

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND
TECHNOLOGY**



**Modeling the Effects of Nutritional Withdrawal on CD4 Cell Count Among
HIV/AIDS Patients Using Generalized Estimating Equations
(GEE).**

By

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Declaration

I hereby declare that this submission is my own work towards the award of the MPhil degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which had been accepted for the award of any other degree of the university, except where due acknowledgment had been made in the text.

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Dedication

I humbly dedicate this research to my lovely mum; Miss Elizabeth Florence Attiah Anku, whose love and prayers kept me focused and motivated and to the Almighty God whose bountiful and unmerited favours saw me through this research and the degree programme.



Abstract

Malnutrition is one of the major complications of HIV/AIDS (Lui et al., 2011). Nutritional intervention programs for People Living with HIV/AIDS (PLWHA) were withdrawn in Kumasi. The aim of this study is to examine the withdrawal effects of the nutritional intervention on CD4+ cell counts and model the CD4+ cell counts before, during and after the initiation of the intervention, given BMI, HB levels and other characteristics of PLWHA. The study was carried out using data from a retrospective cohort study of 501 PLWHA who started ART from 2007 to 2011. Generalized Estimation Equations (GEE) was used to model the CD4+ cell counts before, during and after the initiation of the intervention, given BMI, HB levels and other characteristics of PLWHA. Age, the linear interaction between HB and time, the linear interaction between BMI and time, Group 1 (No Intervention) and Group 2 (Intervention) were all statistically significant predictors of CD4+ cell count at the α level of 0.05. The nutritional intervention positively affected the health outcomes of PLWHA during the nutritional intervention. After the intervention however, there was a significant reduction in the average CD4 cell count of respondents. BMI predicts a positive gain in CD4+ cell count of respondent and there was a positive correlation between the blood haemoglobin levels of the HIV/AIDS patients and their CD4 cell counts. There was an inverse relationship between age and CD4 cell counts of HIV/AIDS patients; which is consistent with other works.

Acknowledgement

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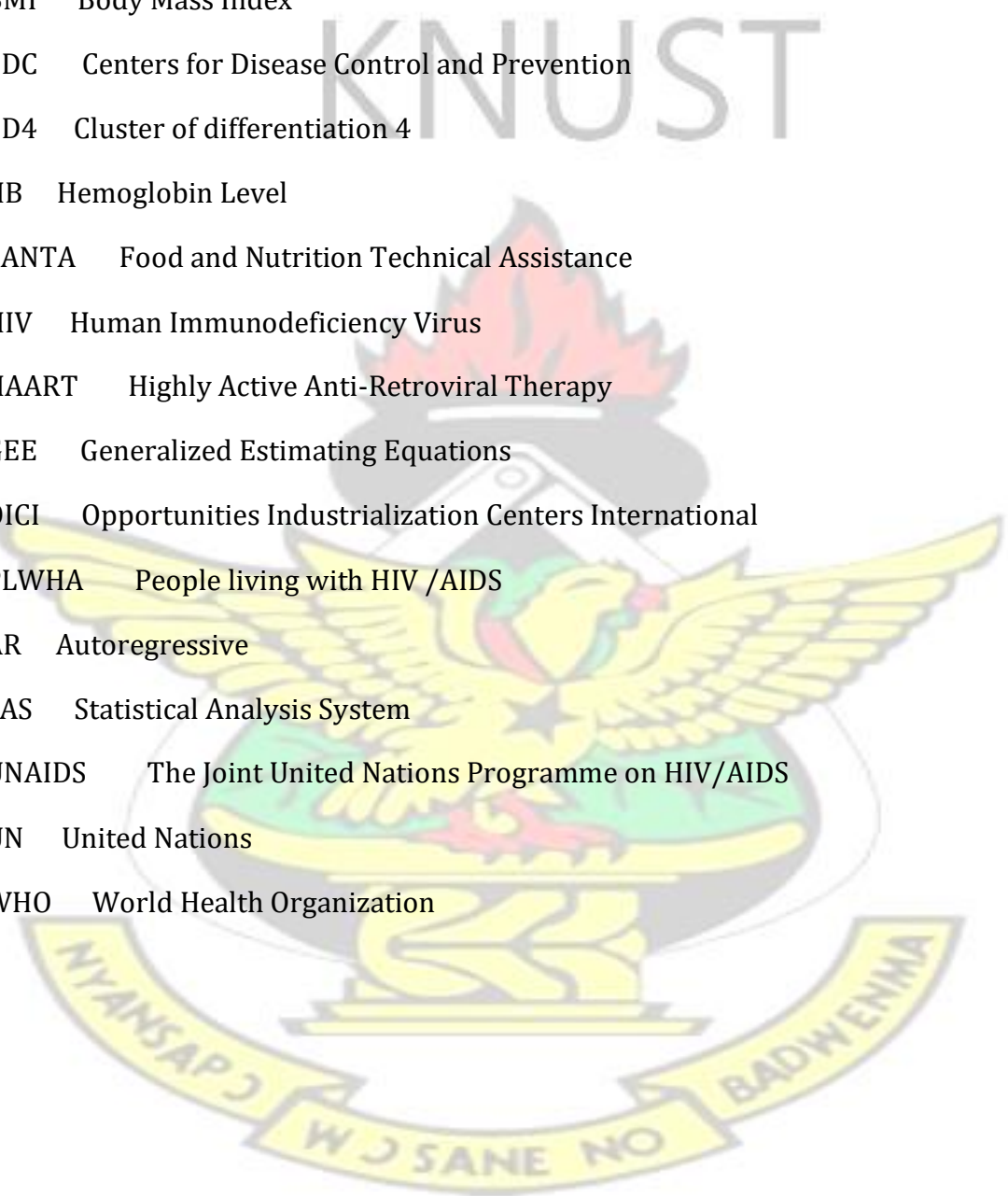
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List of Abbreviations



AIDS	Acquired Immunodeficiency Syndrome
AIC	Akaike Information Criterion
ART	Antiretroviral Therapy
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CD4	Cluster of differentiation 4
HB	Hemoglobin Level
FANTA	Food and Nutrition Technical Assistance
HIV	Human Immunodeficiency Virus
HAART	Highly Active Anti-Retroviral Therapy
GEE	Generalized Estimating Equations
OICI	Opportunities Industrialization Centers International
PLWHA	People living with HIV /AIDS
AR	Autoregressive
SAS	Statistical Analysis System
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UN	United Nations
WHO	World Health Organization

Chapter 1

Introduction

1.1 Introduction

Good nutrition is key to maintaining strength, energy, and a healthy immune system. It is little wonder that many studies have shown that poor nutritional status is highly associated with immunologic damage and adverse health outcomes among people infected with HIV (Liu et al., 2011). Therefore it is noted that nutritional support can reasonably contribute to the clinical success of HIV programs thereby improving the general health condition and quality of life of people living with HIV/AIDS (Argemi et al., 2012).

HIV continues to be one of the most devastating global public health challenge of this present generation, having claimed more than 39 million lives around the world so far (WHO, 2014). World reports have indicated that there were approximately 35.0 million people infected with HIV at the end of 2013 and almost 70% of those infected (about 24.7 million people) live in sub-Saharan Africa (WHO, 2014).

No country, till today, has been able to overcome the HIV/AIDS pandemic in the world (WHO, 2009). More disturbing is the fact that the majority of persons infested with this deadly disease are not aware of their condition (UNAIDS, 2001). Despite all efforts that have gone into prevention and care of HIV/AIDS in Ghana, about 220,000 people are found to be infected with the disease as at 2013 (UNAIDS, 2013).

Since decades, the associations between malnutrition and HIV infection have been the subject of study. Earlier study by Scrimshaw, Taylor, and Gordon (1968) on

the links between malnutrition and infections indicated that most infections were increased in prevalence and severity as a result of malnutrition.

The associations that exist between malnutrition and HIV infection have been termed as synergistic; since it has been established that morbidity and mortality shoot up to a degree more than the sum of the expected individual effect anytime malnutrition occur together (Scrimshaw, 2003).

It is not surprising therefore that malnutrition is one of the major complications of HIV/AIDs. Even though, nutrition has been given little or no proper attention by people infected with HIV/AIDs, it boosts the immune function thereby helping to fight further infections and maximizes the effectiveness of antiretroviral therapy (Obi, et al. 2010). Adequate hydration and increase calories and protein intake are necessary to fight the infection. Good nutrition must start once a person has been diagnosed with HIV to support nutritional deficiencies that occur early in the disease process, since nutritional deficiencies contribute to decreased immunity and disease progression.

Good nutrition coupled with antiretroviral treatment and continual monitoring of body composition changes are thus vital for positive outcomes in PLWHA (Tsehay, 2010). It is hence strange and unfortunate that nutrition intervention programs have been withdrawn from the country and the nutritional support of PLWHA has been neglected by both the care givers and the people living with many of these HIV/AIDs patients.

1.2 Background to the study

The Centers for Diseases Control and Prevention defines HIV as a virus spread through body fluids that affects specific cells of the immune system, called CD4 cells, or T cells. It is this HIV infection that leads to AIDS.

Acquired Immune Deficiency Syndrome (AIDS) was described for the very first time in 1981 by the Centre for Disease Control (CDC) (Dong & Imai, 2012, p. 864) when the United States of America recorded the first case of AIDS in that year. By the end of 2013, according to UNAIDS, there were 35 million [33.2 million-37.2 million] people infected with HIV. Even though the number of new HIV infections is declining, the total number of PLWHA is rising as more people are living longer because of antiretroviral therapy (UNAIDS, 2014).

CD4+ T lymphocyte (CD4) cell counts is an important indicator of HIV progression since HIV/AIDS infects CD4 cells, CD4 count gives an immunologically significant measure for HIV/AIDS progression. The administration of ART for PLWHAs; where nutritional cares and supports form an integral part, is known to significantly increase CD4 count and thereby enhancing the immunity state of patients. Nutritional cares therefore are essential for the clinical success of HIV programs (ART) especially started in developing countries. Repeated measurements are done at several points for each subject in longitudinal studies. Within-Individual repeated measurements of a variable are correlated and observations normally contain missing data, dropouts and measurement error.

Generalized Estimation Equations (GEE) model is one of the most used method for analyzing longitudinal data. In GEE, the mean and covariance structure of the response variable are specified. A correct and appropriate specification results in a consistent and asymptotically normal parameter estimates. Models such as the GEE are mostly used to model CD4 cell count change over time in longitudinal analysis.

