NUTRITIONAL STATUS OF HIV SEROPOSITIVE PATIENTS IN ASHANTI REGION OF GHANA.

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by

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DECLARATION

I hereby declare that this thesis is the result of my own work except for the references cited which have been duly acknowledged. It has never been submitted in substance for the award of any degree of the university.

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ABSTRACT

The relationship between HIV/AIDS and nutritional status has been a distinguishing characteristic of the disease course since the earliest days of the epidemic. Since the introduction of Highly active antiretroviral therapy (HAART), there has been divergent views concerning the nutritional status of HIV patients. Therefore to contribute to the sparse information on the nutritional status among HIV infected patients, a comparative cross sectional study was conducted to compare and investigate the nutritional status of participants on HAART and participants who are HAART naïve. In all two hundred and eighty seven (287) confirmed People Living with HIV/AIDS (PLWHAs) consisting of 179 participants on highly active antiretroviral therapy (HAART) and 108 HAART naïve participants were included in the study. The participants were recruited from the antiretroviral (ART) clinics at ST. Michael's Hospital, Pramso and Bomso specialist clinic, Bomso all in Ashanti region of Ghana. Anthropometry(BMI, MUAC, WHR,TSF, BSF), Complete haemogram, immunological (CD4) and biochemical (albumin, total protein, zinc, transferrin, ferritin, urea, ALP, ALT, AST) analysis were conducted for all the participants. Socio demographic features were collected using an interviewer administered questionnaire. Ethical clearance was obtained from Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. All participants gave informed consent to take part in the study after verbal and written explanation of the methods and risks involved had been given. Out of the 287 HIV/AIDS Participants categorized into HAART naïve and on HAART participants, 108 were highly active antiretroviral therapy (HAART) naïve participants of which 41were males and 67 were females and 179 participants on HAART of which 48 were males and 131 were females. The ages of participants ranged from 18 to 60. No significant difference was observed in the BMI and MUAC between participants on HAART and participants who are HAART naïve though the mean values of these measurements indicated overweight and obesity. HAART naïve participants had a significantly higher WHR (p=0.016) as a measure of central adiposity as compared to participants on HAART. All anemia incidence among participants on HAART were in the Grade 1 anaemia toxicity grade (54.7%; 98/179) whereas in the HAART naïve participants, there were incidence of Grade 2 (13.9%; 15/108) and Grade 3 anemia (16.7%; 18/108). Microcytic hypochromic anaemia was significantly higher in HAART naïve participants (29.2%) as compared to participants on HAART where no case was recorded. Hypozincaemia, hypoalbuminaemia and low transferrin levels were significantly higher among HAART naïve participants as compared to participants who are on HAART therefore putting HAART naïve participants at greater risk of developing nutritional deficiencies. This study is therefore beneficial in the advocacy for complete assessment of nutritional status in HIV partients before and after the initiation of antiretroviral therapy in Ghana.

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"Great is thy Faithfulness O Lord……, they are new every morning." (Lamentations 3:23).

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ABBREVIATIONS

3TC – Lamivudine

ABC – Abacavir

AIDS – Acquired Immune Deficiency Syndrome

ALP – Alkaline Phosphatase

ALT – Alanine Transaminase

ARV – Antiretroviral

AST – Aspartate Transaminase

AZT – Azidothymidine (Zidovudine)

BMI – Body Mass Index

BUN – Blood Urea Nitrogen

CDC – Center for Disease Control

CHRPE - Committee on Human Research Publication and Ethics d4T

– Stavudine

DNA – Deoxyribonucleic Acid

EDTA - Ethylenediaminetetraacetic Acid

EFV – Efavirenz

EIA – Enzyme Immunoassay

FANTA- Food and Nutrition Technical Assistance

FBC - Full Blood Count

FIV – Feline Immunodeficiency Virus

GGT – Gamma Glutamyl Transferase

HAART – Highly Active Antiretroviral Therapy

Hb - Haemoglobin

HCT – Haematocrit
HDL – High Density Lipoprotein

HIV – Human Immunodeficiency Virus

HTLV – Human T-lymphotrophic Virus

IDV – Indinavir

LAV – Lymphadenopathy Associated Virus

LDH – Lactate Dehydrogenase

LDL – Low Density Lipoprotein

MCH – Mean Cell Haemoglobin

MCHC – Mean Cell Haemoglobin Concentration

MCV – Mean Cell Volume

MDA – Malondialdehyde

MHC – Major Histocompatibility Complex

NFV – Nelfinavir

NNRTI – Non-Nucleoside Reverse Transcriptase Inhibitors

NRTI – Nucleoside Reverse Transcriptase Inhibitors

NVP – Nevirapine

PI – Protease Inhibitors

RBC – Red Blood Cell

RNA – Ribonucleic Acid

SEM – Standard Error of Mean

SIV – Simian Immunodeficiency Virus

SOD – Superoxide Dismutase

SQV – Saquinavir

TDF – Tenofovir

TG – Triglyceride

ALC – Absolute Lymphocyte Count

UNAIDS – United Nations Programme on HIV/AIDS

USAID- United States Agency for International Development

VLDL – Very Low Density Lipoprotein

WBC – White Blood Cell

WHO – World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Since its emergence in sub –Saharan Africa in the early 1980's, Acquired Immune Deficiency Syndrome (AIDS) caused by a retrovirus called the Human immunodeficiency Virus, has had a wide distribution and infected millions of people in this region of the world. The AIDS epidemic remains one of the most devastating disease spread in the world today especially in the developing countries. Sub-Saharan Africa (SSA) remains the worst hit region with AIDS as the leading cause of death in the region. About 90% of all HIV positive adults live in the Sub-Saharan Africa region(UNAIDS, 2013; HIV/AIDS, 2014). Despite more than 2 decades of research, a cure remains elusive and the average survival is about 10.3 to 10.8 years without Anti retroviral therapy(Todd *et al.*, 2007).

Results for the epidemiological and demographic plan of the HIV / AIDS epidemic show that the number of persons infected with HIV in Ghana has risen steadily since the start of the epidemic in the mid-1980s. However, Ghana is one of the five countries in sub-Saharan Africa whose HIV prevalence declined by more than 52 per cent between 2001 and 2010 among young people aged between 15-24 (UNAIDS, 2010). In spite of the relatively low prevalence in Ghana, HIV/AIDS has indeed been recognized as a significant public health challenge which also has a tremendous effect on the socioeconomic growth of the nation (Uthman, 2008).

Moreover, the introduction of affordable Antiretroviral Therapy (ART) in Ghana since June 2003 has led to a significant boost in the immunity of persons and an increase in the survival of Ghanaians living with HIV/AIDS (UNAIDS, 2013). Even in the current era of highly active antiretroviral therapy (HAART), weight loss and malnutrition remain significant clinical problems (Grinspoon *et al.*, 2008). Some Anti retroviral therapies, are best given on an empty stomach, some with food and others with a fatty meal. Many give rise to metabolic alterations, such as insulin resistance and glucose intolerance, fat abnormalities (lipodystrophy, hyperlipidaemia), lactic acidosis, liver enzyme abnormalities, anaemia and osteopenia. Therefore HIV/AIDS and nutrition are closely related and could improve or worsen the infection and progression of the disease (Grinspoon *et al.*, 2008).

There seems to be a complex relationship between nutrition and HIV infection. Malnutrition, even without HIV, can compromise the immune system, and CD4 T cells can be decreased in malnourished, HIV-negative individuals (Chantry *et al.*, 2003). HIV/AIDS and malnutrition effects are interrelated and aggravate one another in a vicious cycle. The disease is known to be associated with conditions that result in reduced food intake, interfere with the lining of the gut and affect its ability to digest and absorb nutrients, resulting in metabolic alterations with the subsequent changes in body composition (Ball, 1998). Impairment of nutrient intake and absorption is increasingly associated with HIV infection which results in malnutrition with the associated risks of opportunistic infections thus accelerating progression of HIV into AIDS (Ball, 1998). Malnutrition can weaken the immune system and increase vulnerability to infections and may speed up the progression of HIV disease. HIV may also interfere with the ability to access, handle, prepare, eat and

utilize food, thus increasing the risk of malnutrition among people living with HIV (Chlebowski *et al.*, 1989). Food and nutritional intake can affect adherence to antiretroviral drugs (ARVs) as well as their effectiveness (Chantry *et al.*,2003).

In determining nutritional status in HIV/AIDS Participants, the assessment of quality of life is central to understanding how people's lives are affected by HIV infection. Studies have found a strong association between the CD4 cell count and quality of life (Daniel *et al.*, 2013). Recent studies have shown that death rates are higher among HIV-infected patients presenting with malnutrition, including those receiving ART (Obi *et al.*, 2010). Mostly in sub-Saharan Africa, the availability of foods are influenced by socio-economic factors such as poverty and political instability such as coup d'etats and wars. These factors greatly contributes to the nutritional status of HIV/AIDS Participants and hence the diseases progression (Nerad *et al.*, 2003).

