of AIDS across society and the potential of ART to reduce the burden, the justification for pursuing this agenda is unarguable. HIV and AIDS are affecting every sector of Ethiopian society. At the macro level, the health, agriculture, education, business and industry sectors are all adversely impacted by the disease. Families and communities are likewise affected. The MOH estimate that the annual mortality rate for those in the 15-49 age range will increase from a projected 200,000 without factoring in AIDS to over 350,000 in 2004 (with the AIDS epidemic).Besides these numbers, the impact of AIDS increased absenteeism in the workplace, reduction of productivity, reduced family income, and increased family expenditure on healthcare and burial rituals. In a resource poor country such as Ethiopia, the economic impact of AIDS-related illness and death is severe (AIDS Resource Center *et al* 2005).

In contrast to Ethiopia**,** AIDS-related deaths and illnesses in countries where ART has been available since the mid-1990s have considerably declined (UNAIDS 2004). The experience of developed nations, as well as countries such as Brazil (from middle income countries which produces ART drugs), have proven that ART treatment reduces disease burden and dependence, and increases the function, well-being, and productivity of individuals. This in turn, can help offset some of the consequences of the HIV and AIDS pandemic (AIDS Resource Center *et al* 2005).

2.1.5 HIV/AIDS and CD4 Counts

HIV-1 uses CD4 to gain entry into host T-cells and achieves this through its viral envelope protein known as gp120. The binding to CD4 creates a shift in the conformation of gp120 allowing HIV-1 to bind to a co-receptor expressed on the host cell. These co-receptors are chemokine receptorsCCR5 or CXCR4. Following a structural change in another viral protein, HIV inserts a fusion peptide into the host cell that allows the outer membrane of the virus to fuse with the cell membrane.

HIV infection leads to a progressive reduction in the number of T cells expressing CD4. Medical professionals refer to the CD4 count to decide when to begin treatment during HIV infection. Normal blood values are usually expressed as the number of cells per micro liter (or cubic millimeter, mm3) of blood, with normal values for CD4 cells being 500-1200 cells/mm3. A CD4 count measures the number of T cells expressing CD4. While CD4 counts are not a direct HIV test--e.g. they do not check the presence of viral DNA, or specific antibodies against HIV--they are used to assess the immune system of a patient. Patients often undergo treatments when the CD4 counts reach a level of 350 cells per micro liter in Europe but usually around 500cpm in the US; people with less than 200 cells per micro liter are at high risk of contracting AIDS defined illnesses. The newest National Institute of Health guidelines recommend treatment of any HIV-positive individuals, regardless of CD4 count. Medical professionals also refer to CD4 tests to determine efficacy (www.wikepedia.com)

2.1.6 Antiretroviral Therapy and CD4 Counts

Human immunodeficiency virus (HIV) attacks CD4 T cells primarily. In HIV infection, the CD4 count decreases. Current CD4 count is a strong predictor of the immediate risk of acquired immunodeficiency syndrome (AIDS) or death than HIV RNA level. Antiretroviral therapy (ART) has dramatically reduced the morbidity and mortality associated with HIV infection and has improved the prognosis for people living with HIV AIDS (PLHA) (Mirudula and et al 2012)

2.1.7 HIV/AIDS and weight of patients

Wasting syndrome is included in the current case definition of acquired immunodeficiency syndrome (AIDS), as specified in 1993 by the Centers for Disease Control and Prevention (CDC). The CDC defines wasting syndrome as unexplained weight loss of more than 10 percent, accompanied by fever or diarrhea. The development of wasting syndrome is considered an indicator condition for AIDS.

Wasting syndrome in patients with human immunodeficiency virus (HIV) infection is a multi factorial process that can be associated with a variety of infectious, neoplastic, metabolic and nutritional abnormalities. Early identification and treatment can improve functional status and, perhaps, survival as well. Although not yet thoroughly studied with regard to HIV-related wasting, highly active antiretroviral therapy seems to have significantly reduced the prevalence of wasting syndrome among patients with HIV infection. Nonetheless, treatment failure despite intensive antiretroviral therapy is not uncommon, and HIV disease activity may place patients at risk for significant weight loss.

Profound weight loss commonly occurs late in the course of HIV disease and is associated with a poor prognosis, more rapid disease progression, significant disability and increased mortality. HIV-related wasting is a starvation state characterized by protein–energy malnutrition in which mobilization of fat is accompanied by loss of somatic and visceral proteins. Loss of more than 30 percent of ideal body weight is associated with high mortality and weight loss of as little as 5 percent of the patient's usual weight is associated with more rapid progression of disease. Weight loss, however, is a relatively insensitive indicator of early wasting syndrome, and decreases in certain body compartments, particularly body cell mass, may portend early death if this depletion is not reversed.

Loss of body cell mass can result from a variety of causes but usually is a result of inadequate intake in the context of active opportunistic disease, HIV disease (as indicated by a high viral load), or both. Certain pro-inflammatory cytokines, particularly tumor necrosis factor-α (TNF-α), have also been implicated, although their precise roles in wasting syndrome are still unclear. (William and et al 1999).

2.1.8 ART and Weight of HIV patient

Access to antiretroviral treatment (ART) has expanded rapidly in many moderate-to low-income countries affected by the HIV epidemic. In addition to reducing mortality rates, ART has many favorable effects among people living with HIV, such as improving weight and lean body mass, particularly in patients with greater pretreatment immunological and virological compromise. Several large-scale ART programs in sub-Saharan Africa indicate that malnutrition (low BMI) at the start of ART is significantly and independently associated with subsequent mortality, while weight gain after ART is associated with survival. It is unclear whether this association is causal. Although weight changes appear to parallel the success of ART, it is unknown whether interventions to improve weight prior to or at ART initiation will improve subsequent outcomes (Tang and et al 2011).

2.1.9 Factors associated with CD4 count and weight

Retrospective cohort study of HIV/AIDS patients on ART done by Ketema Kibebew (2011), gives an insight into survival and its determinants in an army hospital setting in Addis Abeba, Ethiopia. The study found low CD4 cell count, advanced WHO clinical stages (III and IV), TB co-infection, opportunistic infections, being bedridden and ambulatory and being employed are statistically significant and strong predictors of mortality of HIV patients under ART.

Bayeh Abera et al(2010) showed female HIV patients had higher mean CD4 cell counts than male ($p < 0.002$) before ART was initiated. This difference could be due to several reasons; HIV associated TB could be the contributing factor for the low CD4 count in males as the proportion of patients having TB was significantly higher in male HIV positive patients than females ($p=0.003$).

Moing L et al (2002) Short-term increase in CD4+ cell counts was associated positively with baseline plasma HIV RNA level; it was higher in patients with CD4+ cell counts of 200–500 cells/mm3, in women, and in antiretroviral naive patients and was lower in patients who were seropositive for HCV. The only baseline characteristic associated with long-term increase in CD4+ cell counts in multivariate analysis was a baseline CD4+ cell count of >500 cells/mm3.

Dinakar Kr et al (2014) showed the mean CD4 counts increment are lesser in the TB co-infection group who are under ART and also the increase in body weight was much more than the increase in the patient who did not have tuberculosis further emphasizing the effect of tuberculosis in decreasing the body weight of the patient.

Tang et al (2011) found several significant predictors of weight gain, particularly in the first six months after ART initiation. Patients with more advanced HIV infection at baseline (CD4 cell counts <200 cells/μL) were more likely to have positive weight changes in the first six months of therapy, likely due to the beneficial effects of ART. However, six months after start of ART, patients with CD4 < 200 cells/μL (a sign of continued immune suppression) had significantly less weight change over the following six months compared to those with CD4 ≥ 200. Another interesting finding from our study is that alcohol intake was associated with negative weight gain in the first six months after ART initiation in the first interval, but not the second.

A study by Reda AA et al (2013) Predictors of Change in CD4 Lymphocyte Count and Weight among HIV Infected Patients on Antiretroviral Treatment in Ethiopia showed a substantial increment in weight and CD4 lymphocyte count among the patients who were taking ART in eastern Ethiopia. The findings indicate that the length of time patients stay on ART and patients' functional status improves both CD4 count and weight gain. Higher age groups, earlier WHO stage, higher CD4 count and high hemoglobin levels were associated with improvements in weight.

A research by Grinspoon S, Mulligan K (2003) in Massachusetts General Hospital showed declines in CD4 lymphocyte count and weight loss among patients on anti-retroviral treatment are associated with increased mortality and morbidity manifesting in the form of deterioration of clinical conditions and decreased functional status in adults.