1.3 Statement of the Problem

A study conducted by Amponsah (2013) on the withdrawal effects of nutritional interventions on the health outcome of PLWHA in Kumasi used descriptive statistics to show that CD4+ cell count, BMI and HB levels of respondents changed during and after the food supplementation

However the changes were not formulated to account for correlations in CD4+ cell counts during and after the nutritional intervention.

1.4 Objectives

The main purpose of this study is

- to examine the withdrawal effects of the nutritional intervention on CD4+ cell counts
- model the CD4+ cell counts before, during and after the initiation of the intervention, given BMI, HB levels and other characteristics of PLWHA, using Generalized Estimating Equations (GEE).

1.5 Methodology

The nutritional support system for PLWHA was withdrawn in the country. One cause of increased mortality in patients starting ART that can be reversed is malnutrition. It has been documented that optimal nutrition no doubt helps boost immune function and maximize the effectiveness of antiretroviral therapy (Obi et al., 2010). Argemi et al. (2012) also buttress the point that nutritional cares are essential for the clinical success of HIV programs started in developing countries.

It is surprising therefore that the nutritional support system of PLWHA in the country has been withdrawn.

The study was carried out using data from a retrospective cohort study in order to assess the associations between nutrition and CD4 count responses in patients starting ART from 2007 to 2011. ART was defined as HAART or non-HAART. Generalized Estimation Equations (GEE) was used to model CD4+ cell counts before, during and after the initiation of the intervention, given BMI and HB levels of PLWHA.

The nutritional status of selected PLWHA was defined by both body weight and body mass index (BMI). BMI was grouped into four (4) according to established criteria: less than 17kg/m² (moderate to severe malnutrition), 17 to 18.5 kg/m² (mild malnutrition), less than or equal to 18.5 to 25 kg/m² (normal nutrition) and greater than 25 kg/m² (overweight and obese) (Ferro-Luzzi et al., 1992).

Measurements of body weight and height were carried out and variables (demographic and clinical) were taken from existing database. Body Mass Index (BMI) was determined and was calculated as the weight (measured in kilograms) divided by the square of the height (measured in metres).

1.6 Justification

The study seeks to compare the health outcomes of PLWHAs during the nutrition support period and the period of withdrawal. It will also provide basic concepts of the relationship between nutrition and CD4 count and thus the survival of patients of HIV/AIDS.

Since nutritional cares are essential for the clinical success of HIV programs started in developing countries (Argemi et al. 2012), the findings of this study

could be useful evidence for the re-implementation of nutrition programs (nutrition/food supplementation support) among patients on ART in the country undertaking by government and other non-governmental organizations.

This is very necessary since the implementation of policies on nutrition support among patients on ART would prove beneficial in reducing HIV-related mortality especially in developing countries (Mageda, et al. 2012). This will also alert decision making bodies on the direction to take in reducing the morbidity, mortality, and the progression of HIV to AIDS.

1.7 Thesis Organization

This study was organized in five chapters. Chapters one comprises of the introduction, background of study, problem statement objective, methodology, justification and thesis organization.

Chapter two reveals both the theoretical and empirical literature about the topic. Chapter three looks at the methodology which mainly is the research purpose, data collection methods, population and its sampling techniques. Chapter four presents the analysis of data and results. The summary of findings, conclusion as well as the recommendations are well presented in the five.

Chapter 2

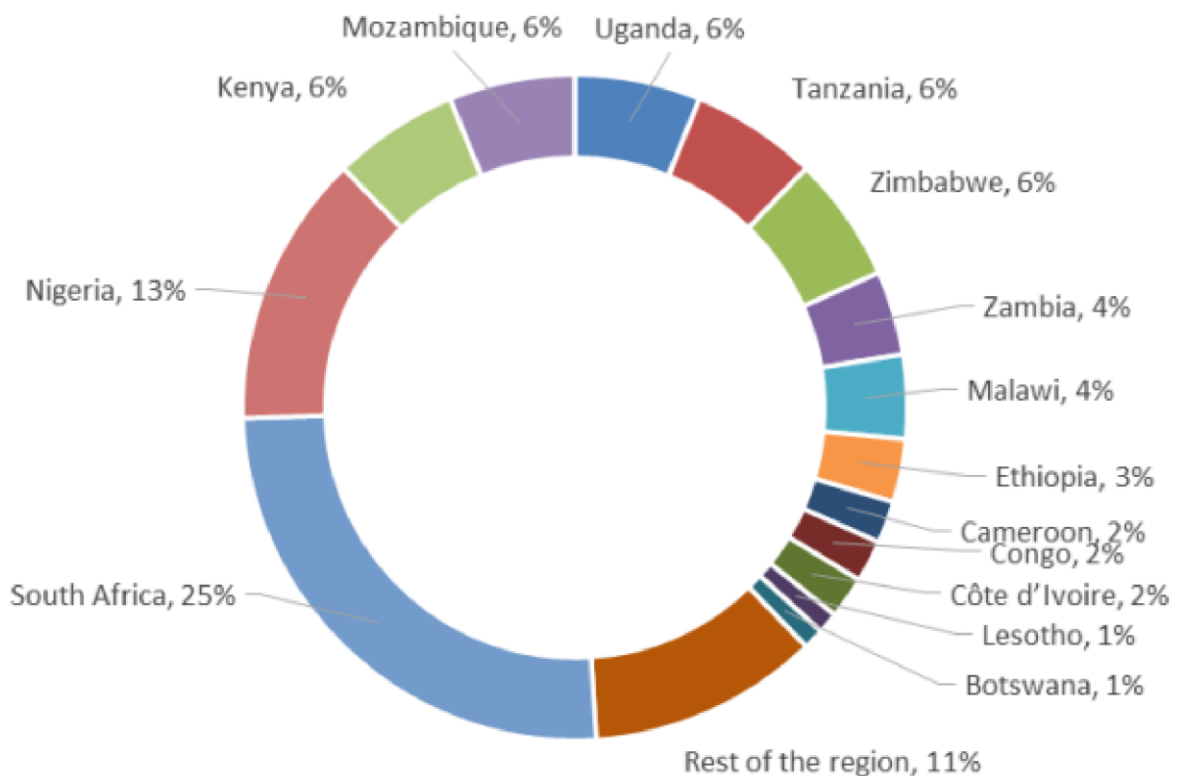
Literature Review

2.1 HIV/AIDS in Africa

There was an estimated 24.7 million [23.5-26.1 million] people living with HIV in the sub-Saharan Africa which is almost 71% of the global total at the end of 2013. Of the estimated 24.7 million, ten countries, namely; South Africa, Nigeria, Kenya, Ethiopia, Malawi, Mozambique, Uganda, Zambia and Zimbabwe. This ten countries account for about 81% of all people living with HIV in the continent and surprisingly, half of those are in South Africa and Nigeria only (UNAIDS, 2013).

Figure 2.1: People living with HIV in sub-Saharan Africa, 2013

Source: UNAIDS 2013 estimates.



2.2 HIV/AIDS in Ghana

In Ghana however, the first AIDS case was in 1986. This deadly disease once diagnosed in the country begins to spread steadily though slowly. Currently, an estimated 150,000 people are now infected with the virus in Ghana as of 2014. It is highest in the Eastern Region of Ghana, followed by the Ashanti Region and lowest in the Northern and Upper West regions of the country (Ghana AIDS Commission, 2013). This reduction can be attributed to the support pledged by the government to ending the HIV/AIDS epidemic in the country (UNAIDS, 2013).

Generally, prevalence is higher in urban areas, mining and border towns and along main transportation routes. According to the Ghana AIDS Commission, heterosexual intercourse is the main mode of transmission of HIV cases in Ghana. Again, the 2003 Demographic and Health Survey indicated that HIV prevalence is quite low among the younger age group whilst infections levels are highest in the middle income and middle educational groups. Specifically, the prevalence by age group is highest from 45 to 49 at 3.3% and lowest from 15 to 19 at 0.8% (Ghana AIDS Commission, 2013).

2.3 Cluster of Differentiation 4 (CD4)

CD4 + T lymphocyte count (CD4 count) is an important indicator of HIV progression to AIDS; because HIV destroys CD4+ T cells. It has been established that there is an inverse relationship between CD4 cell count and the degree of immune suppression (Akinbami et al., 2010). Therefore, CD4 cell count is vital in deciding when to start ART, staging, and monitoring HIV progression and determining clinical success of treatment. Even though, viral load is also a key indicator of HIV/AIDS, CD4 cell count is prioritized over it in situations where

both tests can be carried out due to financial constraints. CD4 cell count thus has become the most common use between the two especially in developing countries due to its affordability (Mellors et al., 1996).

2.4 HIV/AIDS, Nutritional Status and Immune System

The relationship between nutrition and HIV is a vicious cycle which is multifaceted and multidirectional. HIV compromises nutritional status, and poor nutrition further weakens the immune system, increasing susceptibility to opportunistic infections (CRHCS and SARA Project 2001). That is to say, the immune system can be affected by either malnutrition or HIV or both combined (Coetzee, 2013).

HIV infection gradually breaks down the immune system and subsequently leading to recurrent opportunistic infections and death. Jourdan, P. (2005) in their work "Nutrition: A Co-factor in HIV Infection/AIDS Progression" indicated that Malnutrition can contribute to impaired immune response, increase frequency and severity of infections which subsequently result in more rapid disease progression & shortened survival. They went on further to state that malnutrition can result in fatigue, loss of appetite, sense of taste and smell, and decreased quality of life. Figure 1 illustrates the vicious cycle of malnutrition and HIV pathogenesis. Malnutrition also consequently decrease tolerance to therapy and lessen medication efficacy.

According to Baum & Shor-Posner (1998), malnutrition may change the immune function to accelerate disease progression, influence viral expression, and play a

significant role in disease processes and related morbidity and mortality. The functional status and quality of life of PLWHA may also be influenced by malnutrition (Scrimshaw & SanGiovanni, 1997). According to Chandra (1997), nutrition is globally and generally now considered as a key determinant of immune functioning. Hattingh (2005) explained that Humoral immunity and cellular immunity are impaired by both malnutrition and by HIV infection. This is illustrated in Figure 2.2 and Figure 2.3.