Ongoing assessment of nutritional and medical status is crucial to quality nutrition care for every person living with HIV/AIDS. Evaluation of nutritional status generally includes medical history, biochemical indices, anthropometrics, dietary intake or a combination of these (Watson *et al.*, 2006). A number of instruments, including nutrition screening and assessment tools, have been developed and validated for use in HIV/AIDS Participants (Guigoz *et al.*, 1996; Rubenstein *et al.*, 2001; Stratton *et al.*, 2004). No single measurement however, has emerged as a 'gold-standard' in assessing nutritional status, making a diagnosis of malnutrition challenging. However, a combination of the various nutritional screening tools such as Subjective Global Assessment (SGA), Malnutrition

Universal Screening Tool (MUST), etc including other medical and nutritional histories, physical examination, anthropometric measures and biochemical assessment to determine whether an individual is well or malnourished have been proven to be adequate nutrition assessment tools that offer a more comprehensive review of nutritional status (McCaffree, 2003).

The purpose of nutrition screening in HIV/AIDS patients is to identify those people who may be at risk of malnutrition, and where such a risk is determined, then a more comprehensive nutrition assessment and appropriate nutrition intervention is required (ADA, 1994; Carey and Gillespie, 1995) . Nutrition assessment for malnutrition is a more detailed process than screening and many assessment tools require training prior to administration. Biochemical Assessment analysing the micro and macro nutrients is a useful tool to assess the metabolic changes as evident in the clinical assessment (Obi *et al.*, 2010).

1.2 STATEMENT OF PROBLEM

Despite the internationally accepted recommendation that eating a diversity of foods leads to a healthy diet, and is associated with positive health outcomes such as reduced mortality (Friis, 1999), there is inadequate information on nutritional status among People Living with HIV/AIDS; both HAART naive and those on HAART in Ghana. Also little information exists on what factors influence nutritional status among this group. Inadequate dietary intake to meet the increased metabolic demands associated with HIV infection is likely to affect nutritional status in people living with HIV/AIDS (Preble and Piwoz, 2000), further lowering their immunity and hastening disease progression hence increased morbidity and mortality.

Common manifestations of nutrient deficiencies include protein–energy malnutrition, anemia, and other micronutrient status alterations. All persons with HIV/AIDS infection are at greater risk for malnutrition as a result of inadequate intake, weight loss, often due to nutritionally compromising conditions such as nausea, diarrhea, anorexia, fatigue, difficulty in chewing and swallowing during the course of the disease (WHO and HIV/AIDS, 2011). Energy requirements are likely to increase by 10% just to maintain body weight and normal physical activity in asymptomatic HIV-infected adults. During symptomatic stages and particularly during AIDS, these energy requirements increase by 20 - 30%. When enriched with various essential nutrients, food will provide greater energy content to supplement the daily, baseline dietary requirements if the malnutrition and weight loss are to be corrected (Knox *et al.*, 2003).

There are various nutritional interventions in Ghana including counselling on nutrition and the need to adhere to the ART plan. However, there seems to be insufficient information as to whether the counselling provided has improved the nutritional status of these clients. The ability to efficiently manage the food and nutrition implications of ART together with the socio demographic and socio economic influence, are vital features in the success of antiretroviral therapy in developing countries. There seems to be a failure in focusing on drug -food interactions in Ghana among HIV Participants which could reduce drug efficacy, leading to poor adherence to drug regimens and could aggravate side effects, or damage the nutritional status of PLWHA (WHO, 2003). For effective implementation of nutritional support and its integration with the routine highly active antiretroviral therapy there must be a clear picture of the magnitude and associated factors of malnutrition. This is not available at present. Hence current strategies being implemented by the Ghana

Health Service to control nutritional complications in HIV Participants could be potentially unsuccessful and misdirected.

1.3 STUDY HYPOTHESIS

The occurrence of nutritional deficiencies and disorders in HIV/AIDS Participants is more severe in infected Participants who are HAART naïve as compared to those on HAART.

1.4 JUSTIFICATION

Prevention and management of nutritional deficiencies, malnutrition and metabolic abnormalities, related to HIV infection are crucial parts of the comprehensive management of HIV-infected Participants (Sheehan and Macallan, 2000). Good nutrition may give strength and help to maintain and improve performance of the immune system thereby protecting the body against infection and delaying progression of the disease to AIDS. Good nutrition also complements and ensures effective antiretroviral treatment (Nerad *et al.*, 2003). The rationale for considering nutritional assessment in HIV/AIDS infection is that malnutrition contributes to the problems of the HIV/AIDS-infected individual in ways that are independent of immune depletion (Horn and Pieribone, 1996; Kotler, 1996).

Ongoing Anthropometric and biochemical assessment can help identify low levels of certain micronutrients (such as zinc), which are common in HIV due to malabsorption, alterations in metabolism, poor dietary intake, and accelerated nutrient turnover (Powanda and Beisel, 2003). Biochemical assessment can also help identify various types of anaemias that can occur with chronic HIV infection and may help identify treatable nutritional deficiencies (Kelly *et al.*, 2008). Assessment of the nutritional status of HIV Participants in resolving nutritional complications and anticipation of HAART associated complications therefore becomes necessary in the collective management of HIV

infected Participants. This is not well documented in Ghana and thus the urgent need for a complete nutritional assessment in HIV/AIDS patients.

1.5 AIM

To compare and assess the nutritional status among HAART naïve patients and those on HAART in the Ashanti Region of Ghana.

1.6 SPECIFIC OBJECTIVES

1. To assess the association between HAART use and nutritional status in HIV patients.
2. To determine the relationship between CD4 cell count, anthropometric variables, biochemical and haematological parameters of nutritional status in HIV-infected adult.
3. To determine the socio-economic and socio-demographic factors associated with nutritional status of HIV positive adults.
4. To assess the body composition changes and frequency of food intake in HIV seropositive patients.
5. To determine the association between duration of HIV infection and nutritional status.

CHAPTER 2

LITERATURE REVIEW

2.1 ORIGIN AND BRIEF HISTORY OF HIV/AIDS

The advent of the Human Immunodeficiency virus in the late twentieth century has affected millions of people in a worldwide pandemic which has resulted in an increase in mortality rate. There are several theories in regards to the origin of this fatal virus. It is speculated that the virus originated in the 1930s in rural areas of central Africa. However, the Center

for Disease Control (CDC) in September 1982, properly identified and defined the Human Immunodeficiency Virus as the etiologic agent of the Acquired Immune deficiency Syndrome Disease (Control and Prevention, 2012). It is however widely believed that HIV in humans originated from cross-species infections by simian viruses in rural Africa, probably due to direct human contact with infected primate blood. In

1970, American molecular biologist David Baltimore and American virologist Howard Temin independently discovered the enzyme reverse transcriptase, which could be used to identify retroviruses in animals. In 1983, Luc Montagnier, reported the isolation of a human retrovirus which was believed to be the cause of AIDS. The virus was named, lymphadenopathy-associated virus (LAV) which was believed to kill CD4 cells in humans. A year after, Robert Gallo, an American virologist, confirmed the discovery of the virus, but they renamed it human T lymphotropic virus type III (HTLV III). Other studies further showed that these human retroviruses were more closely related and genetically impossible to differentiate and were named Human Immunodeficiency virus

(Korber *et al.*, 2000).

The Human Immunodeficiency virus is believed to have been transferred to man through zoonotic interaction with non human primates such as gorilla and chimpanzees. This believe is generally accepted by most researchers because of the close resemblance of HIV to the Simian immunodeficiency virus (SIV) which is found in the whitecollared monkeys, indigenous to West Africa and chimpanzees in West-Central Africa (Sharp *et al.*, 2001).

There are other theories of the origin of HIV/AIDS which seems to contradict the widely believed scientific evidence. Paramount among these theories is the "HIV/AIDS denialism" which believes that the human immunodeficiency virus (HIV) does not cause

acquired immune deficiency syndrome (AIDS) (Kalichman *et al.*, 2010). Some of these theories reject the existence of HIV, while others accept that HIV exists but say that it is a harmless passenger virus and not the cause of AIDS. Insofar as these theories acknowledge AIDS as a real disease, they attribute it to some combination of sexual behavior, recreational drugs, malnutrition, poor sanitation, haemophilia or the effects of the drugs used to treat HIV infection. Despite its lack of scientific acceptance, HIV/AIDS denialism has had a significant political impact in some parts of the world (Kalichman *et al.*, 2010)

The debate concerning the true origin of HIV/AIDS has continued up to date and seems to be more profound in the 21st century. However, the general ideas held by the medical and scientific community seems to be much substantive and accepted as opposed to the other theories held by the minority group of denialists (Smith and Novella, 2007).

2.2 CLASSIFICATION OF HIV

HIV is a human retrovirus belonging to a large family of ribonucleic acid (RNA) viruses called Lentivirus. The lentivirus family consists of other viruses such as feline immunodeficiency virus, simian immunodeficiency virus, visna virus of sheep, and the equine infectious anemia virus which are associated with diseases of immunosuppression leading to opportunistic infections. These viruses are also associated with causing slow and long incubation period diseases before the manifestation of the illness becomes apparent (Plantier *et al.*, 2009). HIV may be classified into types, groups and subtypes, based on genetic similarities and the numerous virus strains. Molecular analysis of different viral isolates reveals considerable variability in many parts of the HIV genome. (Plantier *et al.*, 2009).