2.2 Longitudinal Modeling

2.2.1 Linear Mixed Model

Many longitudinal studies are designed to investigate change over time in a characteristic which is measured repeatedly for each patient (Laird and Ware 1982). Analyses of multiple observations measured on the same individual over time are different from observations measured on different people. Investigators gather repeated measures or longitudinal data in order to study change in a response variable over time as well as to relate these changes in explanatory variables over time (McCulloch *et al.* 2008). In addition, modeling the true correlation structure becomes significant in the presence of missing value and when the number of observations per subject is not large. There are two types of covariates in longitudinal studies in general. There are time invariant or baseline covariates (e.g. gender) and time varying covariates (e.g. weight). The Linear Mixed Model (LMM) has become the most commonly used tool for analyzing continuous repeated measures data from a sample of individuals in agriculture, biomedical, economical, and social applications. Thus the term `individual' will have different interpretation or meaning for different areas of application. A special case of a linear mixed model is when there are no fixed effects leading to what is called a random effects model (McCulloch *et al.* 2008).For example the units may be patients in a longitudinal study where a measurement of biological laboratory markers such as CD4 count and viral load measures is taken at every six month visits. Thus the patient is measured repeatedly giving rise to a cluster of observations from each patient.

The linear mixed-effects model fits the mean response as a combination of population characteristics (fixed-effects) assumed to be shared by all individuals and subject-specific effects (random-effects) that are unique to a particular individual (Nonhlanhla 2009). By including random-effects in the model, linear mixed-effects models are able to explicitly distinguish between within-subject and between-subject sources of variation. With a linear mixed-effects model it is not only possible to estimate parameters that describe how the mean responses change over time, but it is also possible to predict how an individual’s response trajectories change over time. Mixed-effects models are highly attractive due to their ability to handle missing and unbalanced data reasonably well.

2.2.2 Generalized Linear Mixed Model

Generalized linear mixed models (GLMMs) (Breslow and Clayton 1993) are obtained from generalized linear models (GLMs) (McCullagh and Nelder 1986) by incorporating random effects in to the linear predictors, and include the well-known linear mixed models (LMMs) for normal responses (Laird and Ware 1982) as a special case. These models are useful for modeling the dependence among response variables inherent in longitudinal or repeated measures studies, for accommodating over dispersion among binomial or Poisson responses, and for producing shrinkage estimators in multi-parameter problems. Due to the wide range of applications of GLMMs, these models have received substantial attention during the last decade and are available in the major software packages. The computational burden associated with high dimensional numerical integration has limited past studies of GLMMs to the case of simplified models (e.g., random intercept models), to tractable random effects distributions (e.g., the Gaussian and conjugate distributions such as the beta-binomial and negative binomial models), or to conditional inference for the regression coefficients, conditioning on the random effects (Zeger and Karim 1991).

A variety of novel approaches have been proposed to overcome the computational difficulties, with the goal to improve inference and estimation procedures for the fixed effects in GLMMs.

2.2.3 Multivariate Longitudinal Data

Most of the time one observes more than one outcome at the same time, which is essentially known as multivariate outcomes. These can all be of the same data type, e.g., all Gaussian or all binary, or of a mixed type, e.g., when the outcome vector is made up of continuous and binary components. Statistical problems where various outcomes of a mixed nature are observed have been around for about half a century and are rather common at present. Many research questions can often only fully be addressed in a joint analysis of all outcomes simultaneously. For example, the association structure can be of direct scientific relevance. It is definitely possible for all of these features to occur simultaneously, whereby a multivariate outcome vector, possible of a mixed nature, is measured repeatedly over time. An array of research questions can then be addressed in this way. A possible question might be how the association between outcomes

evolves over time or how outcome-specific evolutions are related to each other (Fieuws and Verbeke 2004).

Fieuws and Verbeke (2004) used a joint random-effects model to evaluate hearing performance at two different frequencies measured repeatedly over time on subjects. The authors specified a bivariate longitudinal model for continuous responses with correlated random intercepts and slopes. Error terms were assumed to be independent conditional on the correlated random effects. The results indicated a discrepancy between the observed data and relations implied by the joint model. However, relaxing the conditional independence assumption by allowing the error terms to be correlated, improved model fit and revealed that the discrepancy was due to inappropriate modeling of the error covariance structure.

Thiebaut *et al*., (2002) used a random-effect bivariate model with correlated stochastic process to investigate the relationship between CD4 and beta-2-microglobulin, two important immunologic measurements in HIV/AIDS research. Another example of joint random-effect models used in psychometric studies is the work by MacCallum *et al.,* (1997). These authors used a multivariate three-level model specified in a fully Bayesian way to study the relationship between accuracy (binary measurement) and speed of test takers (continuous measurement) on response items clustered within subjects who were nested within groups.

Chakraborty *et al*., (2003) obtained estimates of the correlation between blood and semen HIV-1 RNA by using a joint random-effects model. Other examples with longitudinal studies can be found in reference (MacCallum *et al.* 1997). All of these examples refer to situations where the number of different outcomes is relatively low. Although the model formulation can be done irrespective of the number of outcomes to be modeled jointly, standard fitting procedures, such as maximum likelihood estimation, is only feasible when the dimension is sufficiently low or if one is willing to make a priori strong assumptions about the association between the various outcomes.

CHAPTER 3

METHODOLOGY OF THE STUDY

3.1 Data Description

This study used data obtained from a retrospective cohort follow-up study from adult HIV/AIDS patients who have been under follow up from 2000 to 2005 in Asella hospital. Assela hospital is located in south east of Ethiopia in Asella town. All patients that included in this study were HIV/AIDS patients whose age above 15 years and who have been followed at least 3 times. The data for this study consists of 300 individuals with a minimum of 3 and maximum of 12 measurements per individual of adult HIV/AIDS patients.

3.1.1 Study Variables

- Dependent variables

The dependent variables for this study were the level CD4 counts and the weight of a patient with HIV/AIDS.

- Independent variables

The independent variables that were included in this study are summarized in the table below.

Table 1Summary of variables

NO.	Variable	Description	Value/codes
1	Gender	Gender	Male=1,Female=0
2.	WHO stage	Stage I :asymptomatic disease Stage II: mild disease Stage III: advanced disease	Stage I :3 Stage II: 2 Stage III:1

		Stage IV: severe disease	Stage IV: 0
3.	TB diagnosis	Negative or positive	Negative=0 Positive=1
4.	Reported function status	Working: means actively participates in age appropriate activities. Ambulatory: means limited tolerance for activities Bedridden: means no tolerance for activities.	Working = 2 Ambulatory = 1 Bedridden = 0

3.2 Methods Of Data Analysis

3.2.1 Exploratory data analysis

The first step in any model building process is exploratory data analysis. Data exploration is a very helpful tool in the selection of appropriate models. Analyses of longitudinal data compare profiles over time and, indeed, time might be viewed as the primary systematic effect to be investigated. Examining the data for clues about the likely nature of the mean structure, to see how the mean profile changes over time, is essential for specifying the functional form of the mean response of the model. So as to understand the possible relationships among means over time, for balanced data, graphical inspection can be used by connecting the average values computed at each time point separately. If the data is not balanced loess smoothing will be used instead. This will give idea as to how the mean profile evolves over time. The results of this exploration were useful in order to choose a fixed-effects structure for the linear mixed model.

3.2.2 Statistical Models

3.2.2.1 Generalized Linear Model

- General class of linear models that are made up of three components: Random, Systematic, and Link Function

- Random component: Identifies dependent variable (Y) and its probability distribution
- Systematic Component: Identifies the set of explanatory variables (X_1,...,X_k)
- Link Function: Identifies a function of the mean that is a linear function of the explanatory variables

$$g(\mu) = \alpha + \beta_1 X_1 + \cdots + \beta_k X_k$$

- Count data (number of CD4 cells in a sample of blood drawn by a needle from a vein in arm)- Random component has *Poisson* distribution and model is called Poisson Regression
- When Count data have V(Y) > E(Y), model can be Negative Binomial Regression
- Log link (used when μ cannot be negative as when data are *Poisson* counts):

$$g(\mu) = \ln(\mu)$$

$$g(\mu) = \ln(\mu) = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k \text{----------(1)}$$

$$\mu(X_1, \ldots, X_k) = e^{\beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k}$$

When the mean and variance are not equal (over-dispersion), often replace the Poisson distribution replaced with Negative Binomial Distribution.

3.2.2.2 Over Dispersion Model

In the context of count data, consider the assumption that the variance proportional to the mean. Specifically,

Var (Y) =φ E(Y) =φλ

Where φ is over dispersion parameter if φ=1 then the variance equals the mean and we obtain the Poisson mean-variance relationship. If φ>1 then have over-dispersion relative to Poisson. If φ< 1 we would have under-dispersion, but this is relatively rare.

An alternative approach to modeling over-dispersion in count data is to start from a Poisson regression model and add a multiplicative random effect θ to represent unobserved heterogeneity. This leads to the negative binomial regression model. Suppose that the conditional distribution of the outcome Y given an unobserved variable ɵ is indeed Poisson with mean and variance ɵμ, so

$$Y/\theta \sim P(\lambda\theta)$$

In this model the data would be Poisson if only we could observe θ. unfortunately we do not. Instead we make an assumption regarding its distribution and integrate it out of the likelihood, effectively computing the unconditional distribution of the outcome. It turns out to be mathematically convenient to assume that θ has a gamma distribution with parameters α and βr.