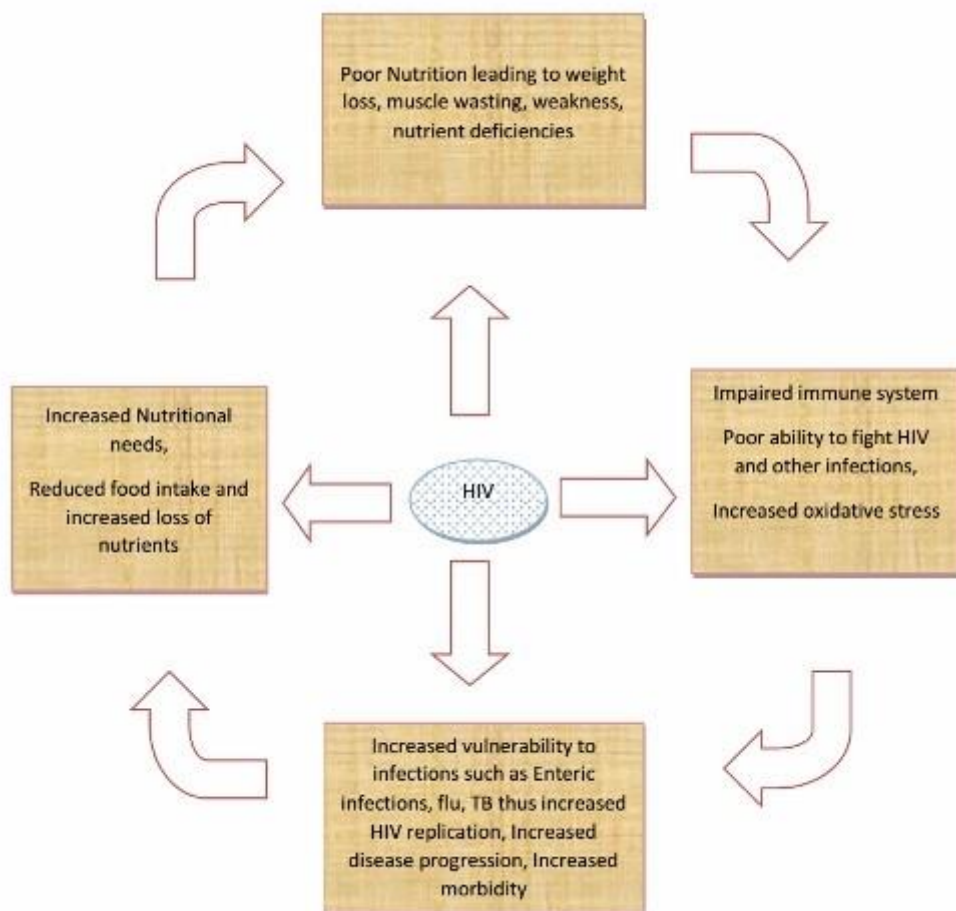


Figure 2.2: Malnutrition and HIV: A vicious cycle Source:
Adapted from RCQHC and FANTA 2003a.

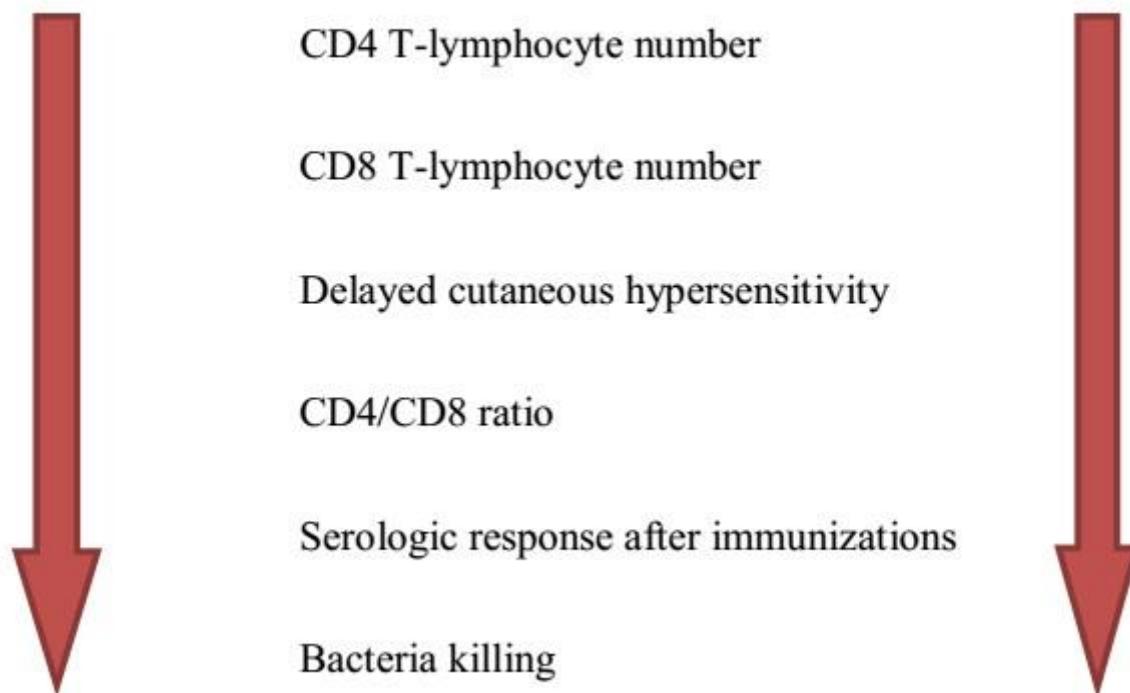


Figure 2.3: The synergistic effects of Malnutrition and HIV on the Immune System (FANTA, 2003)

Appropriate enhancement in nutritional status of PLWHA can help boost their immune system, manage the frequency and severity of symptoms and promote good responses to medical treatment. This therefore reduces the incidence of infections, prevent loss of weight and lean body mass, and delay disease progression (Tsehay, 2010). Nutritional care and support improves PLWHA's quality of life by maintaining strength, comfort, level of functioning, and human dignity (FANTA, 2004). A well-nourished person has a stronger immune system for coping with HIV and fighting illness. Figure 2.4 illustrates how effective nutrition interventions can help transform the vicious cycle of HIV and undernutrition into a positive relationship between improved nutritional status and stronger immune response.

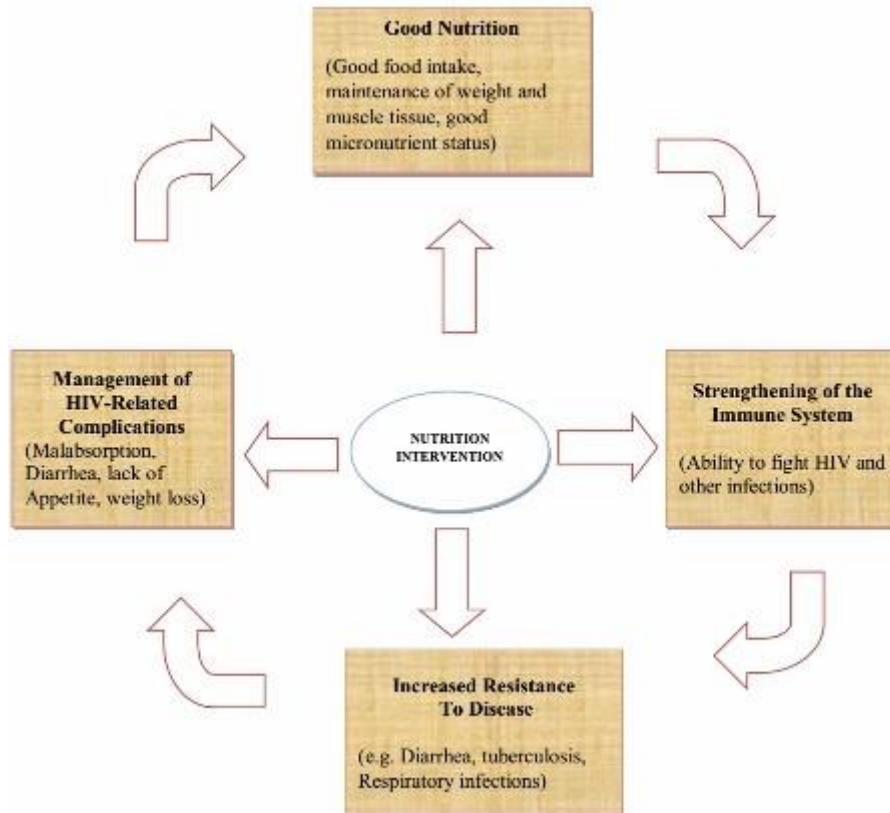


Figure 2.4: Nutrition and HIV: The cycle of benefits from nutrition interventions

Source: Adapted from RCQHC and FANTA 2003a.

2.5 Nutrient Requirements of PLWHA

Adequate nutrition, which is best achieved through consumption of a balanced healthy diet, is vital for health and survival for all individuals regardless of HIV status (WHO 2003). However, PLWHA may have different nutritional requirements than people without HIV/AIDS. Additionally, the nutrient requirements in PLWHA may be different depending on the stage of the infection. Nutrition counseling and care and support interventions therefore, as an urgent priority, needs to be given to PLWHA. This must vary according to nutritional status and the extent of disease progression.

2.5.1 Macronutrient Requirements of PLWHA

Energy

Energy requirements are likely to increase by 10% to maintain body weight and physical activity in asymptomatic HIV-infected adults. During symptomatic HIV, and subsequently during AIDS, energy requirements increase by approximately 20% to 30% to maintain adult body weight WHO (2003). According to Coetzee (2013), there seems to be a direct correlation between a patient's energy intake, weight and CD4 cell count. It is also believed that weight loss coupled with inadequate energy intake is directly associated with faster disease progression (ASSAf, 2007:132).

Protein

According to WHO (2003, 2005), there are insufficient data at present to support an increase in protein intake for PLWHA above normal requirements for health, that is, enough to make up 12% to 15% of total energy intake. Therefore, data are inadequate to support an increased protein requirement associated with HIV infection (Tomkins et al., 2005). WHO (2003) as a result recommends that PLWHA eat the same amount of fat as non-infected people. Nonetheless, in a study conducted by Shabret et al. (1999), it was found that increased protein supplements intake showed a significant body weight and cell mass gain in PLWHA.