2.2.1 Types

There are two distinct types of HIV: HIV-1 and HIV-2. These two types are genetically different but antigenically related forms of HIV. They are distinguished on the basis of genome organization and phylogenetic (evolutionary) relationships with other primate lentiviruses. Each type evolved from a different simian immunodeficiency virus (SIV); HIV-1 is closely associated to the SIV*cpz* strain of the simian immunodeficiency virus found in chimpanzees and HIV-2 is associated with the SIV*sm* strain found in the sooty mangabey (the white-collared monkeys). In contrast to the SIV's, which appear not to harm their natural primate hosts, HIV infection damages the immune system, leaving the body susceptible to infection with a wide range of bacteria, viruses, fungi and protozoa.

HIV-1 is more virulent and the more common type associated with AIDS in the United States, Europe, Central Africa and vast majority of HIV infections globally whereas HIV2 causes a similar disease principally in West Africa and rarely in other parts of the world (Plantier *et al.*, 2009). However both types could be transmitted principally through sexual intercourse, through blood and other fluids and through mother to child transmission.

2.2.2 Human Immunodeficiency virus – 1

HIV-1 appears to be a particularly variable virus, having evolved into a number of groups and subtypes. It comprises of four distinct virus groups: the predominant or major M group, the outlier O group and two new groups, N and P (Burke, 1997). These four distinct groups seems to be a representation of the four separate introductions of simian immunodeficiency virus into humans (Burke, 1997).

Group O is found mainly in the West-Central Africa regions whiles the rarely occurring Group N is restricted to Cameroon since its discovery in 1998. Group P, the most recent discovered strain, was seen in a Cameroonian woman in 2009 (Plantier *et al.*, 2009). Group

M viruses, the more common form worldwide, are further divided into at least ten genetically distinct subtypes, called, clades. These are subtypes A, B, C, D, E, F, G, H, J and K. Recombinant forms of virus are also found in circulation in humans in different geographic regions (Hahn *et al.*, 2000). The worldwide distribution of the clades of HIV 1 Group M is illustrated in the table 2.1:

Table 2.1 Distribution of Subgroups of HIV 1

Group (CLADES)	Region
Subtype A	West and Central Africa
Subtype B	South America(including Brazil, Argentina, United States, Europe, Thailand, Russia
Subtype C	India, Sudan, Southern and Eastern Africa
Subtype D	East and Central Africa
Subtype E	Thailand, Philippines, China, Central Africa
Subtype F	Brazil, Argentina, Eastern Europe, Central Africa
Subtype G	Western and Eastern Africa, Central Europe
Subtype H	Central Africa
Subtype J	Central America
Subtype K	Democratic Republic of Congo, Cameroon

2.2.2.2 Transmission of Subtypes (Clades) Of HIV-1 Group M

Beyond molecular homologies, it is known that the clades also show differences in modes of transmission and progression of disease. Thus E clade is spread predominantly by heterosexual contact, presumably because of its ability to infect vaginal subepithelial Cells. In contrast to the E clade, it is known that , B clade virus grows poorly in the vaginal subepithelial cells and may are transmitted principally through homosexual contact and intravenous drug use. These concept are not conclusively proven and still remains a subject of debate (Essex, 1999; Bhoopat *et al.*, 2001).

Moreover, studies done in Uganda in 2006 on disease progression showed that people infected with the D clade developed AIDS sooner than those infected with the A clade. This evidence suggested that the D clade is more virulent because it is very effective in the binding of immune cells therefore the more effective in immunosuppression (Laeyendecker *et al.*, 2006). Another study conducted in 2007 in Kenya, supported this result in which it was found out that, Kenyan women infected with subtype D had more than twice the risk of death over six years compared with those infected with subtype A (Baeten *et al.*, 2007).

2.2.3 Human Immunodeficiency Virus -2

The human immunodeficiency virus -2 (HIV-2) has a lower prevalence as compared to that of HIV-1 and is believed to have been present in Africa as early as the 1960's (Miyazaki, 1995). HIV-2 is mainly restricted to the West Africa regions and have been reported to have appeared only occasionally in other parts of the world (De Cock *et al.*, 1993). Simian immunodeficiency virus from sooty mangabeys, a type of monkey in West

Africa and HIV-2 are considered to be variants of the same virus. It is believed that HIV2 became established in humans as a result of the zoonotic association with the sooty mangabeys (Hahn *et al.*, 2000). Most research works focuses on HIV-1 more than HIV-2 possibly due to the more prevalent nature of HIV-1.

2.2.3.1 Subtypes

At least six subtypes of HIV-2 has been identified as subtypes A through F. Difference in genetic homolog y among these subtypes are up to 25%. Evidence of infection by subtypes HIV-2 can be detected by enzyme immunoassay (EIA) and Western blot assays. Persons could be co -infected with HIV - 1 and HIV - 2 (De Cock *et al.*, 1993; Gao *et al.*, 1999).

2.2.3.2 Mode of Transmission

The manner of spread of HIV-2 is similar to that of HIV-1. However, the high-risk groups are commercial sex workers and persons with other sexually transmitted diseases such as syphilis, gonorrhea, or herpes simplex type 2. It is known that HIV-2 utilizes the same cellular mechanisms for infection as HIV-1, including the use of CD4 receptors and chemokine co-receptors (Murdoch and Finn, 2000).

2.3 STRUCTURE OF HIV

Human immunodeficiency virus is spherical and contains an electron-dense, cone-shaped core surrounded by a lipid envelope derived from the host cell membrane. Like all viruses, HIV cannot grow or reproduce on its own. In order to make new copies of itself it must infect the cells of a living organism. The virus core contains: a major capsid protein, p24, nucleocapsid protein p7/p9, two copies of genomic RNA, and three viral enzymes (protease, reverse transcriptase, and integrase). Due to the two copies of the RNA genome possessed, the virion particle can be described as diploid. The two molecules are present as a dimer, formed by base pairing between complementary sequences. The regions of

interaction between the two RNA molecules have been described as a 'kissing-loop complex'. An HIV particle is around 100-150 nano meter in diameter (Gamble *et al.*, 1996).

Projecting from the envelope structure, are around 72 little spikes, which are formed from the proteins gp120 and gp41. The viral core is surrounded by a matrix protein called *p17*, lying beneath the virion envelope. A protein p24 forms the core which is usually bullet shaped and is the most readily detected viral antigen and is therefore the target for the antibodies used to diagnose HIV infection in blood screening. Generally, there is one capsid per virion, though virions with two or more capsids have been reported.The three enzymes required for HIV replication, reverse transcriptase, integrase and protease are located in the core. The genetic material, which consists of two identical strands of RNA, is also located in the core (Freed and Martin, 1995)

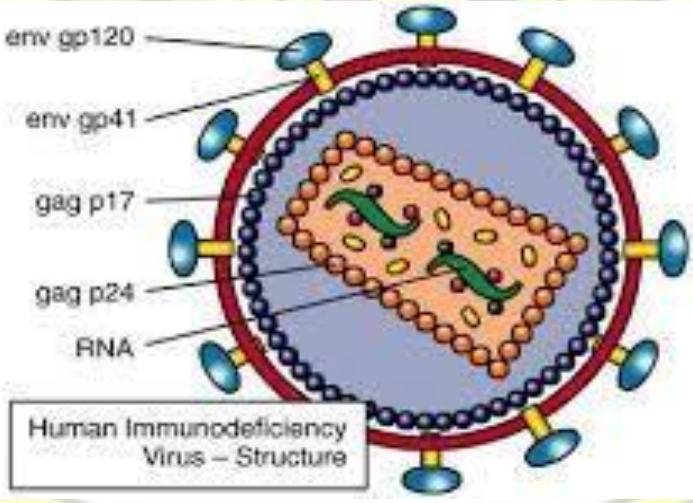


Figure 2.1 Structure of Human immunodeficiency virus 2.4 GENES

The Human immunodeficiency virus genome contains the *gag*, *pol*, and *env* genes, which code for various viral proteins. In addition to these three standard retroviral genes, HIV contains several other genes such as *tat*, *rev*, *vif*, *nef*, *vpr*, *vpu*, *vpt* that regulate the synthesis

and assembly of infectious viral particles. The *nef* protein activates intracellular kinase activity (affecting T-cell activation, viral replication, and viral infectivity) and reduces surface expression of CD4 and MHC molecules on infected cells (Schaub *et al.*, 2007). The progression of HIV infection in vivo is dependent on *nef*. In monkeys, strains of simian immunodeficiency virus with mutated *nef* genes cause AIDS at a markedly decreased rate, and humans infected with a *nef*- defective HIV-1 strain display low viral burden. The product of the *tat* (transactivator) gene, for example, is critical for virus replication, causing a 1000-fold increase in the transcription of viral genes. The *rev* gene (regulator of expression of viral proteins), which functions as a promoter of the export of viral RNA from the nucleus of the cell. The gene *vif* (virus infectivity factor) influences the infectivity of the virus particles and also the release of infectious virus from cells. The gene, *vpu* (viral protein u) is known to enhance the production of virus particles by promoting release of infectious virus from cells. The *vpr* gene (viral protein r) and *vpt*,
(viral protein t) have been described although their function still needs to be elucidated (Schaub *et al.*, 2007). The products of various regulatory genes are important for HIV pathogenicity, and several therapeutic approaches are being developed to block their actions.