This distribution has mean α/βr and variance $\alpha/\beta r^2$, so we take α = βr=1/σ^2, which makes the mean of the unobserved effect one and its variance σ^2.

With this information we can compute the unconditional distribution of the outcome, which happens to be the negative binomial distribution. The density is best written in terms of the parameters βr, α and λ as done below, although you must recall that in our case α = βr=1/σ^2, so there's just one more parameter compared to a Poisson model. (German Rodrguez 2013)

$$\Pr(Y = y) = \frac{\Gamma(\alpha_y)}{y!\,\Gamma(\alpha)} \frac{\beta r^{\alpha} \lambda^{y}}{(\lambda + \beta r)^{\alpha+y}} \qquad \text{---------- (2)}$$

3.2.2.3 Linear Mixed Model (LMM)

Linear mixed models are expansion of linear models (LM) with variance components. Thus the LMM contains both the fixed effect and the random effect parts. LMM will be used to analyze longitudinal data with continuous response variable for this study weight of HIV patients after the initiation of antiretroviral therapy (ART). The Normal LMMs are with form as follows:

$$Y_i = X_i\beta + Z_i b_i + \varepsilon_i \qquad \begin{cases} b_i \sim N_q(0, \sigma^2 I_n) \\ \varepsilon_i \sim N_{ni}(0, \Sigma_i) \\ b_1, b_2 \ldots b_n, \varepsilon_1 \ldots \varepsilon_n \end{cases} \qquad \text{----------(3)}$$

Where,

- Y_i is $n_i \times 1$ response vector for observations of the i^{th} patient.
- X_i is $n_i \times$ p design matrix for the fixed effects for observations of the i^{th} patient.
- β is the p × 1 vector of fixed-effect coefficients.
- Z_i is the $n_i \times$ q model matrix for the random effects for observations of the i^{th} patient.
- b_i is the q × 1 vector of random-effect coefficients for of the i^{th} patient.
- ε_i is the $n_i \times 1$ vector of errors for observations in of the i^{th} patient.
- σ2 is the q × q covariance matrix for the random effects.
- Σ_i is the $n_i \times n_i$ covariance matrix for the errors of the i^{th} patient.
- b_i and ε_i are independent.

$Y_i \mid b_i \sim N(X_i\beta_i + Z_i b_i, \Sigma_i)$, $b_i \sim N(0, D)$,

Marginally, Y is given as

$$Y_i \sim N(X_i\beta, Z_i D Z_i^{'} + \Sigma_i)$$

Methods of Estimation in LMM

Estimation is more difficult in the mixed model than in the general linear model. This is because in mixed model estimation of random effects and covariance structure of the random error is necessary besides to the fixed effect. Both the maximum likelihood (ML) and restricted maximum likelihood (REML) were used for estimation of the parameters in this study. The maximum likelihood estimation method finds the parameter estimates that are most likely to occur given the data. The parameter estimates are derived by maximizing the likelihood function,

which is a mathematical expression that describes the joint probability of obtaining the data expressed as a function of the parameter estimates.

3.2.2.4 Generalized Linear Mixed Model (GLMM)

GLMM are extending the LMMs to have two features:

- One is normality assumption not needed.
- The other is mean do not need to be linear combination of parameters.

Therefore, the researcher used the combination of GLMM and some other model for the Cd4 count response which doesn't fulfill the two features of linear mixed model.

$$g(\lambda) = \ln(\lambda) = \gamma X_{ij} + b_i Z_{ij}$$

$$\Rightarrow \lambda(X_{11}, \dots, X_{jk}) = e^{\gamma X_{ij} + b_i Z_{ij}} \quad \text{----------(4)}$$

Where,

λ: The mean of Y_{ij} which is related to the covariates of X by link function

X_{ij} : Covariates of the ithpatient of the jth time

γ: Regression coefficients of X_{ij}.

Z_{ij} : The covariates of the random effects of the ith patient at jth time

b_{1i}:The random effect which are assumed to be multivariate normal distribution having mean vector 0 and covariance matrix G, i.e. $b_{1i} \sim N(0, G)$

Method of estimation in GLMM: Maximum likelihood (ML) by Laplace approximation technique is used to estimate the parameters. ML estimates standard deviations of the random effects assume the fixed-effect estimates are correct. Such likelihood may involve high-

dimensional integrals that cannot be evaluated analytically so that much software is able to solve such complex manipulation using iteration technique.

3.2.2.5 Poisson Normal Gamma (PNG) Model

Combining ideas from the over dispersion models in section 3.2.2.2 and the Poisson-normal (GLMM) model of section 3.2.2.4, in which the gamma distributed random factor in the log-linear predictor fine tunes the over dispersion whereas the normal random effects to induce association between repeated Poisson data. The Poisson model with normal and gamma random effects can be specified as

$$Y_{ij} \sim poi(\lambda_{ij} = \Theta_{ij} K_{ij}), \text{with } K_{ij} = \exp(X_{ij}\Upsilon + Z_{ij} b_i)$$

$$\lambda_{ij} = \Theta_{ij} \exp(X_{ij}\Upsilon + Z_{ij} b_i) \qquad \text{---------- (5)}$$

Y_{ij} be the jth outcome measured for subject I = 1. . . N, j = 1, . . . ,ni. Where $b_i \sim N(0,D)$, and $\Theta_{ij} \sim$ Gamma(α, βr), X_{ij} and K_{ij} p-dimensional and q dimensional vectors of known covariate values, and γ a p-dimensional vector of unknown fixed regression coefficients.

Methods of Estimation in PNG

Molenberghs et al. (2007) and Molenberghs et al. (2010) marginalized the combined model analytically over the gamma random effect, whereby this partially marginalized model takes the form:

$$f(y_{ij}|b_{1i}, \Upsilon) = \int f(y_{ij}|b_{1i}, \Upsilon, \Theta_{ij}) f(\Theta_{ij}|\alpha_j, \beta_{rj}) d\Theta_{ij}$$

$$f(Y_{ij}|b1i, \Upsilon) = \binom{\alpha_j + y_{ij} - 1}{\alpha_j - 1} \left(\frac{\beta r_j}{1 + K_{ij}\beta r_j}\right)^{y_{ij}} \left(\frac{1}{1 + K_{ij}\beta r_j}\right)^{\alpha_{ij}} {K_{ij}}^{y_{ij}}$$

We integrated out the gamma random effect of the over dispersion we left with the normal random effect for the correlation of the observations.

3.2.2.6 Joint Poisson Normal Gamma Model

Modeling bivariate outcome using joint multivariate random effect models (JMRE) is a popular approach in the medical field. There are a number of conditions where a disease under study is well understood when two outcomes are considered.

For example, in clinical trials the clinician may be interested in the joint evolution of HIV RNA and CD4+t lymphocytes in a cohort of HIV-1 infected patients treated with active antiretroviral drugs. Bellamy (1995) studied the study of the risk factors associated with the progression of osteoarthritis (OA) of the knee. The JMRE model allows the modeling of mixed effects and bivariate outcomes.

This study used a straightforward and efficient method to handle bivariate outcome data where one outcome is continuous and the other is count with over-dispersion. That is one of the objective of this research is to analyze the joint evolution of CD4 count and weight of HIV patients after the initiation of ART.

The joint distribution of the continuous and the over dispersed count data conditioned over the parameters is given by:

$$f_i(y_{ij}, z_{ik} | b_{1i}, b_{2i}, \beta, \Upsilon, \Theta_{ik}) = f_{1i}(y_{ij} | b_{1i}, \beta) * f_{2i}(z_{ik} | b_{2i}, \Upsilon, \Theta_{ik}) \quad \text{---------- (6)}$$

The two normal random effects are correlated which captures the correlation between the two responses and distributed as:

$$\begin{bmatrix} a_{1i} \\ a_{2i} \\ b_{1i} \\ b_{2i} \end{bmatrix} \sim N(0, D)$$

Where, D is unknown positive-definite matrices. This is known as shared parameter in which the association of the two responses is captured. This structure imposes strong assumptions on the relationship between the two response variables. It is very unlikely that the two responses would exhibit complete dependence in the association between the random slopes and between the

random intercepts. One advantage of this model, when the assumption is tenable, is that it drastically reduces the number of random effects that must be estimated when the number of response variables is large. For models with a large number of response variables, estimation would likely be impossible if the shared-parameters (or alternative approach) are not used.

Association of the Evolution (AOE)

One important question that was addressed with a joint mixed-effects model is how the evolution of one response is associated with the evolution of another response ("association of the evolutions"). By definition, the correlation between the evolutions for the two random slopes is given by:

$$r_E = \frac{\text{cov}(b_1, b_2)}{\sqrt{\text{var}(b_1) \times \text{var}(b_2)}} = \frac{\sigma_{b_1 b_2}}{\sqrt{\sigma^2_{b_1} \times \sigma^2_{b_2}}} \qquad \text{---------- (7)}$$

Joint Model Estimation Method

Adaptive Gaussian Quadrature method approximates a given integral by a weighted sum over predefined abscissas for the random effects. A good approximation can usually be obtained with an adequate number of quadrature points as well as appropriate centering and scaling of the abscissas.