Fat

Fat requirements for PLWHA are the same as for non-HIV-infected people. Nevertheless, special advice regarding fat intake might be required for individuals undergoing antiretroviral therapy or suffering from persistent diarrhea (WHO, 2003).

2.5.2 Micronutrient Requirements of PLWHA

The role of micronutrients in health still remains incontestable (WHO, 2003). Consequentially, intake of vitamins and minerals in PLWHA is greatly significant due to their critical roles in cellular differentiation, enzymatic processes, immune system reactions, and other body functions (Piwoz and Preble, 2000). The immune system requires a lot of micronutrients to combat infectious pathogens. As a result, people with insufficient intake of micronutrients have difficulty in resisting infections.

Some observational studies conducted indicate that inadequate intake of micronutrients has been associated with quicker oxidative stress, subsequent acceleration of HIV replication and CD4+ T-cell depletion and mortality (Drain et al., 2007; Semba, 2006; WHO, 2003). Micronutrient deficiencies mostly happens in advanced HIV disease (Tomkins, 2005) which is mostly caused by malabsorption, diarrhea, altered metabolism and insufficient dietary consumption (Semba & Tang, 1999).

Micronutrient deficiency has been associated with further immunopression, oxidative stress, subsequent acceleration of HIV replication and CD4+ T-cell depletion. It has been suggested that PLWHA should use multivitamin-mineral supplement which provides a 100% of the recommended daily allowance (RDA) to prevent faster disease progression and hence reducing the risk of death (ASSAf, 2007, p.144). It must be noted that multivitamin supplementation should not be taken as an alternative to HAART especially in developing countries but as a complementary intervention exercise (Fawzi et al. 2005). WHO (2003) pointed out that inadequate intake of vitamin A, B1, B2, B6, B12, and E coupled with iron, folic acid and zinc can damage host resistance and lymphocyte function.

However, Vitamin A and the minerals iron and zinc can affect negatively the general outcome of PLWHA if taken in large amounts-the recommended amounts (WHO, 2003). Again, large dosages of antioxidants reduces oxidative stress which thus can cause a decrease in CD4 counts and an increase of viral replication in PLWHA (Arendt et al., 2008; Drain et al., 2007; Faintuch et al., 2006). Hence large amounts of micronutrients are not recommended (Coetzee, 2013). Main dietary sources of vitamins and minerals are liver, kidney, lentils, beans, cereals, egg, some fishes, yellow fleshed sweet potato, pumpkin seeds, palm oil, carrot, dark green leafy vegetables, fruits, such as papaya and mango, poultry, shell fish and cheese (FANTA, 2004).

2.6 Body Composition Changes in PLWHA

2.6.1 Changes in Body Weight

Weight loss, commonly referred to as "slim disease" in some African countries (Marston and De Cock, 2004), has been identified as a substantial prognostic factor in HIV infector since the beginning of the deadly epidemic (Kotler et al., 1989). This slim disease appears to be a prominent characteristics of PLWHA in most developing countries especially in Africa where food insecurity is quite high (Piwoz and Preble, 2000). According to Ockenga et al. (2006), weight loss may occur at all stages of the HIV infection. Ockenga et al. (2006) further explained that malnutrition continues to be a major cause of weight loss in PLWHA. Loss in body weight is a major problem in PLWHA since it is closely associated with poor health outcomes and early morbidity (Marston and De Cock, 2004; Fenton & Silverman, 2004:1044).

Because of the high association of weight loss and wasting with mortality, early detection and treatment of weight loss and wasting is very critical to ensuring

longer survival and better quality of life of PLWHA (Wanke et al., 2002). Increase dietary intake may have a positive effect on the weight gain for PLWHA starting antiretroviral therapy. Especially, Olsen et al. (2014) found that the provision of lipid based supplements in the first three months of ART will largely improve gains in body weight. It was also found by Shikuma (2004) that PLWHA usually begin to gain weight when they are put on HAART.

2.6.2 Changes in Body cell mass

Total mass of all the cellular elements in the body constitute metabolically active tissue. Measure of BCM is found to be predictor of survival among PLWHA (Tsehaye, 2010). According to Zaneta et al. (2003), BCM is seen as one of the most vital parameters in assessing body composition because it is generally associated with survival and is primarily made up of muscles and organs which process nutrients and medications. Many studies have indicated that the body cell mass of PLWHA increased after they were exposed to HAART. Also, it was pointed out that the gaining of BCM increases with increase effectiveness of the antiretroviral treatment (Tsehaye, 2010). Shabret et al. (1999) also found out that PLWA tend to increase in body cell mass when they consume food that contain high protein.

2.6.3 Changes in Lean Body Mass

Loss in lean body mass (LBM) is a common problem in PLWHA and is associated with mortality (Melchior et al., 1999; Schwenk et al., 2000). In addition to mortality, Raso et al. (2013) and Kusko et al. (2012) found that low lean body mass can generate into functional limitations devastating outcomes for patients and their families. Tsehaye (2010) concluded that antiretroviral treatment leads to an increase in overall weight and lean body mass in the first ten weeks of treatment. Even though not yet confirmed by empirical research, Agin et al. (2000,

2001) suggested that whey protein can improve the recovery of lean body mass in PLWHA.

2.6.4 Changes in Body Fat

In the absence of adequate energy intake, body fat and protein are used as fuel sources when this happens, body fat loss occurs (Hsu et al., 2005). Hsu et al. (2005) further indicated that body fat oxidation increases in PLWA. It must be noted that the production of hormones that are responsible for the metabolism macronutrients like fat is affected by HIV infection (Piwoz and Preble, 2000). Fat maldistribution and Lipodystrophy Syndrome which is characterized as body fat abnormality have also been associated with antiretroviral therapy in HIV infected persons (George et al., 2009; Pujari et al., 2005). According to Hansen et al. (2004) and Martin et al. (2004), avoiding antiretroviral drugs that have strong metabolic adverse effects may help prevent partially reverse lipodystrophy since medical treatment of lipodystrophy seems not to be effective (Carr et al., 2004).

According to Ockenga et al. (2006), predominant muscle mass depletion-wasting and peripheral fat loss-lipoatrophy should be distinguished by changes in body shape and muscular function.

They also noted that wasting and lipoatrophy may be combined in patients failing on long-term antiretroviral treatment.

2.7 Modeling Longitudinal Data

Longitudinal data sets are made up of an outcome variable y_{it} and a $q \times 1$ vector of covariates, x_{it} , observed at times $t = 1, 2, \dots, n_i$ for subjects $i = 1, 2, \dots, m$. A generalized linear model can be used in the analysis of longitudinal data with a single observation for each subject ($n_i = 1$) (McCullagh and Nelder, 1983).

However, with repeated observation, the correlation among values for a given subject must be taken into consideration. An assumption of the standard Generalized Linear Model is that observation are uncorrelated. Statistical researches thus have come up with a wide range of more accurate approaches to the analysis of longitudinal data. One of the most widely used among the approaches is the Generalized Estimating Equations (GEE) models (Zeger & Liang, 1986).

2.7.1 Generalized Estimating Equation (GEE) Models

The Generalized Estimating Equation model is a major statistical method used in the analysis of longitudinal data especially in clinical studies (Diggle et al., 2002). GEE indicates how the average response variable of a subject changes with covariates while allowing for the correlation between repeated measurements on the same subject over time. Essentially, Generalized Estimating Equation models extend generalized linear models to the case of correlated data.

Hence, GEE models have become a popular statistical tool in longitudinal analysis of data. They are referred to as marginal models and are used to model the regression of y on x and also model within-subject dependency separately. GEE models have the form;

$E(y_{it}) = X_{it}\beta$, thus yielding linear regression, random-effects regression, or other regression-related models, depending on what we assume for the correlation structure (StataCorp, 2013).

To fit a Generalized Estimating Equation (GEE) model, one needs to specify the link function to be used, the distribution of the dependent variable and a working correlation structure for the repeated measurements of the dependent variable.

The link transformation function models the expected value of the marginal responses for the population as a linear combination of the covariates. Hence the link function expresses the dependent variable as a vector of the parameter estimates in an additive model form (McCullagh & Nelder, 1989). The identity link is the basic link function; used for normally distributed data. It requires no transformation of μ before the matrix of the covariates are constructed.

The response variable's distribution must also be specified. Normally, a Generalized Estimating Equations allow the user to specify from the exponential family of distributions of which are the normal, binomial, and the poisson distributions. Liang and Zeger (1986) stated that for the parameter estimates to have a sampling distribution that is approximately normal, the specification of the variance function does not need to be exactly stated. Generalized Estimating Equation (GEE) takes correlation for within subject correlations into account by specifying a working correlation structure for the repeated measurements. The specification of the correlation structure depends on the nature of the data collected. The correlation structure can be Independent (naive analysis), Exchangeable (compound symmetry, as in rANOVA), Autoregressive, Stationary M-dependent or Unstructured (no specification, as in rMANOVA). Pan (2001) noted that the goal of choosing a working correlation structure is to efficiently estimate the parameter estimates (β_s).

The independent correlation structure assumes that correlation between time points is independent and has the form;

$$\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

Exchangeable correlation structure is used when the within subject observations are equally correlated. Horton & Lipsitz (1999) noted that an exchangeable correlation matrix should be used in studies where there is no logical ordering for observations within a cluster (such as when data are clustered within subject but not necessarily collected over time). The exchangeable correlation structure has the form;

$$\begin{bmatrix} 1 & \rho & \rho & \rho & \rho \\ \rho & 1 & \rho & \rho & \rho \\ \rho & \rho & 1 & \rho & \rho \\ \rho & \rho & \rho & 1 & \rho \\ \rho & \rho & \rho & \rho & 1 \end{bmatrix}$$

Autoregressive correlation structure is assumed when repeated measures are mostly strongly correlated when close together in time and least correlated when furthest apart in time.

$$\begin{bmatrix} 1 & \rho_2 & \rho_3 & \rho_4 & \rho_5 \\ \rho_2 & 1 & \rho_3 & \rho_4 & \rho_5 \\ \rho_3 & \rho_2 & 1 & \rho_4 & \rho_5 \\ \rho_4 & \rho_3 & \rho_2 & 1 & \rho_5 \\ \rho_5 & \rho_4 & \rho_3 & \rho_2 & 1 \end{bmatrix}$$

is the form of an autoregressive correlation structure (AR1).