2.5 HIV LIFE CYCLE

2.5.1 *Entry*

Like most retroviruses, HIV can only replicate or reproduce in the human cells. Before this could take place there is the need for entry of the virus into the body. HIV infection can occur through oropharyngeal, cervical, vaginal and gastrointestinal mucosal surfaces, even in the absence of mucosal disruption. The interaction of HIV with the hosts immune system is a complex mechanism. The virus uses surface proteins of the immune system

cells to gain entry (Cladera *et al.*, 2001). The entry of HIV into cells requires the CD4 molecule, which acts as a high-affinity receptor for the virus. CD4, the surface glycoprotein, is the name badge of a particular group of helper T lymphocytes. The HIV envelope gp120 must also bind to other cell surface molecules (*coreceptors*) to facilitate cell entry; Two cell surface chemokine receptors, CCR5 and CXCR4 play the role of facilitating the cell entry of the virus. After binding of the HIV envelope gp120 to the CD4 molecules, there is a conformational change that exposes a new recognition site on gp120 for the CXCR4 and CCR5 coreceptors for binding. Another viral glycoprotein, gp41, then undergoes conformational change causing fusion of the viral envelope with the plasma membrane of the T cell. The contents of the HIV particle are then released into the cell, leaving the envelope behind (Cladera *et al.*, 2001; Clapham and McKnight, 2001).

2.5.2 Reverse Transcription and Integration

Once the virus is internalized, the viral genome undergoes reverse transcription, leading to formation of complementary DNA (cDNA). The HIV enzyme reverse transcriptase converts the viral RNA into DNA, which is compatible with human genetic material (Moore and Chaisson, 1999). The converted DNA is then transported to the cell's nucleus, where it is spliced into the human DNA by the HIV enzyme integrase forming an HIV cDNA known as provirus.

2.5.3 Transcription and Translation

After integration, the provirus may remain nontranscribed for months or years, and the infection becomes *latent;* much of intracellular HIV can remain silent for a long time before being stimulated into activity. HIV genes are treated in much the same way as human genes by conversion into messenger RNA using human enzymes. The messenger

RNA is then transported outside the nucleus and is used as a blueprint for producing new HIV proteins and enzymes ((Emerman and Malim, 1998; Cladera *et al.*, 2001).

2.5.4 Assembly, Budding and Maturation

Activation of viral synthesis leads to release of new infective particles from the host cell surface by budding. Budding virions utilize host cell membrane to help form the outer virion envelope. A protease enzyme encoded by the *pol* gene of HIV plays a vital role at this stage of the HIV life cycle by chopping up long strands of protein into smaller pieces, which are used to construct mature viral cores. The enzyme protease Newly matured HIV particles are ready to infect other cells and begin the replication process all over again and in this way the virus quickly spreads through the human body (Emerman and Malim, 1998).

2.6 PROGRESSION OF HIV INFECTION

Untreated HIV infection is generally classified under six main stages: primary infection, dissemination of virus to lymphoid organs, clinical latency, elevated HIV expression, clinical disease, and death. The duration between primary infection and progression to clinical disease averages about 10 years. Within days after the first exposure to HIV, viral replication can be detected in the lymph nodes. This replication leads to viraemia, during which high numbers of HIV particles are present in the patient's blood, accompanied by an acute HIV syndrome that includes a variety of nonspecific signs and symptoms typical of many viral diseases such as flu-like symptoms of fatigue and malaise (Freed and Martin, 1995). The virus disseminates throughout the body and infects helper T cells, macrophages, and dendritic cells in peripheral lymphoid tissues. During this period of the disease, the immune system remains competent at handling most infections with opportunistic microbes and clinical symptoms are few or no symptoms. Therefore, this phase of HIV

disease is called the clinical latency period. Although the majority of peripheral blood T cells do not harbor the virus, destruction of CD4+ T cells within lymphoid tissues steadily progresses during the latent period, and there is a significant drop in numbers of circulating CD4 T cells during this time (Sierra *et al.*, 2005).

More than 90% of the body's approximately 10^{12} T cells are normally found in lymphoid tissues, and it is estimated that HIV destroys up to 1 to 2×10^9 CD4+ T cells every day. Early in the course of the disease, the body may continue to make new CD4+ T cells, and therefore CD4+ T cells can be replaced almost as quickly as they are destroyed. However, at this stage, up to 10% of CD4+ T cells in lymphoid organs may be infected, but the number of circulating CD4+ T cells that are infected at any one time may be less than 0.1% of the total CD4+ T cells in an individual. Eventually, over a period of years, the uninterrupted cycle of virus infection and T cell death leads to a continual decline in the number of CD4+ T cells in the lymphoid tissues and the circulation. CD4+ T cells play a central role in regulating the immune response: they produce a plethora of cytokines, chemotactic factors, and hematopoietic growth factors such as granulocytemacrophage colony-stimulating factor. Therefore, loss of this "master cell" has ripple effects on virtually every other cell of the immune system and disrupts cellular functions sufficiently to cause death of infected cells. In the absence of treatment, most Participants with HIV infection develop AIDS after a chronic phase lasting 7 to 10 years. The typical adult patient with AIDS presents with fever, weight loss, diarrhea, generalized lymphadenopathy, multiple opportunistic infections, neurologic disease, and secondary neoplasms (Phillips *et al.*, 1992; Sierra *et al.*, 2005).

The development of signs and symptoms of AIDS typically parallels laboratory testing for CD4 lymphocytes. The Center for Disease Control (CDC) classification of HIV infection stratifies Participants into three categories on the basis of CD4+ T-cell counts:

Participants with more than 500 cells/μL, those between 200 and 500 cells/μL, and those less than 200 cells/μL. Participants with more than 500cells/μL are generally asymptomatic and a reduction in the total CD4 lymphocyte count below 500/μL signals the development of symptomatic clinical AIDS. A drop below 200/μL not only defines

AIDS, but also indicates severe immunosuppression and a high probability for the development of AIDS- related opportunistic infections and/or neoplasms. The risk for death from HIV infection above the 200/μL CD4 level is low (Phillips *et al.*, 1992; Gallant *et al.*, 1994; Bozzette *et al.*, 1995).

There are other laboratory findings to indicate the progression of the HIV disease to AIDS. These findings include HIV p24 antigen positivity, increased serum bet a2- microglobulin (B2- M), elevated serum IgA, or increased neopterin levels in serum, cerebrospinal fluid, or urine. However, the best laboratory measure for determination of the progression of AIDS is the level of HIV - 1 RNA in peripheral blood. HIV- 1 RNA levels is independent of the CD4 lymphocyte count and of age in adults (Sterling et al. ,

2001). The set point levels of HIV -1 RNA correlate with the ti me to development of AIDS. The set point can range from <50 to 1,000,000 copies/mL. Persons with a higher set point tend to lose CD4 cells more rapidly and progress to AIDS more quickly. Levels of HIV-1 RNA can remain at a steady state for months to years, but usually fall with time. Persons with >100,000 copies/mL are 10 times more likely to progress to AIDS in 5 years.

For persons in the top quartile (>36,270 copies/mL) the median time to development of AIDS is 3.5 years (Sterling *et al.*, 2001).

2.7 HIV TRANSMISSION

Transmission of HIV occurs under conditions that facilitate the exchange of blood or body fluids that contain the virus or virus-infected cells. Thus, the major routes of HIV infection are sexual contact, parenteral inoculation, and passage of the virus from infected mothers to their newborns. Sexual transmission is by far the major mode of infection worldwide, accounting for more than 75% of all cases of HIV transmission. Transmission of HIV can occur from male to male, male to female, and female to male. Female to female transmission remains extremely rare, though women with same- sex contact are also often bisexual and have additional risk factors for HIV infection (Rich *et al.*, 1993; Bevier *et al.*, 1995). Clearly, all forms of sexual transmission are aided and abetted by the concomitant presence of other sexually transmitted diseases that cause genital ulcerations, including syphilis, chancroid, and herpes simplex virus. Gonorrhea and Chlamydia also act as cofactors for HIV transmission, primarily by increasing the seminal fluid content of inflammatory cells (Lamptey, 2002).

Another important secondary means of spread of HIV disease is through blood or blood products. Parenteral transmission of HIV is well documented in three different groups: intravenous drug abusers being the largest group, hemophiliacs receiving factor VIII or IX concentrates, and random recipients of blood transfusion. Among intravenous drug abusers, transmission occurs through shared needles, syringes, or other paraphernalia contaminated with HIV-containing blood (Perkins *et al.*, 1987).