First, we make use of the partial marginalization for parameter estimation of the count component, as presented in Molenberghs et al. (2010). By this we refer to integrating the likelihood over the gamma random effects only, leaving the normal random effects untouched. The corresponding conditional probability for the PNG model is given by:

$$f(Z_{ik}|b_{1i}, \Upsilon, \Theta_{ik}) = \int f(Z_{ik}|b_{1i}, \Upsilon, \Theta_{ik}) f(\Theta_{ik}|\alpha_k, \beta_{rk}) d\Theta_{ik}$$

$$f(Z_{ij}|b1i, \Upsilon) = \binom{\alpha_j + z_{ij} - 1}{\alpha_j - 1} \left(\frac{\beta r_j}{1 + K_{ij}\beta r_j}\right)^{z_{ij}} \left(\frac{1}{1 + K_{ij}\beta r_j}\right)^{\alpha_{ij}} {K_{ij}}^{z_{ij}}$$

Since conditionally the two outcomes are independent so we have the joint PNG model:

$$f_i(y_{ij}, z_{ik}|b_{1i}, b_{2i}, \beta, \Upsilon, \Theta_{ik}) = \left\{2\pi^{\frac{-n_i}{2}} |\Sigma i|^{\frac{-1}{2}} \exp\left(\frac{-1}{2}(Y_i - X_i\beta - Zib1i)^T \Sigma i^{-1}(Y_i - X_i\beta - Zib1i)\right)\right\} * \prod_k f(z_{ik}|b_{1i}, \Upsilon, \Theta_{ik})$$

SAS software were used to estimate the parameters by Adaptive Gaussian Quadrature with procedure PROC NLMIXED which selects the number of quadrature points adaptively by evaluating the log-likelihood function at the starting values of the parameters.

Variable selection technique: -To select significant variables, first the main effect and main effect by time interaction were incorporated to the initial candidate model. After that, avoid non-significant variables one by one starting from the most non-significant terms which is called backward variable selection technique (Pinheiro and Bates 2002).

Model Comparison Technique

In order to select the best and final model which is appropriately fits with the given longitudinal data, it is necessary to compare the different models by using different techniques and methods. Hence, models are compared with Akaki Information Criteria (AIC), the Bayesian Information Criteria (BIC), and the Likelihood ratio test methods for nested were used at 5% level of significance.

AIC = -2log L + 2p

BIC=-2log Likelihood + n Par log (N),

Where, -2 logL is twice the negative log-likelihood value for the model

P: - is the number of estimated parameters.

npar: -denotes the total number of parameters in the model

N: - is the total number of observations used to fit the model. Smaller values of AIC and BIC reflect an overall better fit.

3.3 Software

For the sake of analysis statistical SAS software procedure PROC NLMIXED used.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

4.1. Baseline Information and Descriptive Statistics

A total of 300 adult HIV patients with a minimum of three and maximum of thirteen measures of CD4 count, weight and other covariates per individual of HIV patients were included for this study. This variables were measured from starting at the baseline that is zero month in ART then measured after six month on ART it continues measuring them by 6 month gap. The baseline characteristics and descriptive statistics of patients are displayed in table 2 below. Out of these HIV patients, 180(60%) were females and 120(60%) were males. At baseline 31(10%) of the patients were WHO STAGE I, 81(27%) were WHO STAGE II, 158(53%) were WHO STAGE III and 30(10%) were WHO STAGE IV. The baseline TB status of HIV patients were 69(77%) had positive result the rest 231(23%) had a negative result. The functional status of HIV patients were 197(65%) working class, 86(29%) were ambulatory and 17(6%) were bedridden. The average baseline CD4 cell counts per mm3 of blood and weight was 197.15 and 51.5Kg with standard deviation of 154.27 and 9.16, respectively. The minimum and maximum CD4 count were 11 and 1153, respectively, and the minimum and maximum weight were 24 and 84, respectively.

Table 2 Baseline demographic and clinical characteristics of HIV data, Assela 2014

VARIABLE		Frequency(n)	Percent (%)
Sex	Male	120	40
	Female	180	60
WHO STAGE	Class I	31	10
	Class II	81	27
	Class III	158	53
	Class IV	30	10
TB diagnosis	Positive	69	77
	Negative	231	23
Functional status	Working	197	65
	Ambulatory	86	29
	Bedridden	17	6

4.2. Separate Analysis of CD4 count and weight of patients

Without considering the association of the two outcomes, separately analyzed the two responses.

4.2.1. Exploratory Data Analysis

Plots are very important to visualize the pattern of CD4 count and weight overtime before model building; different plots have been explored that expose the patterns relevant to the scientific question about the progress of CD4 count and weight of HIV patients.

4.2.1.1. Exploring Individual Profile plots of CD4 count and weight over time

As shown in figure 1a, the variability CD4 count between individuals seems smaller at baseline and appears to increase over time. Furthermore, considerable variability is observed within each subject. Similarly, figure 1b depicts a between and within subjects variability in weight of HIV patients, both implying that the between and within subject specific differences cannot be ignored.