The unstructured structure is however used when no constraints are placed on correlations. It has the form;

$$\begin{bmatrix} 1 & \rho_{12} & \rho_{13} & \rho_{14} & \rho_{15} \\ \rho_{21} & 1 & \rho_{23} & \rho_{24} & \rho_{25} \\ \rho_{31} & \rho_{32} & 1 & \rho_{34} & \rho_{35} \\ \rho_{41} & \rho_{42} & \rho_{43} & 1 & \rho_{45} \\ \rho_{51} & \rho_{52} & \rho_{53} & \rho_{54} & 1 \end{bmatrix}$$

stated even if the correlation is not specified correctly, GEE models are robust to the misspecification of the correlations structure (Liang & Zeger, 1986). With the

estimated working correlation matrix R (of the n repeated measures) and the diagonal matrices A_i ($n \times n$ diagonal matrix with $V(\mu_{ij})$ as the j th diagonal element), the GEE have the form;

$$\mu(\beta) = \sum_{i=1}^n D'_i [V(\hat{\alpha})]^{-1} (y_i - \mu_i)$$

$$D_i = \frac{\delta \mu_i}{\delta \beta}.$$

) = 0; where $\hat{\alpha}$ is a consistent estimate of α and

Generalized Estimating Equation models are based on the quasiliikelihood theory and not maximum likelihood as in Generalized Linear Models (McCullagh and Nelder 1989). Hence, some statistics are not valid under GEE. For instance, even though the likelihood ratio test may be more trustworthy than the Wald test because of its better statistical properties, it is not available and hence the Wald test is rather used. Also, Akaike's information criterion (AIC; Akaike 1974), a widely used method for model selection in GLM, is not applicable to GEE.

GEE assumption regarding missing data is that missing data are missing completely at random (MCAR) as compared with MAR of the full-likelihood estimation models. GEE also has the following assumptions; 1. the dependent variable is linearly related to the predictors 2. Errors are correlated 3. The responses are $Y_1, Y_2, \dots, Y_n$ are correlated or clustered. A variation of GEE known as GEE2 has been introduced (Prentise, 1988 ; Zhao and Prentise, 1990) that demands that the mean model and the correlation structure be specified correctly. This variation has not gain much efficiency as was the reason behind its introduction

(Liang et al., 1992).

2.8 Nutritional Support and Intervention in

HIV/AIDS

The role of nutrition in the treatment of HIV/AIDS infection is widely known and cannot be overemphasized. Nutritional intervention must be started once HIV is

diagnosed (Babameto & Kotler, 1997), and should include nutrition counseling. Optimal nutrition no doubt helps boost immune function and maximize the effectiveness of antiretroviral therapy (Obi et al., 2010). Argemi et al. (2012) also buttress the point that nutritional cares are essential for the clinical success of HIV programs started in developing countries. Ockenga et al. (2006) stated that nutritional intervention aims to:

1. improve nutritional status
2. decrease functional impairment from malnutrition (muscular fatigue, bedridden state)
3. improve tolerance to antiretroviral treatment
4. alleviate gastrointestinal symptoms of HIV illness (nausea, diarrhea, bloating),
5. Improve quality of life of the patients and their families.

Dong and Imai (2012) further explained that the aim of nutrition intervention should not only focus on improving the nutritional status of the patient but must only focus on improving the nutritional knowledge of the patient, thereby enhancing their sense of empowerment. Hu et al. (2011) suggested that Nutrition evaluation and support should be considered an integral parts of national and community HIV/AIDS treatment and care guidelines.

2.8.1 Nutritional Support and Intervention Program in Ghana

Among the few donor organizations in the country who provide nutritional support PLWHA is the Opportunities Industrialization Centres International (OICI). Part of OIC Ghana's program is to provide nutritional support in the form of soya fortified wheat, sorghum, oil etc for orphanages and people living with HIV/AIDS (www.oici.org). Key among the institutions that benefited from this

nutritional support were; Bomso Clinic, SDA Hospital, Kwadaso, Bekwai Government Hospital, Adom HIV/Management Association, Step by Step Orphanage Centre and Liberty Foundation. Some PLWHAs who were put on the OICI nutritional intervention however were later withdrawn (Amponsah, 2013).

KNUST



Chapter 3

Methodology

3.1 Introduction

This chapter looks at a description of the research design, research tools and procedure used and ethical consideration. The research methodology describes the procedures used to achieve the study objectives.

3.2 Study Method and Design

The study was carried out using data from a retrospective cohort study in order to examine the withdrawal effects of the nutritional intervention on CD4+ cell counts and model the CD4+ cell counts before, during and after the initiation of the intervention, given several independent variables of PLWHA who started ART from 2007 to 2011. ART was defined as HAART or non-HAART.

The nutritional status of selected PLWHA was defined by both body weight and body mass index (BMI). BMI was grouped into four (4) according to established criteria: less than 17kg/m^2 (moderate to severe malnutrition), 17 to 18.5kg/m^2 (mild malnutrition), less than or equal to 18.5 to 25kg/m^2 (normal nutrition) and greater than 25kg/m^2 (overweight and obese) (Ferro-Luzzi et al., 1992).

Measurements of body weight and height were carried out and variables (demographic and clinical) were taken from existing database. Body Mass Index (BMI) was determined and was calculated as the weight (measured in kilograms) divided by the square of the height (measured in metres).

Analyses were conducted using STATA, version 12.1 (StataCorp LP, College Station, TX) SAS (release 9.4), with a significance level of $\alpha = 0.05$. Descriptive

statistics of continuous variables was carried out to indicate the minimum and maximum CD4+ cell counts and the distribution of the HIV-Positive factors. The correction matrix was used to examine the correlation between the repeated measurements and important relationships determined.

Generalized Estimation Equations (GEE) model the CD4+ cell counts before, during and after the initiation of the intervention, given BMI and HB levels of PLWHA.

Generalized Estimating Equations

Liang and Zeger (1986) and Zeger & Liang (1986) extended the generalized linear model to allow for correlated observations since there exist correlations between observations on a given subject. This extension is referred to as Generalized Estimating Equations (GEE) model. The GEE model makes use of the Quasilikelihood which specifies only the first two moments, mean (μ) and variance $v(\mu)$. The Quasi-likelihood also specifies a link function $g(\mu)$ which links the mean to a linear predictor.

Generalized Estimation Equations Approach

Univariate Case

Let Y_i be the outcome on the subject Y with $\mu_i = E(Y_i)$ and a variance function $V(\mu_i)$ where $i = 1, 2, \dots, n$. Let x_{ij} be the value of the explanatory variable j . Given a link function g ,

$\eta_i = g(\mu_i) = \sum_i \beta_j x_{ij} = x_i' \beta$ is the linear predictor. Then quasi-likelihood pa-

rameter estimates ($\hat{\beta}$) are the solutions of the quasi- score equations:

$$\mu(\beta) = \sum_i \frac{\delta \mu_i}{\delta \beta} V(\mu_i)^{-1} (y_i - \mu_i) = 0, \text{ where } \mu_i = g^{-1}(x_i' \beta). \text{ Where } \frac{\delta \mu_i}{\delta \beta_j} = \frac{\delta \mu_i}{\delta \eta_i} \frac{\delta \eta_i}{\delta \beta_j} = \frac{\delta \mu_i}{\eta_j} x_{ij} \text{ and } y_i \text{ has a natural exponential family distribution.}$$

Longitudinal Case

Again, let y_i be the outcome on the subject Y with $y_i = (y_{i1}, y_{i2}, \dots, y_{iT_i})^0$ and $\mu_i = (\mu_{i1}, \mu_{i2}, \dots, \mu_{iT_i})^0$, $\mu_{it} = E(Y_{it})$ and a variance function of Y ; $v(\mu_i)$.

Let $A_i = n \times n$ be an diagonal matrix with $V(\mu_{ij})$ as the j th diagonal element.

Define $R_i(\alpha) = n \times n$ working correlation matrix of the n repeated measures. The working variance-covariance matrix for y_i equals

$V(\alpha) = \phi A_i^{1/2} R_i(\alpha) A_i^{1/2}$, where $V(\alpha) = \phi R_i(\alpha)$ for normally distributed outcomes.

The GEE estimator of β is the solution of $\mu(\beta) = \sum_{i=1}^n D_i' [V(\hat{\alpha})]^{-1} (y_i - \mu_i) = 0$;

where $\hat{\alpha}$ is a consistent estimate of α and $D_i = \frac{\delta \mu_i}{\delta \beta}$. In the normal case,

$\mu_i = x_i \beta$, $D_i = x_i$, $V(\hat{\alpha}) = \phi R_i(\hat{\alpha})$, $(\beta) = \sum_{i=1}^N X_i' [R_i(\hat{\alpha})]^{-1} (y_i - X_i \beta) = 0$;

and

$$\hat{\beta} = \left[\sum_{i=1}^N X_i' (R_i(\hat{\alpha}))^{-1} X_i \right]^{-1} \left[\sum_{i=1}^N X_i' (R_i(\hat{\alpha}))^{-1} y_i \right].$$

The estimate of β are calculated iteratively by the reweighted Least-Squares, given $R_i(\alpha)$ and ϕ . The estimates calculated are quasi-likelihood because the solution depends on the mean and variance of y . The estimates of β and the Pearson residuals are used to consistently estimate α and ϕ . The Pearson residuals are calculated by;

$$r_{ij} = \frac{(y_{ij} - \hat{\mu}_{ij})}{\sqrt{V[\hat{\alpha}]_{ij}}}$$

The square root of the diagonal elements yield the standard errors of β ; $V(\beta)$.

There are two types of in GEE;

Naive or Model-based Estimator

$$V(\hat{\beta}) = \sum_1^N \left[D_i' \hat{V}_i^{-1} D_i \right]^{-1}.$$

Sandwich or Empirical or Robust Estimator

$$V(\hat{\beta}) = M_0^{-1} M_i M_0^{-1}$$

where $M_0 = \sum_1^N D_i' \hat{V}_i^{-1} D_i$ and $M_i = \sum_1^N D_i' \hat{V}_i^{-1} (y_i - \hat{\mu}_i) (y_i - \hat{\mu}_i)' \hat{V}_i^{-1} D_i$

The model-based and empirical estimates becomes equal when $V = (y_i - \hat{\mu}_i)(y_i - \hat{\mu}_i)'$. This is achieved when a correct working correlation structure is specified.