Acquisition of HIV through congenital infection perinatally or in infancy is another route of transmission. Mother-to-infant vertical transmission is the major cause of pediatric AIDS. Three routes are involved: in utero, by transplacental spread; intrapartum, during delivery; and via ingestion of HIV-contaminated breast milk. Of these, the transplacental and intrapartum routes account for most cases. Vertical transmission rates worldwide vary from 25% to 35%, with a 15% to 25% rate reported in the United States; higher rates of infection occur with high maternal viral load and/or the presence of chorioamnionitis, presumably by increasing placental accumulation of inflammatory cells(UNAIDS, 2008; HIV/AIDS and HIV/AIDS, 2009)

2.8 GLOBAL HIV STATISTICS

It is estimated that the number of people living with HIV worldwide at the end of the year 2013 was 35 million [32.2 million – 38.8 million], an increase of 4.6% compared with the estimate published in 2008 (33.4 million [31.1 million–35.8 million]). An estimated 2.1 million [1.9 million–2.4 million] individuals worldwide became newly infected with HIV in 2013 and the estimated deaths due to AIDS-related illness was 1.5 million [1.4 million – 1.9 million], having declined by 35% down from 1.7 million [1.5 million–1.9 million] in 2011 (HIV/AIDS, 2011; HIV/AIDS, 2014).

Sub-Saharan Africa recorded the highest number of people living with HIV with an estimation of 24.7 million [23.5 million–26.1 million] people, this number represents 71% of all people who are living with HIV in the world. Asia and the Pacific had the next largest population of people living with HIV, at an estimated 4.8 million [4.1 million–5.5 million] people (HIV/AIDS, 2014).

An estimated 19 million people of the 35 million people living with HIV today do not know that they have the virus whiles 3.2 million of the 35 million people living with HIV in

2013 were children (<15 years old). Moreover, an estimated 240,000 children (<15 years) in 2013 were newly infected. Most of these children live in sub-Saharan Africa and were mostly infected by their HIV-positive mothers during pregnancy, during delivery or post partum through breastfeeding. The number of newly infected children in 2013 have fallen by 58% since 2001 (HIV/AIDS, 2014)

2.8.1 HIV Statistics in Ghana

Ghana recorded its first HIV/AIDS case in March 1986 and by the end of 1987, there were 107 HIV/AIDS positive cases. As of the end of 2003, the estimated adult prevalence of HIV was 3.1% with an estimated number of 350,000 people living with HIV/AIDS in Ghana and 30,000 AIDS-related deaths (USAID, 2003). In the year ended 2013, the estimated total number of people living with HIV/AIDS was 220, 000 [170,000-300,000] representing 1.3% of the estimated Ghanaian population. The percentage of Infected people in Ghana in 2013shows a decline in the percentage of people infected in 2011 which was 1.5%. Out of the total estimated number of Infected people in 2013, the estimated number of children (<15 years) living with HIV/AIDS was 35,000 [25,00046,000], total adult estimated number, was 190,000 [140,000-250,000] of which 110,000 [87, 000-150,000] were adult women. Estimated deaths due to this viral disease as at the end of 2013 was, 10,000 [5,000-18,000] (WHO, 2003; HIV/AIDS, 2014)

HIV prevalence rate in Ghana varies in different regions in the country. Prevalence is generally high in mining and border towns, and along main transportation routes as well as urban areas where population is dense. The lowest prevalent rate is found in the Upper West and Northern Regions with 0.8% each of the total estimated infection whiles the highest prevalence is found in the Eastern Region with 3.7%. Ashanti Region is the second

most prevalent region with 3.2% with Greater Accra Region being the third prevalent with 2.7% (HIV/AIDS, 2014).

HIV-1 constitutes 92% of HIV cases in Ghana; whiles 7.4% of reported HIV cases are dual infections with HIV-1 and HIV-2 and only 0.5% of HIV cases were exclusively HIV-2. About 80% of HIV cases are transmitted principally through heterosexual intercourse with mother-to-child transmission and transmission through blood and blood products accounting for another 15% and 5% respectively (Kelly *et al.*, 2008; HIV/AIDS, 2014).

2.9 ANTIRETROVIRAL THERAPY

There has been several efforts and trials by the scientific community to discover a vaccine and complete curative approach to the Human Immunedeficiency virus since its emergence; However, after the expenditure of much effort, currently, there is no sign on the horizon of an HIV vaccine and cure suitable for mass immunization and administration (Dybul *et al.*, 2002). This may be attributed largely due to the ability of the virus to rapidly evolve multiple antigenic variants as a result of its high mutation rate. Currently, Management of HIV/AIDS patients is done through the administration of antiretroviral therapy which was first introduced in 1986. HIV/AIDS management through antiretroviral drugs principally aims at the suppression of the viral replication. These drugs are usually administered in combination and the combination of several of such drugs, typically three or four is termed highly active antiretroviral therapy (HAART) (BHIVA Writing Committee and BHIVA Executive Committe, 2001). Since its emergence, antiretroviral agents has changed the prognosis of the HIV/AIDS disease. Results with combination therapy have been successful and have turned HIV infection into a chronic, treatable disease. Prolonged suppression of viral replication can be achieved, along with

restoration of immune function. HAART is therefore recommended for the treatment of all patients with HIV/AIDS as a lifelong activity needing distinctive strategies to ensure its effectiveness and prevent development of drug resistance and minimal side effects. Current drug regimens are often complicated and cannot be tolerated by all patients and may lead to a number of treatment failures, therefore, patients are involved in the choices of the combination therapy to ensure effective management. When HAART is discontinued or there is treatment failure, active viral replication rebounds (WHO, 2014).

2.9.1 Classifications of Antiretroviral Drugs

Different classes of antiretroviral drugs are based on their interactions with certain targeted enzymes that are virus specific and these are potential targets for the drugs.

These classes of antiretroviral drugs, act at different stages of the HIV life-cycle.

2.9.1.1 Nucleotide and Nucleoside Reverse Transcriptase Inhibitors (NRTIs) This class of antiretroviral drugs act by inhibiting reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation. Drugs in this class includes zidovudine (AZT), stavudine (d4T), lamivudine (3TC), emtricitabine (FTC), didanosine (ddl), abacavir (ABC) and tenofovir (TDF) (Weller and Williams, 2001).

2.9.1.2 Non -Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

This class of drugs acts by binding directly to reverse transcriptase and disrupting the enzyme's catalytic site. Most drugs in this class are inducers, substrates or inhibitors to varying degrees of the liver cytochrome P450 enzymes. Examples include nevirapine (NVP) and efavirenz (EFV) (Weller and Williams, 2001).

2.9.1.3 Protease Inhibitors (PIs)

Drugs in this group acts by inhibiting the viral protease, an enzyme used by HIV to cleave nascent proteins for final assembly of new virons. Inhibition of the protease yields

noninfectious virus particles. Examples are saquinavir (SQV), indinavir (IDV) and nelfinavir (NFV) (de Soultrait *et al.*, 2002).

2.9.1.4 Integrase Inhibitors

Integrase Inhibitors obstructs the activity of the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. An example is raltegravir (de Soultrait *et al.*, 2002).

2.9.1.5 Entry Inhibitors of Fusion Inhibitors

These drugs interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. They have much lower activity against HIV-2. Examples includes Maraviroc and enfuvirtide (Kilby *et al.*, 1998).

2.9.1.6 Maturation Inhibitors

These drugs act by inhibiting the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24). These viral particles have a defective core, and hence releasing virions which consists mainly of non-infectious particles . Currently, there are no drugs in this class, however, two are under investigation; bevirimat (Panacos Pharmaceuticals) and Vivecon (Kilby *et al.*, 1998; Weller and Williams, 2001).

2.9.2 Initiation of HAART

Combinations of antiretrovirals create multiple obstacles to HIV replication to keep the number of virions as low as below limits of detection and reduce the possibility of a superior mutation.

The starting point of the antiretroviral drug treatment guideline has changed many times with a more conservative approach. According to the World Health Organisation,

HIVinfected adults and adolescents should start ART when HIV infection has been confirmed and any of the following conditions is present (AIDS, 2008);

Clinically advanced HIV disease;

- WHO Stage IV HIV disease, irrespective of the CD4 cell count;
- WHO Stage III disease with consideration of using CD4 cell counts less than 350 cells mm^{-3} to assist decision making;
- WHO Stage I or II HIV disease with CD4 cell counts < 200 cells mm^{-3}

A number of detailed clinical evaluation comprising of clinical history, assessing the clinical stage of the infection, laboratory test and the patient's consent is needed prior to the initiation of HAART. For pregnant women, where the CD4 count is greater than 350, it is required to place them on ARV prophylaxis starting from 14 weeks for the purpose of PMTCT (AIDS, 2008).