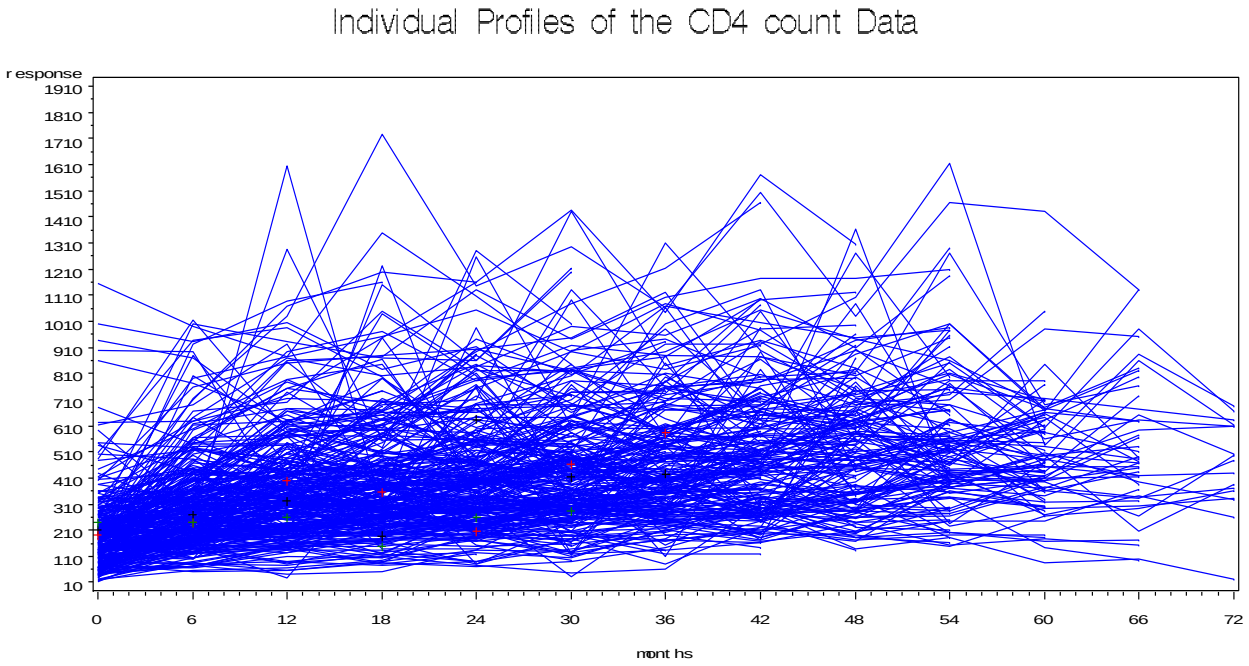

Figure 1a. Individual profile plot for CD4 count of HIV patients

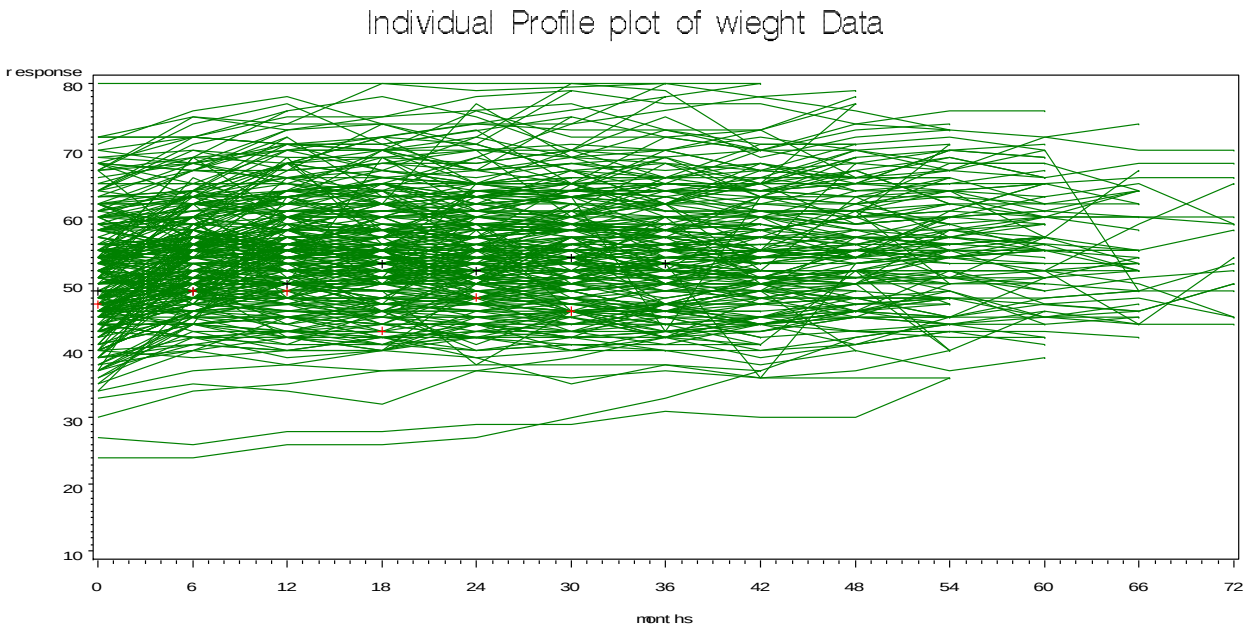

Figure1b. Individual profile plot of weight of HIV patients.

4.2.1.2. Mean profile Plots of CD4 count and weight of HIV patients.

Figure 2a depicts the average profile plot of CD4 count seems nonlinear so it is better to check whether there is quadratic time effect or not by including the quadratic time effect on the model and compare with the one that exclude the quadratic effect. On the other hand Figure2b shows the average profile plot for weights resembles to have linear relationship with time.

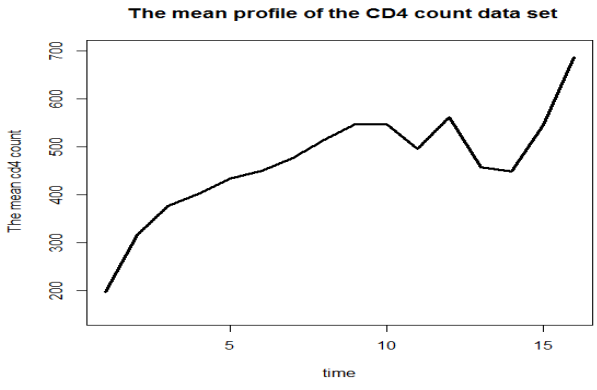

Figure2a mean profile plot of CD4 count

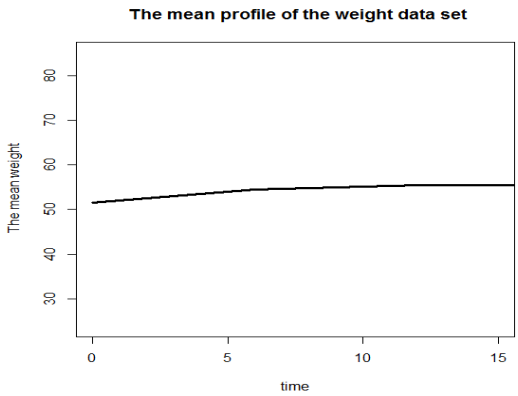

Figure2b mean profile plot of weight

4.2.1.3. Mean profile Plots of CD4 count and weight of HIV patients by sex

Figure 3a shows that the average CD4 count of HIV patients for female is higher that of male. On the other hand figure3b shows the average weight of HIV patients for male is higher than female.

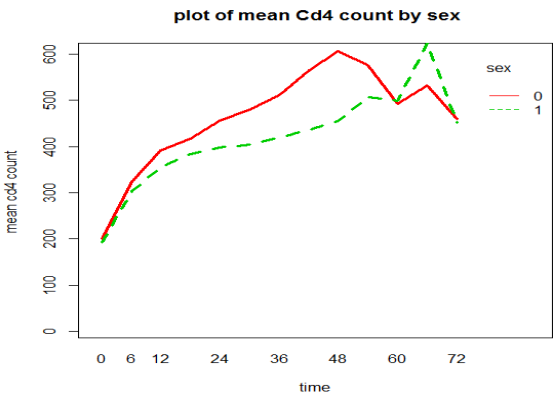

Figure3a average profile plot of CD4 count by sex.

Figure3b average profile plot of weight by sex

- Female=0 and male=1

4.2.2 Separate Mixed Model Analysis of weight

4.2.2.1 Selection of fixed effect for weight

The aim of this section is to select a set of fixed effect to fit a linear mixed model for weight.

To select the fixed effect components of the response variable, weight, including all covariates and interaction terms with time without considering the corresponding different random effects were fitted below: Let weightij denote the jth weight of the ith patient at time tij,. Where i indexes the subjects i= 1, 2 ,.., 300 and j indexes the time visit for subject i, j= 1, 2, ...,ni. ni represents the overall visits of subject i. Hence, the fixed effects model with linear time effect for weight is given by:

$$weight_{ij} = \beta_{10} + \beta_{11} sex_i + \beta_{12} VARF_i + \beta_{13} VARW_i + \beta_{14} VART_i + \beta_{15} time_{ij} + (\beta_{16} sex_i + \beta_{17} VARF_i + \beta_{18} VARW_i + \beta_{19} VART_i) time_{ij} + \beta_{120} time * time + \varepsilon_{ij}$$

Where: $weight_{ij}$=weight of patients sex=gender of patients VARF: - functional status of HIV patients VARW: WHO stage of patients VART= TB status of patients. $\beta_{10}, \beta_{11}, , , , \beta_{1p}$:- Are the fixed effect coefficient parameters ε_{ij}= error term.

Table 3 Fixed effect estimation with interaction terms for weight of HIV patients.

Variable	estimate	P value
Intercept	52.6564	<0.001
Sex	6.3051	<0.001
VARF	-3.4191	<0.001
VARW	0.2821	0.4834
VART	-2.644	0.0175
Time	0.0103	0.001
Sex*time	-0.0456	0.0194
VARF*time	-0.0031	0.9088
VARW*time	0.0056	0.6766
VART*time	0.0895	0.4423
time*time	-0.00006	0.9043

From the outputs in table 3 , we can observe that except WHO stage and quadratic time all the covariates are statistically significant, but all the interaction terms except time by sex are statistically insignificant. Thus, the insignificant terms should be removed from the model starting with the most insignificant one of which is the interaction term WHO stage by time with p-value of 0.9088. The model was then refitted after removing the interaction term WHO stage by time and the AIC dropped from 19667.9 to 19661.9 indicating a better fit. The model was fitted again and the categorical covariate quadratic time was still insignificant. The next step is to remove the covariate quadratic time with the p-value of 0.9677. The model was fitted again and the AIC dropped. By doing the same procedure sex functional status, TB status, time and sex by time interaction term were retain as fixed effect with random intercept and random slope as random effect. The final model for weight is given by:

$$weight_{ij} = \beta_{10} + \beta_{11} sex_i + \beta_{12} VARF_{ij} + \beta_{14} VART_i + \beta_{15} time_{ij} + \beta_{16} sex_i time_{ij} + a_{10} + b_{11} time_{ij} + \varepsilon_{ij}$$

4.2.2.2 Selection of Fixed Effects for CD4 Count

To select the fixed effect for cd4 count, all covariates and interaction terms without considering the corresponding different random effects model were fitted. Let cd4countij denote the jth CD4 count of the ith patient at time tij,. Where i indexes the subjects and j indexes the time visit for subject i, ni represents the overall visits of subject i. Hence, the full fixed effects model is given by:

$$CD4count_{ij} = \beta_{20} + \beta_{21}sex_i + \beta_{22}VARF_i + \beta_{23}VARW_i + \beta_{24}VART_i + \beta_{25}time_{ij} + (\beta_{26}sex_i + \beta_{27}VARF_i + \beta_{28}VARW_i + \beta_{29}VART_i)time_{ij} + \beta_{220}time * time + \varepsilon_{ij}$$

Where: $CD4count_{ij}$=cd4 count of patients sex=gender of patients VARF: - functional status of HIV patients VARW: WHO stage of patients VART= TB status of patientsβ_{10}, β_{11}, , , , β_{1p}:- Are the fixed effect coefficient parameters ε_{ij}= error term.

Table 4. the first result of the fixed effect estimation with the interaction terms for CD4 count.