The empirical estimator gives a consistent estimator of $V(\hat{\beta})$ even if the working correlation structure is wrongly specified.

The following are the major correlation structure that can be specified:

Independent correlation structure assumes that correlation between time points

$$\text{is independent. } R_{uv} = 1 \text{ if } u=v, \text{ otherwise } = 0.$$

$$\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

Exchangeable correlation structure is specified when the within subject observa-

tions are equally correlated. $R_{uv} = 1$ if $u=v$, otherwise =

$$\begin{bmatrix} 1 & \rho & \rho \\ \rho & 1 & \rho \\ \rho & \rho & 1 \end{bmatrix}$$

Autoregressive correlation structure is specified when repeated measures are mostly strongly correlated when close together in time and least correlated when furthest

$$\text{apart in time. } R_{uv} = 1 \text{ if } u=v, \text{ otherwise } = \rho^{|u-v|}.$$

$$\begin{bmatrix} 1 & \rho & \rho^2 \\ \rho & 1 & \rho \\ \rho^2 & \rho & 1 \end{bmatrix}$$

Unstructured structure is specified when no constraints are placed on correla-

$$tions. R_{uv} = 1 \text{ if } u=v, \text{ otherwise } = \begin{bmatrix} 1 & \rho_{12} & \rho_{13} \\ \rho_{21} & 1 & \rho_{23} \\ \rho_{31} & \rho_{32} & 1 \end{bmatrix}$$

GEE Model Parameters (Definitions)

Regression Parameters (β): The regression parameters express the relationship between the covariates and the outcome variable. For the analysis of this study, only the regression parameters are of primary interest.

Correlation Parameters (α): They express the within-cluster correlations of the response variable. This is achieved by the specification of a correlation structure. These parameters are considered as nuisance parameters and are not of primary interest even though they add to the accuracy of the model

Scale Factor (φ): this parameter accounts for the extra variation of the response variable Y. the variation can be an underdispersion or overdispersion. It assumes 1 or estimated from the data.

The GEE Model

The Generalized Estimating Equations model for this study is given by;

$$Y_{ij} = \beta_0 + \beta_1 G_1 + \beta_2 G_2 + \beta_3 G_3 + \beta_4 age_i + \beta_5 BMI * t_{ij} + \beta_6 HB * t_{ij} + \epsilon_{ij}$$

$$i=1,2,...,n, j=1,2,3 \text{ and } \epsilon_{ij} \sim (0, I_n \sigma^2)$$

$$E(Y_{ij}) = \beta_0 + \beta_1 G_1 + \beta_2 G_2 + \beta_3 G_3 + \beta_4 age_i + \beta_5 BMI * t_{ij} + \beta_6 HB * t_{ij}$$

$Y_{ij} = \beta_0 + \beta_1 G_1 + \beta_2 G_2 + \beta_3 G_3 + \beta_4 age_i + \beta_5 BMI * t_{ij} + \beta_6 HB * t_{ij}$ Where G1, G2 and G3 are Group 1 (before intervention), Group 2 (during intervention) and Group 3 (after intervention) respectively.

Statistical Tests in GEE Model

The likelihood ratio test cannot be used since GEE models are based on quasiliikelihood theory and not the maximum likelihood. The score test which is

based on the generalized estimating score - like equations is one of the tests that can be used.

Let $L(\beta)$ be the likelihood function and $l(\beta) = \log[L(\beta)]$ be the log-likelihood. Then the score function needs to be calculated in order to find the maximum likelihood estimates (MLEs). The score function, $U(\beta)$, is the derivative of the loglikelihood with respect to β .

$$U(\beta) = \frac{\delta}{\delta\beta} \log L(\beta) \text{ where;}$$

$$\begin{aligned} E_x[U(\beta)] &= \int \frac{\delta}{\delta\beta} \log L(\beta) f(x/\beta) \delta x = \int \left\{ \int \log f(x/\beta) \right\} f(x/\beta) \delta x \\ &= \int \frac{\{\delta f(x/\beta) \delta\beta\} f(x/\beta) \delta x}{f(x/\beta)} = \int \frac{f(x/\beta) \delta x}{\delta\beta} dx \\ &= \frac{\delta}{\delta\beta} \int f(x/\beta) \delta x = 0 \end{aligned}$$

$$V_x(U(\beta)) = E_x[U(\beta)^2] = E_x \left[\left(\frac{\delta}{\delta\beta} \log L(\beta) \right)^2 \right] = I_n(\beta) \text{). Where } I_n \text{ is the usual expected Fisher information.}$$

To test $H_0: \beta = \beta_0$ vs $H_1: \beta \neq \beta_0$; then the Score test, $S(\beta)$ is

$S(\beta) = \frac{[U(\beta_0)]^2}{I_n(\beta_0)}$. When the null hypothesis is true, $S(\beta_0) \rightarrow \chi^2$, with $df = 1$. The square root of the score statistic makes it a standard normal distribution. The Wald test is the most commonly test of significance of an individual predictor used since parameters estimates for GEE models are asymptotically normal. It can be used to test $H_0: \beta = \beta_0$ vs $H_1: \beta \neq \beta_0$ and can be calculated by;

$W = \frac{[\hat{\beta} - \beta_0]^2}{1/I_n(\hat{\beta})} = I_n(\hat{\beta})[\hat{\beta} - \beta_0]^2$ where $\hat{\beta}$ is the MLE. When the null hypothesis is true, $W \rightarrow \chi^2$, with $df = 1$. Similarly, the wald-like confidence interval can be calculated by;

$$\hat{\beta} \pm Z * \sqrt{1/I(\beta)}$$

Pearson Chi-Square Goodness of fit Test

For a generalized estimation equations model with y_i as the responses, weights w_i , fitted means $\hat{\mu}_i$, variance function $v(\mu_i)$ and dispersion ϕ , the Pearson goodness

of fit statistic is;

$$\chi^2 = \sum \frac{w_i (y_i - \hat{\mu}_i)^2}{v(\mu)} \quad (\text{McCullagh \& Nelder, 1989}).$$

If the model is fitted correctly, $\chi^2 \sim \chi^2$ with the model's residuals degrees of freedom.

3.3 Variables

CD4 cell count (cells/ μ L) with repeated measurements; before, during and after the food supplement intervention, linear time effect of BMI, linear time effect of HB level and age at the start of the study are other variables.

3.3.1 Response Variable

CD4+ cell count (cells/ μ L) is the outcome or response variable in this study.

3.3.2 Covariates

The covariates for this study are listed below:

- I Age in years (at start of ART)
- II Hb*time (Linear interaction between Hb and time)
- III BMI*time (Linear interaction between BMI and time)
- IV G1
- V G2
- VI G3

3.4 Study Area and Population of Interest

The Chronic Care Centre of the SDA Hospital Kwadaso has about five hundred registered HIV/AIDS patients as at January 2011. Some services offered by the S.D.A hospital are general In and Out-patient department, family planning, surgery, Ante-Natal services, and ART services.

The HIV/AIDS centre of the Bomso Clinic has about fifty HIV/AIDS patients. Some of the services rendered by the hospital are general medical care, surgical procedures, paediatrics, Out-Patients Care, In-Patients Cares and the Treatment of HIV/AIDS - (VCT, PMTCT, ART).

3.4.1 Study Population, Sample Size and Sampling Procedure

The study population consisted of HIV-Positive patients above 18years who had initiated ART between 2007 and 2011 and were receiving health care at the S.D.A hospital's Chronic Care Centre, Kwadaso, and the Bomso Clinic, Bomso. A sample size of 501 was selected for the study which includes 490 PLWHA, and 11 health workers (8 counsellors, and 3 Nutrition Officer).

3.4.2 Limitations

The following limitations were experienced during the study.

1. Accuracy of the secondary data used for this study is probable to have been affected because of limited and poor documentation in most of the facilities.
2. All respondents were assumed to be HIV/AIDS patients since information regarding their actual status (HIV or AIDS) was not given.

Chapter 4

Data collection, Analysis and Results

4.1 Introduction

Results of the study are described and the analyses of the data are presented. Analyses were conducted using STATA, version 12.1 (StataCorp LP, College Station, TX) and SAS (release 9.4) with a significance level of $\alpha = 0.05$. The results are presented in tables and graphs.

4.2 Characteristics of the Study Population

The study population's characteristics (n=501) are described below. From Table 4.1, the mean age was 35 years and the minimum age was 20 years. The maximum age was found to be 61 years. Thirty-five (35) years was the most frequent age of the respondents.

Table 4.1: Age and CD4+ Cell Count Characteristics of PLWHA

Variable	Obs	Mean	Std. Dev	Min	Max
Age	501	35.325	8.077	20	61
CD4 Counts Before Intervention (cells/ μ L)	501	223.190	156.476	1	1068
During Intervention (cells/ μ L)	501	367.443	154.423	10	1391
After Intervention (cells/ μ L)	501	356.946	139.667	70	1300

A total of 501 patients were observed and their CD4+ cell counts measured from the start of ART, during the food supplement intervention and after patients were taken off the food supplement intervention. The mean CD4+ cell counts over each time period is as indicated in Table 4.1. The minimum CD4+ cell counts before the food supplement intervention is 1 (cells/ μ L) whilst and the maximum CD4+ cell count was 1068 cells/mm³. Ignoring the variations in CD4+ counts, the average CD4+ count of PLWHA increased from 223.1 (cells/ μ L) before the intervention to 367.4 (cells/ μ L) during the intervention and decreased substantially to 356.9 (cells/ μ L) after the intervention. The minimum CD4 +cell count now rose from to

1 (cells/ μ L) to 10 (cells/ μ L) and finally to 70 (cells/ μ L) and the maximum CD4+ cell count also increased from 1068 (cells/ μ L) to 1391 (cells/ μ L) and to 1300 (cells/ μ L) after the food supplement intervention. These results indicate that the food supplementation positively affected the CD4+ cell counts of respondents that were put on it and each one had their CD4+ cell count increased during the intervention. The correlation structure is illustrated in Table 4.2.