2.9.3 Adverse Effects

Antiretroviral therapy regimen has a number of unwanted effects which may be experienced by patients taking antiretroviral agents. These effects vary based on the combination of ARV drugs taken, ethnicity, individual and interaction with other drugs, including alcohol (Jamjian and McNicholl, 2004). Hypersensitivity to some drugs may also occur in some individuals. several common adverse effects experienced by Participants taking some antiretroviral drugs includes alopecia (baldness), anaemia, asthenia (loss of body strength), diarrhoea, dizziness (vertigo), flatulence, abdominal pains, headache, hepatitis, xeroderma (dry skin), xerostomia (dry mouth), hyperbilirubinaemia, hypercholesterolaemia, dyslipidemia, hyperlipidemia,

hyperpigmentation (of nails, palms or soles), ingrown nails, insomnia, jaundice, lipodystrophy, liver failure, flu-like syndrome, renal dysfunction, malaise, mental confusion, migraines, mitochondrial toxicity, mood swings, myalgia, myalgic encephalomyelitis (chronic fatigue syndrome), myopathy, nausea, neutropoenia (low number of white blood cells), nightmares, oral ulcers, pancreatitis, paresthesia (numbness), peripheral neuropathy, rash, renal failure or insufficiency, somnolence (drowsiness), change in taste perception, loss of appetite and vomiting (Jamjian and McNicholl, 2004).

2.10 HIV AND NUTRITION

HIV and nutrition are closely related to each other and may exacerbate one another in a vicious cycle. Despite developments in medical treatment, nutrition remains a key component in managing HIV patients because, many HIV-related conditions affect and are affected by the body's nutritional status. Nutrients play a big role in immune functions and serves to strengthen and protect the immune system as well as the many generalized aspects of host defense. Nutrients that play significant role in immune function include: protein, total energy, lipids, amino acids, vitamins and minerals (Baum *et al.*, 2000).

Macronutrients such as proteins play roles as structural components of tissues as well as antibodies, cytokines, acute-phase proteins and components of the complement pathways. Therefore alterations in these macronutrients could lead to immunologically important changes in enzymes dependent activation, antioxidant protection, antibodymediated virus neutralization and intercellular communication through cytokines (Keen and Gershwin, 1990). In the case of HIV patients, any immune impairment due to the viral disease may strongly lead to poor nutrition and inadequate nutrition leads

to immune dysfunction which worsens the effect of HIV and contributes to more rapid progression to AIDS, thus creating a vicious cycle. From the point of infection, HIV acts by replicating inside the white blood cells through the stages of the window period, through sero-conversion to asymptomatic and symptomatic phases. To eliminate the infection, the immune system plays a significant role in identifying and destroying this infection. The cells that mediate immunity include lymphocytes. Among these, CD4 cells are critical to the immune system. Both the immune system and the levels of the nutrients are correlated with the progression of the disease. This implies that inadequate nutrition results in increased replication of HIV and the former is a result of HIV itself (Keen and Gershwin, 1990).

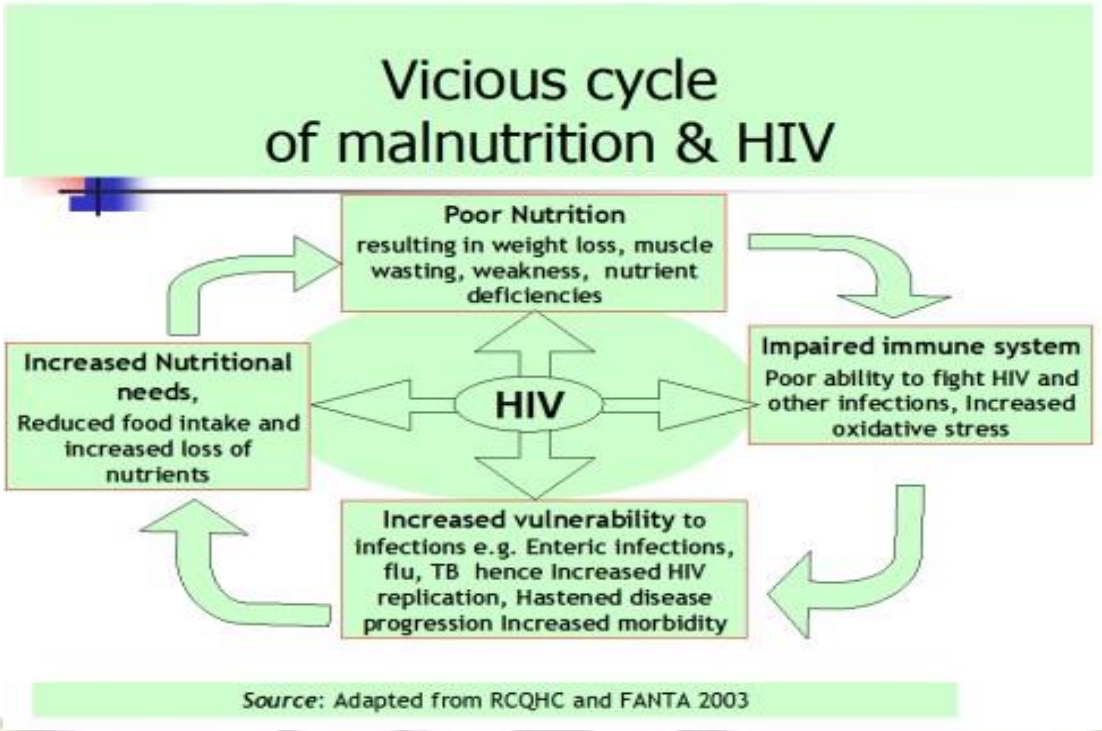
Moreover, during HIV infections, there is an acceleration of the release of pro oxidants, cytokines, and other reactive oxygen species that results in the increase utilization of antioxidants such as vitamins E, C, beta-carotenes and micronutrients such as iron, zinc, selenium, manganese and copper (Friis, 1999). A negative balance between these pro oxidants and antioxidants leads to oxidative stress which further damages the immune cells, proteins and enzymes, thus accelerating HIV replication (Schwarz, 1996).

As basal metabolic rate increases due to increased utilization of micro and macro nutrients to attack the HIV infection, the body mobilizes fats and proteins later on resulting in weight loss, muscle wasting, weakness and nutrient deficiencies. In advanced stages in HIV infection, opportunistic infections occur due to a decreased immune function. These opportunistic infections may present symptoms such as diarrhea, fever, vomiting, anorexia, mouth sores and lesions that interferes with ingestion, digestion and absorption and necrosis of the gastro intestinal tract set in. Poor nutrient absorption prevents the body

from using the nutrients provided by foods and contributes to energy and nutrient losses, which will increasingly hinder the capacity of people living with HIV/AIDS to meet their increased nutritional needs. If malabsorption of nutrients is not properly addressed, the deficit in energy and nutrients will increase and further weaken the person and their immune system and speed up the progression of the disease (Schwarz, 1996).

Therefore, the nexus between HIV/AIDS and inadequate nutrition is a typical example of the vicious cycle of immune dysfunction, infectious diseases, and malnutrition. As illustrated in figure 2, the immune system could be weakened when there is inadequate nutrition and this could increase vulnerability to infections and may speed up the progression of HIV disease (Hart, 2006).

Figure 2.2 The vicious cycle of malnutrition and HIV



Evaluation and enhancement of patients' nutritional status may help correct or compensate for deficiencies in cases such as weight loss or nutrient deficits, and may be a key treatment modality for certain conditions such as dyslipidemia, and may help to maintain good health and immune function (Hart, 2006).

2.11 MALNUTRITION

Malnutrition is classically defined as the health disorders due to too much or too little food energy or nutrients. Thus malnutrition includes over nutrition and under nutrition (WHO, 1995). Over nutrition is excessive intake of nutrients, mostly macronutrients and calories which usually results in obesity and overweight and increases risk of many chronic diseases. However in most cases, the term malnutrition is used to refer to under nutrition where there are not enough calories, macro and micronutrients usually resulting in underweight which are most prevalent in HIV/AIDS patients (WHO, 1995).

2.11.1 Undernutrition

Under nutrition is the lack of food energy and nutrients to satisfy physiological requirements. It is the sub-optimal supply of a nutrient that interferes with an individual's growth, development or maintenance of health. Under nutrition could be further subdivided into two classifications namely; Protein Energy malnutrition and micronutrient deficiencies. General signs and symptoms of inadequate nutrition include: loss of fat (adipose tissue), oedema, breathing difficulties, depression, hypothermia (abnormally low body temperature), pancytopenia, longer recovery times from infections, impotence, reduced muscle mass (WHO, 1995).

2.11.2 Protein Energy Malnutrition.

This form of under nutrition arises from inadequate energy and protein supply to cells in body to satisfy physiological requirements. Protein is essential for the proper production and functioning of the hemoglobin and red blood cells. In Protein Energy Malnutrition there is reduction in cell mass and energy requirenment which may lead to hypochromic normocytic anaemia. Symptoms of Protein Energy Malnutrition include low weight, stunted growth and oedema. Kwashiorkor and marasmus are the most common clinical presentations of Protein Energy malnutrition. (Hsu *et al.*, 2005).