Variable	estimate	P value
Intercept	2.3018	0.0595
Sex	-0.0317	0.0168
VARF	-0.04319	<0.0001
VARW	0.01741	0.1105
VART	-0.2083	<0.0001
Time	0.01525	0.0709
Sex*time	-0.0020	0.035
VARF*time	-0.002143	0.0867
VARW*time	-0.00018	0.6162
VART*time	0.008595	0.0767
time*time	-0.00015	<0.0001

By excluding the most insignificant variable that is for this analysis as we can see in table 4 WHO stage by time (p value=0.6162) were the most non-significant so we exclude it and refit the model. The second non-significant estimator is functional status by time with p value=0.0828 exclude this variable we refit the model. Therefore in this study sex, functional status, WHO stage, TB

status, time and quadratic time used as fixed effects with random intercept and random slope as random effects for the CD4 count. The final model:

$$CD4count_{ij} = \beta_{20} + \beta_{21}sex_i + \beta_{22}VARF_{ij} + \beta_{23}VARW_{ij} + \beta_{24}VART_{ij} + \beta_{25}time_{ij} + \beta_{220}time * time + a_{20} + b_{21}time_{ij} + \varepsilon_{ij}$$

4.2.3. Joint and separate Analysis of cd4 count and weight of HIV patients.

Previously, the two outcomes are analyzed separately for purpose of identifying associated risk factors for the progress of CD4 count and weight separately and then include on the joint analysis those factors to investigate the joint evolution and association of CD4 count & weight, and associated risk factors for the progress of the two end points by considering a joint linear mixed effects model.

Table 5 joint and separate analysis result of CD4 count and weight of HIV patients

Separate analysis of cd4 count and weight			Joint analysis of CD4 count and weight	
Effect	Estimate (SE)	P-value	Estimate (SE)	Pvalue
For CD4				
Intercept	97.8721(0.06498)	<.0001	97.4651(0.0538)	<.0001
sexF	1.0960(0.000667)	<.0001	0.5960(0.0004)	<.0001
Fun. statusA	1.1826(0.000641)	<.0001	0.6826(0.00062)	<.0001
Fun statusW	1.4390(0.000573)	0.0002	0.9390(0.0004)	0.0008
WHO stg I	0.9121(0.000731)	0.0008	0.4121(0.00061)	0.0021
WHO stg II	0.9105(0.000732)	<.0001	0.4105(0.00072)	<.0001
WHO stg III	0.8868(0.000741)	<.0001	0.3868(0.00065)	<.0001
TB statusN	1.8768(0.000486)	<.0001	1.3768(0.0004)	<.0001
time	1.0529(0.000681)	<.0001	0.5529(0.00051)	<.0001
time*time	0.9999(0.000699)	<.0001	0.4999(0.00004)	<.0001
Alpha	17.0033(0.00084)	<.0001		
$\sigma_{a_{20}}$	1.6267(0.0033)	0.001		
$\sigma_{b_{21}}$	1.3246(0.0011)	0.003		
For weight				
Intercept	51.6283(0.00024)	<.0001	51.1166(0.0001)	<.0001
sexF	-6.2855(0.0001)	0.0001	-6.7855(0.0001)	<.0001
Fun. statusA	1.1441(0.000031)	<.0001	0.6441(0.00002)	<.0001
Fun statusW	5.5621(0.000429)	0.0001	5.0621(0.000329	0.0024

TB statusN		2.2373(0.000219)	0.0003	1.7373(0.0002)	0.0001
time		-0.02682(0.003)	<.0001	-0.5268(0.0001)	<.0001
Sex*time		0.04488(0.00031)	<.0001	-0.4551(0.0003)	<.0001
sigma		11.36731(0.0442)	0.0001		
$\sigma_{a_{10}}$		0.1057(0.004)	0.002		
$\sigma_{b_{11}}$		0.2665(0.0102)	<0.0001		
Common parameters	$\sigma_{b_{10},b_{20}}$			0.1237(0.024)	<.0001
	$\sigma_{b_{..},b_{..}}$			0.0707(0.16)	0.0039
	$\sigma_{b_{20},b_{11}}$			0.2190(0.172)	0.0083
	$\sigma_{b_{..},b_{..}}$			0.1784(0.004)	<.0001
	Rho (ρ)			0.5054(0.012)	<.0001

4.2.3.1 Results of Joint mixed effect model

A joint mixed effect model for the two outcomes was fitted. This model is the same as the separate model except the sets of random intercepts and slopes for each response are now correlated. This model was fitted allowing for a linear time effect for each covariate that was selected as a fixed effect in the separate mixed model. The subject specific random intercepts and random slopes were fitted to account for within-subject correlations.

As shown in table 3, the fixed-effect intercept 97.46(0.05) represents an estimate of the average CD4 count at baseline excluding all covariates in the model. Likewise, the fixed-effect intercept 51.11(0.0001) represents an estimate of the average weight at baseline excluding all covariates in the model. Among all covariates, sex and time were negatively associated with weight that means when the time increases after sometime the weight decreases made with (P-value<0.0001). Sex was significantly associated with both CD4 count and weight of HIV patients; female patients had 1.8(0.0004) higher CD4 count than Male, female patients had 6.78(0.0001) lower weight than male. Similarly, Functional status and TB diagnosis was significantly associated with both CD4 count and weight , thus, patients under worker status(VARFW) had 2.53(0.0004) points higher CD4 count relative to the patient who is bedridden, patient who is under worker status had relatively 5.06(0.0003) higher weight than patients under bedridden status. In the same way, TB status of patients also had a significant association with both weight and CD4 count. Patient whose TB diagnosis was negative had

relatively 3.93 (0.0004) higher CD4 count than whose TB diagnosis was positive. Patients whose TB diagnosis was negative had 1.73(0.0002) higher weight than positive TB diagnosis patients. Likewise, sex by time interaction has a significant association only with the weight outcome. WHO stage had significant association with CD4 count only. Patient with WHO stage I (VARWI) had 1.5 (0.0006) relatively higher CD4 count than patient who were at WHO stage four.

4.2.3.2 Associated (common) effect parameters

By referring table 3, based on 300 subjects, a substantial correlation (ρ=0.5054, S.E. =0.012) between the CD4 count and weight within the same subjects is noted. The covariance for subject specific random intercept of CD4 count and weight with ($\sigma_{b_{10},b_{20}}$ =0.1237(0.024)) and the covariance for subject specific random slopes of CD4 count and weight with ($\sigma_{b_{11},b_{21}}$ =0.1784(0.004)). With the joint mixed effect model for the two outcomes, it is possible to investigate how the evolution of cd4 count associated with the evolution of weight. Hence, the association of the evolution (AOE) is to be estimated 0.5054(S.E. =0.012, p-value<0.0001).

4.2.3.3 Results of separate mixed effect model

Technically, the separate models were fitted for the two outcomes, CD4 count and weight together but assuming that ρ = 0, which is entirely equivalent to fitting the models separately or independently interpretations for the models those modeled independently for CD4 count & weight is entirely equivalent to that of separate models by assuming ρ = 0.

As shown in table 3, the fixed-effect intercept 97.87(0.06) represents an estimate of the average CD4 count at baseline excluding all covariates in the model. Likewise, the fixed-effect intercept 51.62(0.0002) represents an estimate of the average weight at baseline excluding all covariates in the model. Among all covariates, sex and time were negatively associated with weight that means when time increases the weight decreases with (P-value<0.0001). Sex was significantly associated with both CD4 count and weight outcomes; female patients had 2.97 (0.0006) points higher CD4 count over evolution of Male, female patients had -6.28(0.0001) lower weight than male. Similarly, Functional status and TB diagnosis was significantly associated with both CD4

count and weight , thus, patients under worker status(VARFW) had 4.17 points higher CD4 count relative to the patient who is bedridden, the same thing for weight patient who is under worker status are relatively have 5.56 higher weight than under bedridden status. In the same way, TB status of patients is also has a significant association with both weight and CD4 count. Patient whose TB diagnosis was negative has relatively 6.48 (0.0004) CD4 count than whose diagnosis was positive. The same thing for weight has 2.23(0.0002) higher than positive TB diagnosis patients. Likewise, sex by time interaction has a significant association only with the weight outcome. WHO stage has significant association with cd4 count only. Patient with WHO stage I (VARWI) have 2.48 (0.0007) relatively higher CD4 count than patient who were WHO stage four.

4.2.3.4 Comparison of separate and joint mixed effect models

Now that both separate and joint mixed effect models have been considered and parameter estimates for the separate and joint models are summarized in table 3. As we have seen CD4 count and weight show positive and statistically significant relationship as evidenced by the correlation of the random effects in joint mixed models so, it was nice to see the two outcomes jointly and also separately then compare and select the best one is crucial. The significant covariates that are included in the separate model were also significant in the joint model. Likelihood comparison shows a convincing improvement in model fit using joint mixed model (-2LL=546797.361) since for the separate analysis (-2LL=547878.628) which is larger than that one. Comparing the separate and joint models, although parameter estimates for both outcomes are nearly equivalent, small changes are observed in parameter estimate of some covariate in joint analysis.

4.2.3.5 Model diagnostic checking

Diagnostic checking and Residual plot for fixed effects: - Different diagnostic checking plots for the final separate mixed linear models of weight and CD4 count are presented in appendix I. According to figure 4 plot of fitted versus standardized residuals for weight, even if there are some outliers, it was indicated that the variability of the errors were almost nearly constant. That means the errors did not deviate far from each other. Furthermore, according to the probability

plots which were shown on figure 5 for weight the normality assumption was supported through the upward nearly straight line of normal plot. Similarly, based on the normal probability plots of random effects with subject specific random intercepts and random slopes those are shown on figure 6, even if it seems a slight deviation of normality at the bottom tail on the random slope (Time) for CD4 count that is not that much worse deviation. Hence, there is no problem with normality assumptions of both random intercepts and random slopes for both & models and the normality assumption are almost fulfilled.