Table 4.2: Correlation matrix on Repeated CD4+ cell counts

	CD4B	CD4D	CD4A
CD4B	1		
CD4D	0.8068	1	
CD4A	0.7451	0.8424	1

Correlation between CD4+ cell count before and during was highly significant and the CD4+ cell counts have a correlation that is very high for observations close together in time. The correlation however tends to reduce with increasing time separation between the CD4 counts. That is to say that, there is a correlation between CD4+ cell counts but this correlation weakens with distance between measurements. Hence, although an HIV-infected patient's CD4+ cell count depended on his/her past CD4+ cell count, the strength of the relationship was much stronger with immediate CD4+ cell count and vice versa.

Table 4.3: BMI Characteristics of HIV-Infected Patients

Variable	Obs	Mean	Std. Dev	Min	Max
BMIB	501	18.948	2.533	.000048	26.5625
BMID	501	23.62703	2.456781	14.67876	31.99217
BMIA	501	20.49984	2.7808777	.000048	29.6875

The mean BMI of respondents was 18.9 kg/m^2 before the intervention but it increased to 23.6 kg/m^2 during the intervention and reduced to 20.5 kg/m^2 after the withdrawal of the intervention. This means that the nutritional status of respondent improved significantly during the food supplementation. The nutritional status of the PLWHA that were put on the intervention however

decreased by a mean BMI of 3.13 kg/m^2 . Descriptive Statistics on nutritional status; defined as

BMI (Categories) are displayed in the tables below.

Table 4.4: BMI before intervention

	Frequency	Percent	Cum. Freq.
overweight and obese	2	0.40	0.40
normal nutrition	263	52.50	52.89
mild malnutrition	197	39.32	92.22
moderate to severe malnutrition	39	7.78	100

Table 4.5: BMI During Intervention

	Frequency	Percent	Cum. Freq.
overweight and obese	153	30.54	30.54
normal nutrition	334	66.67	97.21
mild malnutrition	6	1.20	98.40
moderate to severe malnutrition	8	1.60	100

Table 4.6: BMI After Intervention

	Frequency	Percent	Cum. Freq.
overweight and obese	15	2.99	2.99
normal nutrition	412	82.24	85.23
mild malnutrition	58	11.58	96.81
moderate to severe malnutrition	16	3.19	100

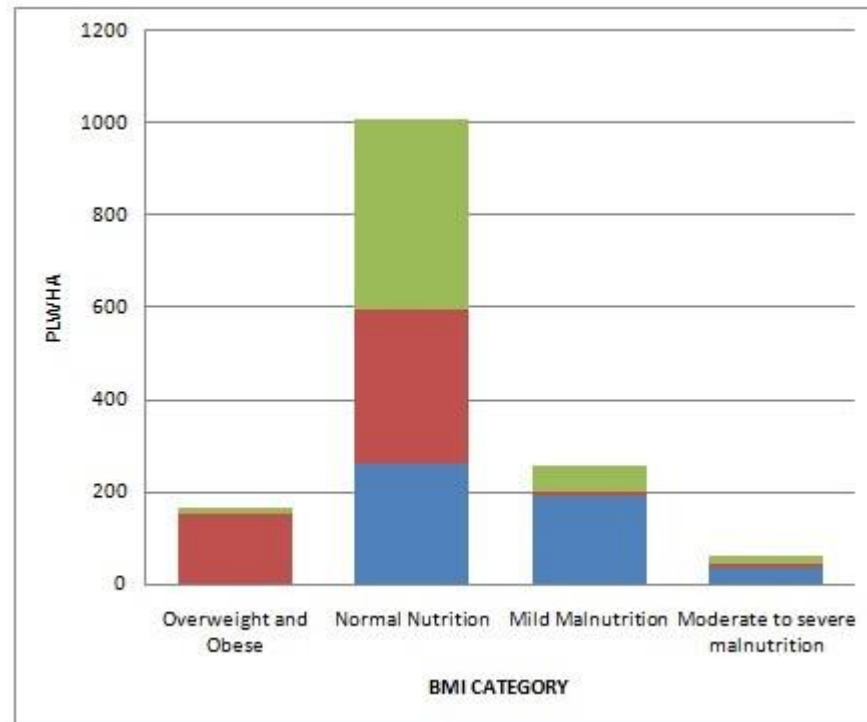


Figure 4.1: A stacked graph of BMI categories over the periods.

Before the intervention about half of the respondents (47%) were having mild to severe malnutrition as indicated in Table 4.4. However, only 3% of the PLWHA were having mild to severe malnutrition during the intervention (Table 4.5). This implies that there was a reduction of 44% of PLWHA who were having mild to severe malnutrition during the food supplementation. The 3% of PLWHA mild to severe malnutrition during the intervention increased to 14% after the PLWHA were withdrawn from the food supplement intervention. Majority (82%) of the patients tends to have a normal nutrition after the food supplement intervention as compared to 67% of the patients during the intervention and 53% of the patients before the intervention (Figure 4.1).

Table 4.7: HB Characteristics of HIV-Infected Patients

HB (g/dl)	Mean	Std. Err	Min	Max
Before	9.577	1.630	3.3	16
During	10.857	1.458	7	17.9
After	10.405	1.278	7	17.0

The mean hemoglobin level of respondents increased during the intervention is shown in Table 4.7. The mean HB level of respondent increased significantly from

9.58 g/dl before the food intervention to 10.86 g/dl during the food supplementation. However, there was a slight decreased in the average hemoglobin level of respondent after the intervention.

Paired t-test

The paired t-test was conducted to confirm the results in Table 4.1; that there was a change in the mean CD4 cell count before and after the intervention and during and after the intervention. The paired t-test was used since we have two related observations per subject (before the intervention and after the intervention) and (during the intervention and after the intervention)

$$H_0 : \mu_{before} = \mu_{after}$$

$$H_a : \mu_{before} \neq \mu_{after}$$

Table 4.8: Paired t-test for CD4 count Before and CD4 count After Intervention

Variable	Obs	Mean	Std. Dev	Std. Err	[95% conf. Interval]	
CD4A	501	356.946	139.667	6.239	344.6866	369.2056
CD4B	501	223.190	156.476	6.9908	209.4445	236.9246
diff	501	133.7565	4.775206	106.8836	124.3745	143.1384
mean(diff)=	mean	(CD4A-CD4B)				t=28.0106
$H_0 : \text{mean}(\text{diff}) =$	0					df=500
$H_a : \text{mean}(\text{diff}) <$	0	$H_a : \text{mean}(\text{diff}) \neq 0$		$H_a : \text{mean}(\text{diff}) > 0$		
$Pr(T < t) =$	1	$Pr(T > t) = 0.0000$		$Pr(T > t) = 0.0000$		

The difference of the mean CD4 count (After - Before) is approximately 134 at t=28.0106. The p-value (0.000) < α (0.05) indicates that the mean difference (134) is significantly larger than 0. The null hypothesis is therefore rejected and we conclude that the average CD4+ cell counts before the intervention and after the intervention are different (mean difference is greater than zero)

Again, from Table 4.9, the difference of the mean CD4 count (After-Before) is 10.497 at t=2.8050. Since the p-value (0.0026) < α (0.05), we reject the null

hypothesis and conclude that the average CD4+ cell counts during the intervention Table 4.9: Paired t-test for CD4 count During and CD4 count After Intervention

Variable	Obs	Mean	Std. Dev	Std. Err	[95% conf. Interval]
CD4D	501	367.44310	154.4228	6.899103	353.8883 380.9979
CD4A	501	365.9461	139.667	6.239	344.6866 369.2056
diff	501	10.49701	3.74228	83.76356	3.144474 17.84954
mean(diff)=	mean	(CD4D-CD4A)			t=28.0106
$H_0: \text{mean}(\text{diff}) =$	0				df=500
$H_a: \text{mean}(\text{diff}) <$	0	$H_a: \text{mean}(\text{diff}) \neq 0$		$H_a: \text{mean}(\text{diff}) > 0$	
$Pr(T < t) =$	.9974	$Pr(T > t) = 0.0052$		$Pr(T > t) = 0.0026$	

and after the intervention are different (mean difference is greater than zero). The paired t-test results confirmed the results in Table 4.1 which indicated that the food supplementation significantly increased CD4+ cell counts of the PLWHA that were put on it. The withdrawal of the nutritional intervention subsequently affected the mean CD4+ cell counts of respondents negatively and thus caused a decrease in CD4+ cell counts of PLWHA who were on the intervention.

4.3 Generalized Estimating Equations Model

Table 4.10: AR (1) Correlation Matrix

	CD4B	CD4D	CD4A
CD4B	1.0000	0.8433	0.7112
CD4D	0.8433	1.0000	0.8433
CD4A	0.7112	0.8433	1.0000

The correlations decrease over time as assumed by AR (1) working correlation and are similar to the correlation of the observed. From the Table 11 below, the AR (1) parameter is 0.8236 which shows the AR (1) correlation structure is correctly specified.

Table 4.11: Covariance Parameter Estimates

Cov Parm	Subject	Estimate
AR(1)	ID	0.8236

NOTE: The scale parameter for GEE estimation was computed as the square root of the normalized Pearson's chi-square.

Table 4.12: GEE Naive (Model-Based) Standard Error Estimates

Parameter	Estimate	Standard Error	[95% Conf. Limits	Z	<i>Pr</i> > Z
Scale	140.8382				< .0001
Intercept	-172.887	45.2350	-261.546 -84.2278	-3.82	0.0001
Group 1	272.8647	26.1064	221.6970 324.0323	10.45	< .0001
Group 2	182.1614	11.0217	160.5593 203.7634	16.53	0.0000
Group 3	0.0000	0.0000	0.0000 0.0000	0.0000	0.0000
Age	-1.6534	0.7220	-3.0685 -0.2382	-2.29	0.0220
Hb*time	15.9896	1.0077	14.0146 17.9646	15.87	< .0001
BMI*time	1.5029	0.3874	0.7436 2.2622	3.88	0.0001

The empirical standard errors and model based standard errors (Table 4.12) are similar, indicating that the working correlation structure is adequate. G1, G2, age, HB*time and BMI*time were all statistically significant.