2.11.3 Micronutrient deficiency

Micronutrient deficiency arises from inadequate vitamin and mineral supply to cells in body to satisfy physiological requirements. This includes deficiencies in micronutrients such as zinc, selenium, calcium, iron, vitamin A, vitamin D, vitamin C, niacin, thiamine

and iodine. The table below shows the clinical presentations of the various micronutrient deficiencies (Hänsch and Mendel, 2009).

Table 2.2 Micronutrients functions and Deficiencies

Vitamins	Principal function	Deficiency effect
VitaminA	Enhances Vision	Night blindness
VitaminD	Increases Calcium absorption and bone formation	Rickets and osteomalacia
Niacin	Component of NAD cofactor in metabolism	Pellagra
Folic acid	Intermediary in metabolism	Megaloblastic anaemia
VitaminB12	Co factor in nucleic acid synthesis	Megaloblastic anaemia
VitaminC.	Collagen formation	Scurvy, anaemia
Selenium	Component of glutathione peroxidase, antioxidant	Keshan Disease
Iodine	Thyroid hormone synthesis	Goitre, thyrotoxicosis
Calcium	Coagulation and bone metabolism	Osteoporosis, rickets
Zinc	Protein and nucleic acid synthesis	Growth retardation.
Copper	Transport and metabolism of iron	Wilsons disease

2.11. 4 Factors affecting Nutritional status in HIV/AIDS Patients

Factors that have been found to be associated with malnutrition in HIV/AIDS patients include: Socio economic factors, socio demographic factors, health related factors and antiretroviral therapy.

2.11.4.1 Socioeconomic factors

A number of studies conducted shows that nutritional status is influenced by socioeconomic factors especially in Africa where poverty and famine is on the ascendency ((De Waal and Whiteside, 2003; Torheim *et al.*, 2003; Savy *et al.*, 2005). Occupation and household income as proxy indicators for socio-economic status has been found to be strongly associated with access to adequate food intake/food security (Ajani *et al.*, 2006). Quantitative results from a US survey to establish the relationship between income and food access and insecurity indicated that lower income respondents were more likely to experience food insecurity and inadequate nutrition whereas high income respondents are more likely to experience quality access to food and adequate nutrition (Vozoris and Tarasuk, 2003). Another study by (Turnell, 2004) on socioeconomic patterning of food access and purchasing shows that those employed in blue-collar (manual) occupations and residents of low income households purchased fewer types of fruit and vegetables, and less regularly, than their higher status counterparts in white collar profession.

2.11.4.2 Sociodemographic factors

Access to variety of quality food has been found to be associated with urban residence (Clausen *et al.*, 2005). Other studies have established that urban residents have higher consumption frequencies for all food categories than rural residents (Holcomb, 1995)and that urbanization is accompanied by an improvement in micronutrient intakes (Vorster *et al.*, 2005). In related studies, married individuals have been found to consume more servings of snacks/desserts, but fewer servings of alcoholic beverages than those who were unmarried (Deshmukh-Taskar *et al.*, 2007). There is also evidence of an obvious difference in nutritional status between the ethnic groups and religion. Muslim

women have been found to have less access to quality food varieties than Christian women (Savy *et al.*, 2005). In 2005 a study by Sebastian established that higher education is strongly associated with quality household food access and security. Higher education is associated with regular consumption of a wider variety of foods than less educated individuals (Holcomb, 1995; Cordain *et al.*, 2005).

2.11.4.3 Health Related Factors

Studies show that food intake is associated with existing health conditions of the individual. A cross sectional study in Abidjan with 100 HIV-infected respondents at different stages of the infection showed that dietary intakes of HIV-infected respondents are aggravated by clinical conditions such as anorexia, catabolism, chronic infection, fever, nausea, vomiting, diarrhea, mal-absorption, metabolic disturbances and depression which are mostly due to opportunistic infections (Strathdee *et al.*, 2006). Further studies with 119 respondents to assess the correlation between micronutrients intakes and immune status in HIV infected respondents established that Vitamin A, and D intakes were correlated with increase in CD4+ count (De Luis *et al.*, 2001). However in a South African Hospital, a cross-sectional study with eightyone HIV/AIDS respondents in different stages of disease, found that there was no association between disease stage and nutritional status or more advanced disease and micronutrient deficiencies (Dannhauser *et al.*, 1999), although it is confirmed that

HIV/AIDS respondents from this population were malnourished.

2.11.4.4 Anti-retroviral Therapy

Side effects of antiretroviral therapy are known to influence intake of food. Although most of the antiretroviral drugs require adequate amount of food intake for optimal reactions,

several side effect presented by these drugs may in turn influence the food intake of the individual. Side effect of antiretroviral drug largely depends on the type of antiretroviral therapy. Common side effect of antiretroviral therapy include: Diarrhoea, vomiting, nausea, loss of appetite, lipodystrophy, dizziness, skin rash and glucose intolerance (Ammassari *et al.*, 2001). These effect may lead to reduction of the quality of food intake as well as frequency of food intake leading to body wasting. However, a recent study conducted in Maryland indicates that being overweight or obese are now more prevalent than wasting (Crum-Cianflone *et al.*, 2008).

2.12 NUTRITIONAL ASSESSMENT IN HIV/AIDS POSITIVE ADULTS

Nutritional assessment in HIV/AIDS patients is necessary to determine nutritional interventions and antiretroviral therapy regimen. There are several nutritional assessment tools such as Subjective Global Assessment (SGA) and Malnutrition Universal Screening tool (MUST). However, there is no single measurement that has emerged as a

'goldstandard' in assessing nutritional status, making a diagnosis of malnutrition challenging (Carey and Gillespie, 1995).

For a comprehensive assessment of nutritional status especially in HIV/AIDS patients, a combination of the various assessment tools has been found to be more useful and thus recommended. Generally nutritional assessment for HIV/AIDS patients includes: Anthropometry, Diet history and intake, Clinical assessment and history, Social history, Biochemical and haematological laboratory assessment (Shevitz and Knox, 2001).

2.12.1 Anthropometry

Anthropometry is a comparative measurement of the body, which is used to study and understand body composition and physical variation in nutritional assessment. Common anthropometric measurements used in nutritional assessment of HIV/AIDS adults include: height weight, Body mass index (BMI), waist/hip ratio, percentage of body fat, as well as skin fold measurements such Mid upper arm circumference (MUAC), triceps skin fold (TSF), Biceps skin fold (BSF), mid arm muscle circumference (MAMC) and wrist circumference. Other measurements are subscapular , and suprailiac skin folds and total upper arm area. BMI is known to express body weight in relation to height and classifies individuals as underweight ($<18.5 kg/m^2$, normal weight ($18.5-24.9 kg/m^2$), overweight ($25-29.9 kg/m^2$) or obese ($\geq 30 kg/m^2$) (WHO, 1995). Moreover, waist to hip ratio is also known to express the distribution of body fat or central obesity whereas skin fold measurements at three or four body sites is known to be useful in estimating the percentage body fat (WHO, 1995). Anthropometry in HIV/AIDS adult patients is very useful in determining

weight and fat loss or gain as well as response to antiretroviral therapy regimen and nutritional interventions (Shevitz and Knox, 2001).

2.12.2 Diet History and intake

A food record of seven (7) days is considered the "gold standard" of dietary intake assessment. This shows the quality of food taken over a period of seven days, the frequency of diet, and the quantification of the micro and macro nutrients depending on the quantity of food consumed as compared to the standard recommended daily allowance (RDA). This measurement is known to give a reliable information concerning the quality of food consumed to determine sufficiency or deficiency of nutrients in the body (Lee *et al.*, 2008).

Moreover a 24 hour diet recall is another assessment for dietary intake. This measurement takes into consideration the quantity, frequency and quality of diet over the last 24hrs of the patient. Though less accurate as compared to the seven day diet history due to day to day dietary variations, the 24 hour diet recall is known to produce substantive information concerning the general nutritional status of the patient. In all cases questionnaire and data sheets are administered (Lee *et al.*, 2008). Both measurements measures the amount and frequency of food intake but do not measure the absorption of the food in the gastrointestinal tract (Lee *et al.*, 2008)

2.12.3 Clinical History and Assessment

This component of nutritional assessment includes a medical history and a physical examination to identify signs of or contributors to malnutrition. Vital areas in the clinical assessment include physical appearance, evaluation of opportunistic infections and comorbid conditions, occurrence of diarrhea, vomiting, symptoms of gastrointestinal

disturbances, malabsorption, oedema, ascites and medications. These signs are known to provide crucial information in nutritional assessment as these signs are common in malnourished patients. The various side effect of medications including antiretroviral agents play a significant role in determining the nutritional assessment and intervention of HIV/AIDS patients. Other medications that may have an effect nnutriional status are Alcohol use, herbal medications and marijuana use (Shevitz and Knox, 2001; McCaffree, 2003).