4.2.3 Discussion

In this thesis, two methods were considered for fitting two response variables measured longitudinally, a separate mixed effects model and a joint model. Since a joint model building usually starts from separate models for each component, initially each data are analyzed separately. Such separate analysis is preferred for several reasons. Firstly, it helps to specify the mean response of the model. Secondly, the random effects and fixed effects to be included in the linear mixed effect model can be easily determined, and thirdly initial values to be provided for the joint models can be obtained. In separate mixed model separate analysis of Cd4 count and weight were carried out. Before fitting the mixed model separately for each out comes, exploring the data analysis have been done to understand the data structure and determine the relevant modeling approaches.

From individuals profile plot, we observed the existence of variability in both Cd4 count and weight within and between individuals. The exploratory analysis result for mean structure also suggested that on average, both Cd4 count and weight measures slightly increasing over time, but the rate of increasing is high in Cd4 count than weight. This is consistent with the results of Reda AA et al (2013), used separate mixed model analysis which indicate that the duration of ART was an important predictor of improvements in CD4 lymphocyte count and weight which implies overtime the Cd4 count and weight of HIV patients are increasing .

From the joint and separate analysis it was obtain actively working patients had higher CD4 lymphocyte count and weight compared to those that were bedridden which is the same with the

result of Reda AA et al(2013) that is functional status of HIV patients has a significant effect on the two responses.

WHO stage was not significantly associated with weight which is not consistent with the results of Reda AA et al (2013) showed higher age groups, earlier WHO stage, higher CD4 count and high hemoglobin levels were associated with improvements in weight.

It was also obtained that female patients have high improvement in CD4 count than male patients this is consistent with the findings of Moing L et al (2002) who found Short-term increase in CD4+ cell counts was higher in women patients.

The other finding was patients with TB negative was have higher CD4 count and weight than patients with TB positive this result is same with the result of Dinakar Kr et al (2014) showed the mean CD4 counts increment are lesser in the TB co-infection group who are under ART and also the increase in body weight was much more than the increase in the patient who did not have tuberculosis further emphasizing the effect of tuberculosis in decreasing the body weight of the patient.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In this study two methods were considered for fitting two response variables measured longitudinally. The result shows both the separate and joint analyses are consistent. But, the joint model is the simplest (fewer complexes) model compared to the separate model because its standard error of the parameter estimates is smaller. And also, the joint model has a smaller log likelihood value which indicates that it fits the data better than the separate model. Hence, the joint model is not only the simplest model but also it results a better fit to the data.

Based on separate analysis; the evolution of CD4 count and weight were significantly differ with respect to time, sex, functional status and TB status of HIV patients. In this analysis time interaction with sex was not significantly associated with CD4 count but was significant with weight. On the other side WHO stage had no significant association with weight but had significant association with CD4 count HIV patients. Moreover, on average CD4 count and weight increases over time after patients initiated antiretroviral therapy.

Based on joint analysis; it is consistent with the separate model on the significance of the fixed effects and slight difference in the estimation and standard error of the fixed effect. But in this analysis sex by time interaction term which is significantly associated with weight was negative.

On both separate and joint analysis time is negatively associated with weight outcome which implies after some time as time increases the weight decreases.

5.2. Recommendation

In Ethiopia currently anti-retroviral treatment is expanding all over the country for HIV patient. But it is not enough only giving a treatment to patients under a follow up clinic, also it is important to know factors that contribute to the progression of the CD4 count and weight. In this study, the progression of Cd4 count and weight was found to be different in all patients due to time, WHO stage TB status sex and Functional status of HIV patients therefore, the concerned body should give special attention for those patients under sever condition like patients under WHO stage IV, TB status positive and who are bedridden are in need.

Further studies are required to improve the progression of CD4 count and weight together with the necessary variables since they are some of the indicator of the efficacy of the treatment. In addition, governmental and non-governmental body gives awareness for health workers to record all the necessary variables during follow up time to see the change of the disease within and between subjects overtime using longitudinal data analysis. Even though, separate model is most common practice for researchers to model several outcomes involved in a disease process, the joint model is also able to address the same questions as separate model with more accuracy (smaller standard errors) while addressing additional questions that may be of great interest to the researcher, such as the AOE and the EOA of the responses. Thus, fitting joint model is recommended for researches to any types of multivariate response variable. In this study, it is focus on only two response variables, for future work, one might want to look at modeling more than two response variables over time.

5.3 Limitation of the study

The investigator intended to study in brief about the joint evolutions of CD4 count and weight over time with associated covariates. However, there were a lot of constraints starting from extraction of data up to the end of the works. Some of them are listed as following:

- The first limitation was lack of enough literature and materials with regards to joint mixed effect model on CD4 count and weight of HIV patients. And also very difficult to

get model building about the over dispersed and correlated count data since CD4 count is count random variable with over dispersion.

- The positive-definiteness constraint was a major obstacle in modeling covariance matrices due to model over-parameterization.
- Another some potential risk factors or covariates which may have high influence on evolution of CD4 count and weight which were mentioned in some literatures are not available.