Table 4.13: Score Statistics For Type 3 GEE Analysis

Source	DF	Chi-Square	<i>Pr</i> > <i>ChiSq</i>
Group	2	224.39	< .0001
Age	1	4.65	0.0310
Hb*time	1	67.49	< .0001
BMI*time	1	14.11	0.0002

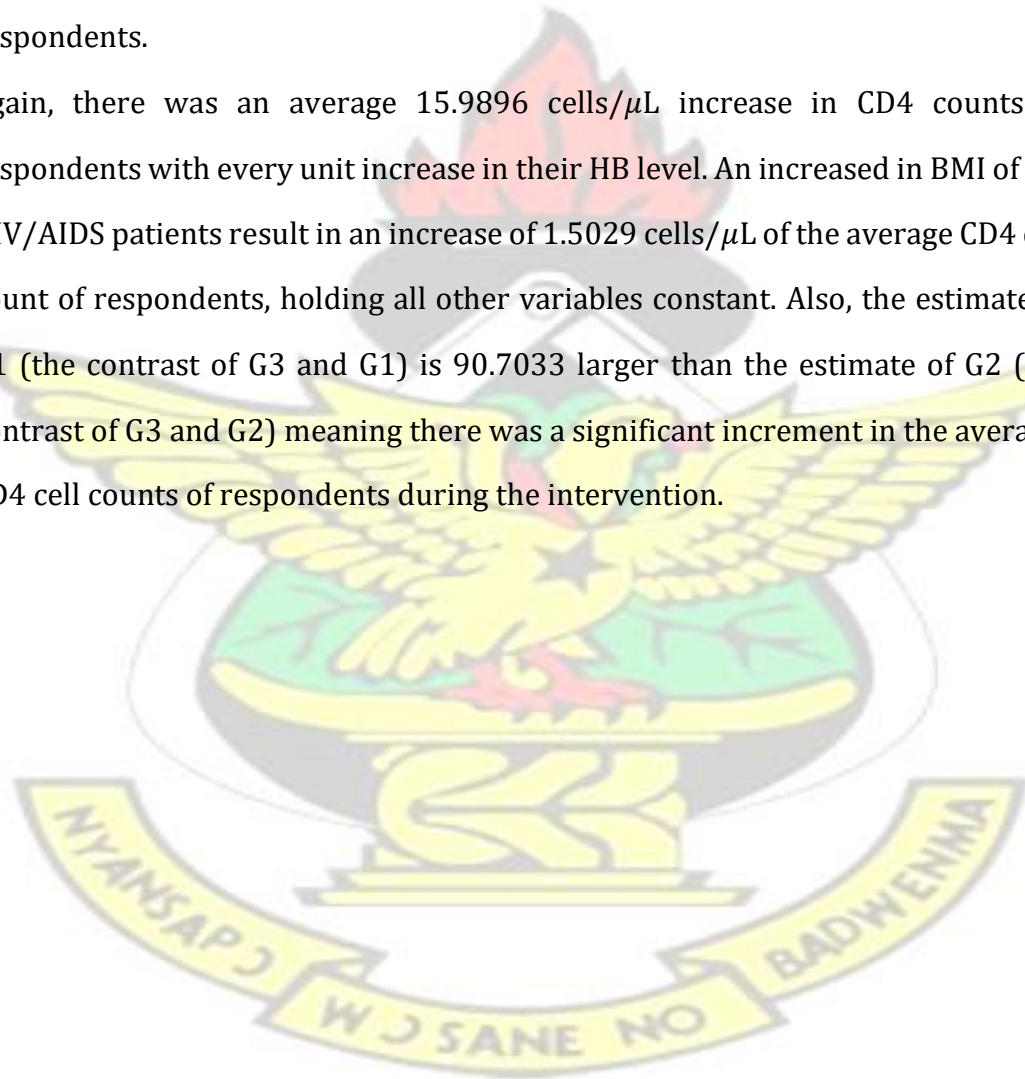
The type 3 analysis is for testing the significance of the group effects. It indicates the p-values for the null hypothesis that there are no effects of Group (G1 and G2), age, HB*time and BMI*time on the CD4 cell counts of the HIV/AIDS patients. From Table 4.13, the effect of Group, age, HB*time and BMI*time were also significant at the α level of 0.05.

Thus the resulting equation for the GEE model is;

$$CD4 \text{ Cell Count} = -172.887 + 272.8647G1 + 182.1614G2 - 1.6534Age + 15.9896Hb * time + 1.5029BMI * time.$$

There was an average reduction of 172.887 cells/ μ L in the CD4 cell counts of respondents after the withdrawal of the nutritional intervention, holding all other variables constant. There is an inverse relationship between the age of respondents and their CD4 cell counts. An increase in the age of respondents is associated with a 1.6534 cells/ μ L decrease in the mean CD4+ cell counts of respondents.

Again, there was an average 15.9896 cells/ μ L increase in CD4 counts of respondents with every unit increase in their HB level. An increased in BMI of the HIV/AIDS patients result in an increase of 1.5029 cells/ μ L of the average CD4 cell count of respondents, holding all other variables constant. Also, the estimate of G1 (the contrast of G3 and G1) is 90.7033 larger than the estimate of G2 (the contrast of G3 and G2) meaning there was a significant increment in the average CD4 cell counts of respondents during the intervention.



Chapter 5

Conclusions and Recommendations

5.1 Introduction

HIV-infected patients especially those on ART are prone to malnutrition due to inadequate dietary intake, appetite loss, nutritional losses, metabolic changes, and increased requirements for both macro- and micro-nutrients (Louise et al., 2009). Hence, as pointed out in other studies, increasing and integrating nutritional supplementation which improves access to food into ART programs will improve adherence and maximize the benefits of ART therapy (Byron et al., 2008) and thus enhancing the general outcome of patients on ART. It is on this score that nutritional interventions for PLWHAs, such as that of the Opportunities Industrialization Centres International (OICI), provided enhanced care and management of the disease. The extent of the withdrawal effects of nutritional interventions for PLWHAs is not known in the country at the time. This chapter presents discussion of the results of the analyses done on PLWHAs who were put on OICI nutritional intervention and which was later withdrawn.

5.2 Discussion of Results

5.2.1 Characteristics of the Study Population

HIV/AIDS is no respecter of age even though the sexually active age groups of both sexes (male and female) are the most affected. The average age of the respondents was 35 years in this study. This age group falls within the projected range of 15-49 years of adult population in Ghana that is largely affected by the HIV/AIDS (Ghana AIDS Commission, 2013).

Females are known to be more susceptible to HIV/AIDS a trend which shows that young women and adolescent girls are disproportionately vulnerable and at high risk (UNAIDS, 2014). The findings of this study revealed a consistent trend with majority (60%) of the PLWHAs were females.

5.2.2 Effect on the nutritional support on the outcome of the PLWHA

BMI was found to be a significant predictor of CD4 cell count and it predicts greater gains in CD4+ cell counts. This finding is in contrast to the results in the Crum- Cianflone et al. (2010) study, which indicated that obese patients have smaller CD4+ cell count gains. The significance of the linear interaction of BMI and time on the CD4 cell counts of respondents signifies the importance of the food supplementation intervention for the clinical success of HIV programs started in the country. The mean BMI of respondents was 18.9 before the intervention but it increased significantly to 23.6 during the intervention. There was a reduction in the mean BMI of respondents of 3.1 after the withdrawal of the intervention. The reduction can reduce the length of survival of patients since wasting assessed by reduced BMI is associated with an increased risk for death among both men and women (Mupere et al., 2012).

It was also discovered that majority (cum. freq of 97%) of respondents tend to have normal nutrition and overweight and obese during the intervention; according to the established classification of BMI. This can be compared with the cumulative frequency of 52.9% of respondents with normal nutrition and overweight classification before the intervention.

Since appropriate enhancement in nutritional status of PLWHA helps boost their immune system, manage the frequency and severity of symptoms and promote

good responses to medical treatment (Obi et al., 2011), there was an increase in the mean CD4+ cell count during the intervention. CD4+ T lymphocyte (CD4) cell counts is an important indicator of HIV progression since HIV/AIDS infects CD4 cells, CD4 count gives an immunologically significant measure for HIV/AIDS progression. The administration of ART for PLWHA; where nutritional cares and supports form an integral part, is known to significantly increase CD4 count and thereby enhancing the immunity state of patients. The study showed that there was a significant reduction in CD4 cell count of respondents after the intervention.

Also, an HIV-infected patient's CD4+ cell count depended on his/her past CD4+ cell count, the strength of the relationship was much stronger with immediate CD4+ cell count and vice versa. This suggests that a higher initial CD4+ cell count would result in a better rate of recovery of HIV-infected patients on ART. This is consistent with findings of Viviane et al (2009). It is unfortunate therefore that majority of patients start ART at a late disease stage characterized by wasting syndrome, lower CD4 cells and hence higher mortality rates (Mageda et al. 2012).

The study showed that the linear interaction of HB and time is strongly associated with CD4 cell count. An increase in the HB of respondents causes a larger increase in the mean CD4 cell count of respondents. This positive correlation between the blood haemoglobin level of respondents and their respective CD4 cell counts confirms the findings of other studies (Obirikorang et al., 2009; Qazi, 2013 etc) in the past.

The study confirmed the inverse relationship between age and CD4 cell counts of HIV/AIDS patients. Younger HIV/AIDS patients have a higher CD4 cell counts and the older an HIV/AIDS patient gets the lower his/her CD4 cell counts. This inverse correlation is consistent with other works (Viard et al., 2011).

5.3 Conclusion

The nutritional intervention positively affected the health outcomes of PLWHA during the nutritional intervention. After the intervention however, there was a significant reduction in the average CD4 cell count of respondents. BMI predicts a positive gain in CD4+ cell count of respondent and there was a positive correlation between the blood haemoglobin levels of the HIV/AIDS patients and their CD4 cell counts. There was an inverse relationship between age and CD4 cell counts of HIV/AIDS patients. The increasing effect of the nutritional intervention on the outcome of PLWHA sets the tone for consistent provision of nutritional interventions for PLWHAs.

5.4 Recommendation

It is recommended that the government, high-income countries and international donor agencies should consider continuing the provision of nutritional cares and food supplements to HIV/AIDS infected patients in Ghana and other developing countries to ensure the success of ART programs.

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Appendix A

Table 5.1: CD4+ cell Count (Frequency) Category Characteristics

CD4 Count Category	CD4 Before	CD4 During	CD4 After
< 50	59	1	0
50-99	63	7	3
100-199	105	44	28
200-249	65	41	51
250 and above	209	408	419