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Appendix I

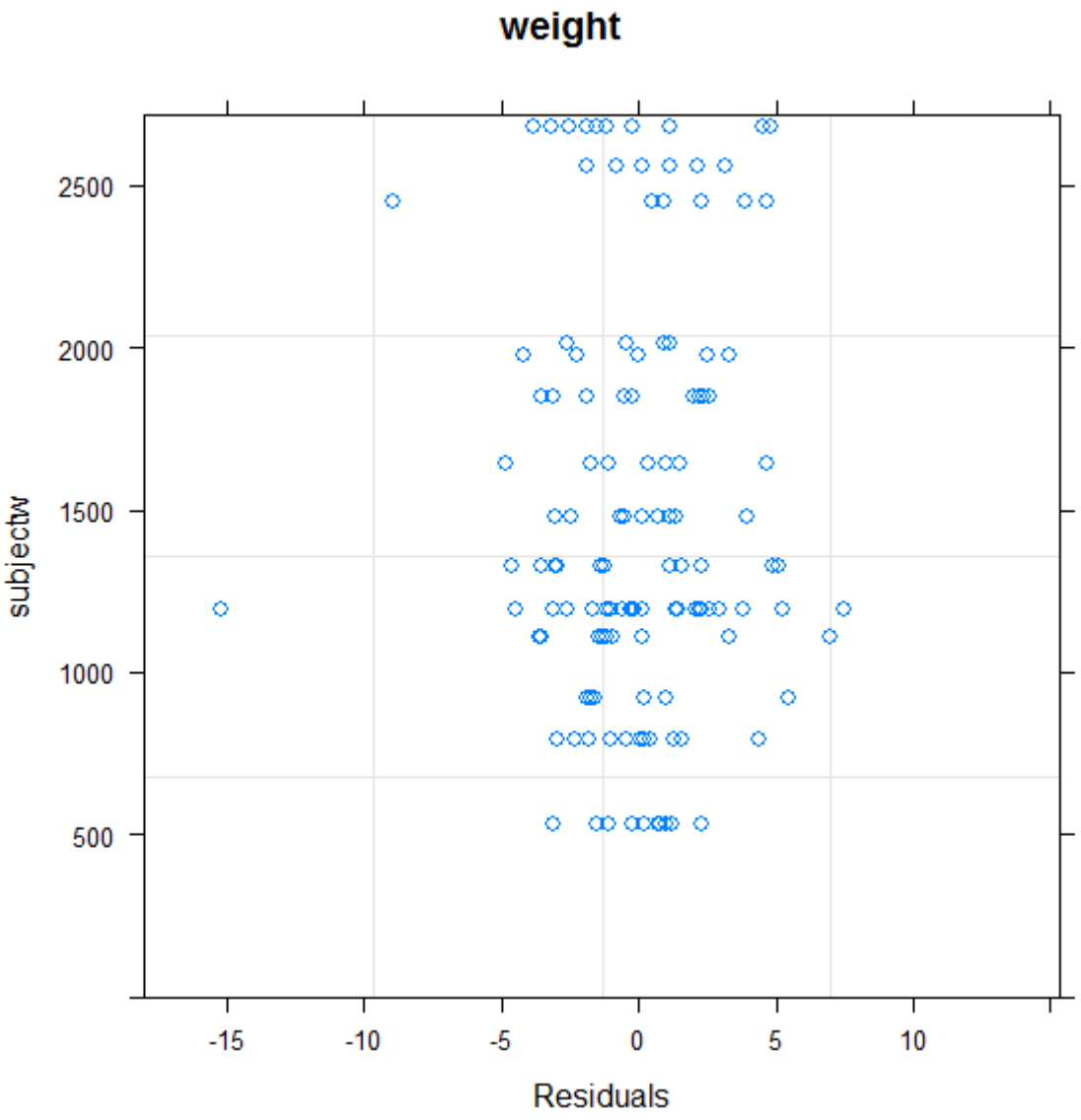

Figure 4 Residual Vs observed subjects

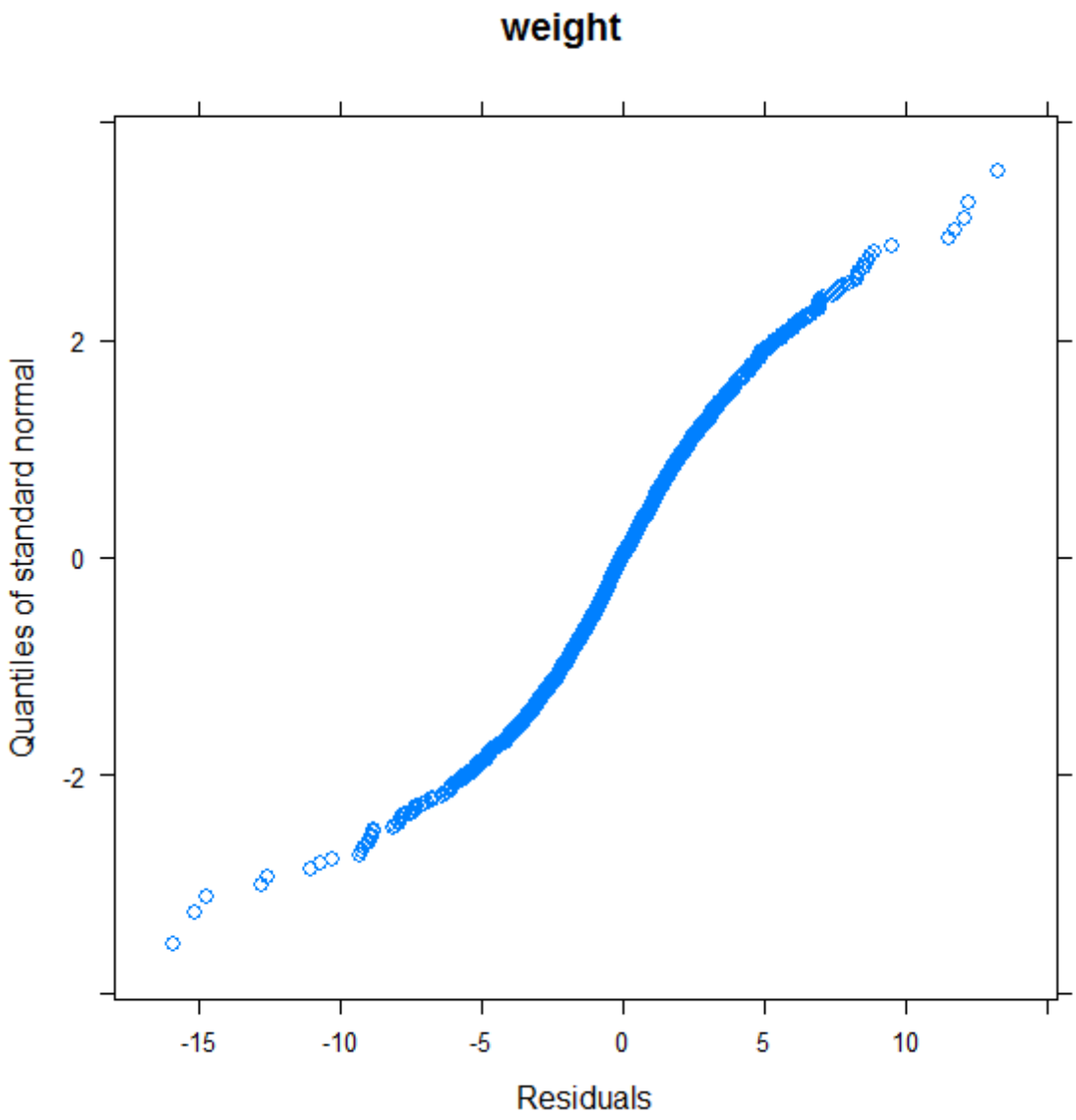

Figure 5. QQ plot for the residual

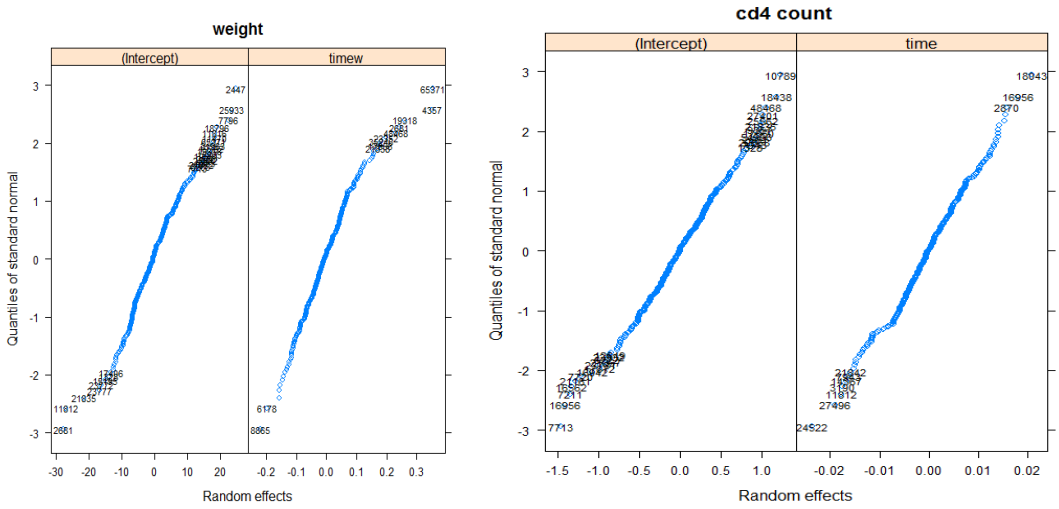

Figure 6 QQ plot for random effects for weight and CD4 count.

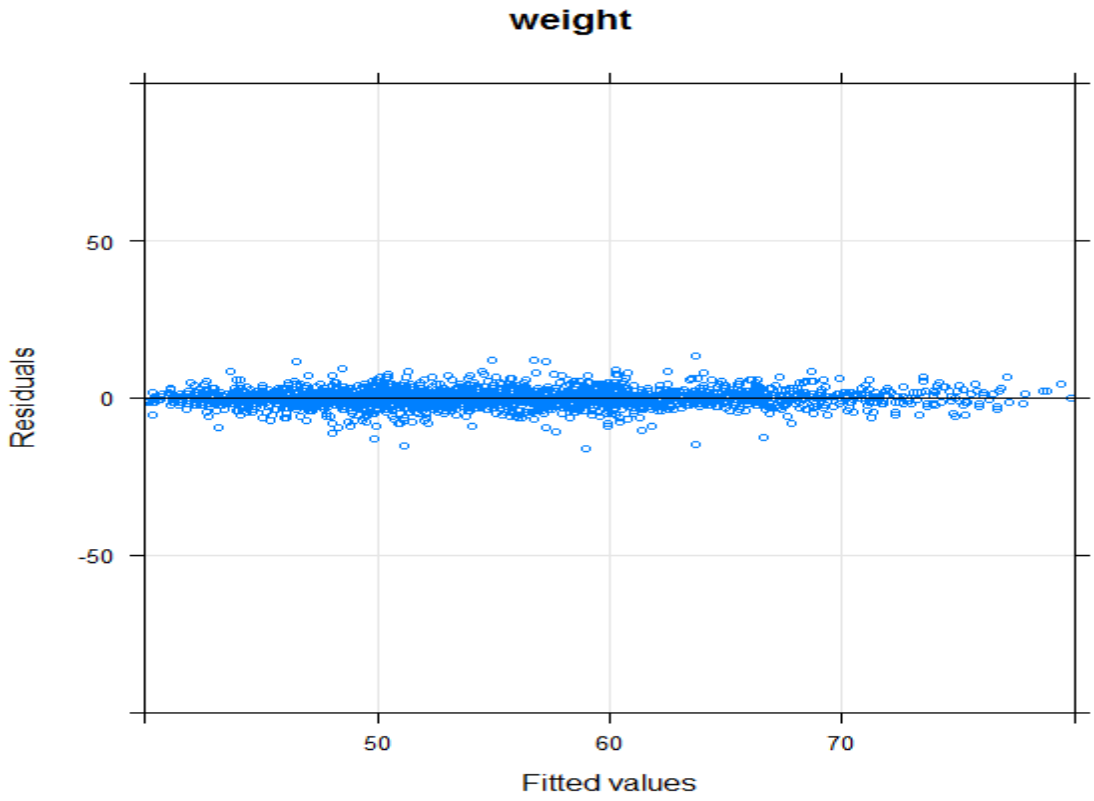

Figure 7 residual Vs fitted for weight.