2.12.4 Social History

The social history of patients takes into consideration the assessment of certain factors and resources that may affect an individual's ability to obtain, prepare, and eat food in evaluating nutritional risk factors. Key areas under social history includes; occupation, education level, living and cooking arrangements, mental status due to stigmatization, as well as age, sex, level of physical activity and daily living activities (Knox *et al.*, 2003; Strathdee *et al.*, 2006). All these factors are known to affect an individuals accessibility to quality food and intake. Research has shown that the stigma associated with the HIV/AIDS disease, may prevent people being employed in organisations and hence affecting their financial status leading to poor economic state and its subsequent effect on access to quality food intake. This however may depend on the level of education and the general health of the patient (Narayan-Parker, 1999; Lundberg *et al.*, 2000).

2.12.5 Biochemical and haematological laboratory assessment

The laboratory provides measures of biochemical and hematological assessment that are used to complement the medical history, social history and the dietary history in assessing the general nutritional status of HIV/AIDS patients.

2.12.5.1 Biochemical parameters

Biochemical assessment measures macro and micronutrients as well as liver function and lipid profile tests that are required in nutritional assessment. Common tests usually includes albumin, prealbumin, magnesium, vitamin levels such as Vitamins B12 and B6, trace elements such as zinc, selenium and copper, cholesterol, triglycerides, fasting glucose, CD4, CD8, virus load of HIV, renal function, and liver enzyme levels (McCaffree, 2003; Lee *et al.*, 2008). Studies by Chlebowski et al. showed that serum albumin levels predicted survival. Micronutrient deficiencies are common in HIV infection. Deficiencies in serum vitamin A, vitamin B, selenium, and zinc, in particular, have been associated with progression of HIV Infection. Thus, measurements of serum proteins and micronutrients can predict outcome and may identify correctable deficiencies (Chlebowski *et al.*, 1989; Süttmann *et al.*, 1995).

Lipodystrophy, or the syndrome of fat redistribution, which could be measured by lipid profile tests including cholesterol, triglycerides, has been described in HIV infection and may be related to antiretroviral therapy. Lipodostrophy is known to increase the risk of HIV patients to cardiovascular diseases. Regional measures of fat is required to detect changes in fat distribution and to plan intervention strategies (Carr *et al.*, 2004; Kaufman *et al.*, 2007).

A low serum albumin level may indicate poor nutrition, and has been shown to predict both death and length of stay in hospitalised HIV-positive patients. Other factors that are known to cause low albumin levels in HIV patients are liver disease with decreased protein synthesis, renal disease with protein loss (albuminuria), enteric infections with chronic diarrhoea and malabsorption. In addition, as an acute-phase reactant, a low albumin level may simply behave as a marker of an active inflammatory state in HIV/AIDS patients (Wood and Souba, 1997; Nerad *et al.*, 2003).

Moreover, Liver-related disease has become a significant cause of death of patients on long-term Anti retroviral therapy such as nevirapine as well as alcohol abuse. Elevated transaminases, such as ALT and AST are known to accompany liver damage. Additionally, elevated Alkaline phosphatise (ALP) and gamma-gluteryl transferase (GGT) are known to suggest an infiltrative process including the presence of hepatic granulomas such as Tuberculosis or cotrimoxazole-induced hepatitis in HIV/AIDS patients (Kreisberg, 1995; Mocroft *et al.*, 2005).

2.12.5.2 Haemtological parameters

Haematological measurements primarily seeks to determine nutritional anemias in HIV/AIDS patients. Parameters most commonly used in haematological nutritional assessment includes; full blood counts (including hemoglobin, MCV, MCH, MCHC and differential white cell counts), serum iron, total ironbinding capacity, transferrin, ferritin and folic acid (Semba and Gray, 2001).

Common nutritional deficiencies associated with anaemia are iron, folate and vitamin B12. However the most common cause of anaemia in HIV-infected patients is 'the anaemia of chronic disorders', an anaemia seen in many chronic inflammatory or infective conditions

which may not be necessarily related to nutrional deficiency. Several studies conducted shows that the hemoglobin and hematocrit are generally decreased in both cases of nutritional anaemias and in 'the anaemia of chronic disorders'(Semba and Gray, 2001; Weiss and Goodnough, 2005).

A measure of the size of the red cell, known as Mean Corpuscular volume (MCV) is usually normal in the anaemia of chronic disorders (normocytic anaemia), while in iron deficiency the red cells are known to be small and the MCV is less than normal (microcytic anaemia). Studies conducted shows that in folate and Vitamin B12 deficiencies, the MCV will usually be elevated (macrocytic anaemia) (Boelaert *et al.*, 1996; Spivak, 2000). In patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology. Vitamin B12 deficiency may result from malabsorption in the ileum or from gastric pathology caused by an patients (Harriman *et al.*, 1989).

Other causes of anaemia that are occasionally present includes; red cell haemolysis, drug-induced toxicity which is caused mostly by antiretroviral agents such as zidovudine and combivir, bone marrow infiltrate (e.g. tumour or infection such as TB) or infection with HIV itself. The hematological laboratory data is therefore known to be crucial in nutritional interventions associated with anemias. Further studies has shown that not every HIV-infected person may require iron supplementation because most have an excess of storage iron (ferritin), and added iron may be harmful and thus the urgent need for heamatological investigations before nutritional interventions. Nevertheless during pregnancy and lactation supplementation with iron, folate and multivitamins is generally given to both HIV-infected and non-infected women. Moreover, low total White blood cell count as well

as neutropenia and decreased lymphocyte count have been recorded in immunocompromised Participants which may initiate a visious cycle of HIV/AIDS and malnutrition (Boelaert *et al.*, 1996; Gangaidzo *et al.*, 2001; Semba and Gray, 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY SITE

This cross sectional study was conducted at the antiretroviral (ART) clinics at Bomso specialist clinic, Bomso and ST. Michael's Hospital, Jachie all in the Ashanti Region of Ghana. Ashanti Region is centrally located in the middle belt of Ghana and lies between longitudes 0.15W and 2.25W, and latitudes 5.50N and 7.46N. As the third largest region in Ghana, it covers an area of about 24,389 square kilometers. The region's population is

at 2002 was 3,612,950, representing 19.1 per cent of the country's population. The capital town, Kumasi is cosmopolitan in nature with inhabitants hailing from all over the country and accounts for nearly one-third of the region's population.

Bomso Specialist clinic is a private owned clinic located at Bomso, approximately, 10 Kilometers from Kumasi. It serves a catchment area of approximately 60,000 people between Ayigya, kentinkrono, Sesanso and people living around KNUST campus who are predominantly rural occupants. Main economic activities are farming and trading with few staff and students of KNUST in the area. Vegetation of the catchment area is mainly tropical rain forest with plantain, cassava and maize being the major crops produced. Literacy is still relatively low in the area.

ST. Michael's Hospital is a catholic mission Hospital located at Pramso, west of Kumasi in the Ashanti Region of Ghana. Twi is the predominant language of the people. It serves a catchment area of approximately 135,000 between Kumasi, Dunkwa and the people around the lake District (Lake Bosomtwe) who are predominantly rural in setting. The main economic activities are fishing and farming with Plantain, cassava, yam and cocoyam as the major crops produced.

3.2 STUDY POPULATION

A total of 287 confirmed HIV positive adults aged 18 to 60 years were recruited in the study. This consisted of 108 highly active antiretroviral therapy (HAART) naïve Participants of which 41were males and 67 were females and 179 Participants on HAART of which 48 were males and 131 were females. HAART use was defined as receipt of one

or two nucleoside reverse transcriptase inhibitors (NRTI) and one nonnucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI).

3.2.1 Inclusion criteria

- Participants should be confirmed to be positive for HIV (HIV-1, HIV-2 or both)
- Participants should not be pregnant at the time of sampling
- Naïve participants should not be on any medication (antibiotics, vitamin supplements, contraceptive pills and tuberculosis (TB) treatment) for at least three months prior to the time of sampling.
- Participants should be clinically stable with no active or symptomatic opportunistic infections.
- Participants on HAART should be on a backbone therapy of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors for at least 3 months.

3.2.2 Exclusion Criteria

- Participants taking alcohol or smoking marijuana or cocaine were exempted from the study.
- HIV Participants who are severely ill including those in the In Patient Department.

3.3 SAMPLE COLLECTION AND PREPARATION

A total volume of 10 ml of venous blood was collected from the antecubital vein of each particpant under aseptic conditions. A volume of 5 ml of the blood sample was placed into vacutainer plain tube and allowed to clot. The sample was then centrifuged at 3000×g

for 10 minutes and the serum separated were immediately used for the determination biochemical assays.

The remaining 5 mls of blood sample was dispensed into two vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA); 2 mls and 3 mls of blood were dispensed respectively. The 2 mls sample was used for immunological analysis (CD4 estimation) and the 3 mls sample used for haematological analysis.

3.4 ASSAY PROCEDURES

3.4.1 Immunological analysis

Immunological assay was performed to determine the absolute cell count of CD4 Tlymphocyte in unlysed whole blood by the use of the Becton Dickinson (BD) FACSCount system (Becton Dickenson and Company, California, USA).

.1.1 Principle of operation

FACSCount is a complete self- contained system incorporating instrument, reagents, controls, and software. It uses whole blood, eliminating lyse and wash steps. The FACSCount System consists of the following key components: FACSCount Instrument and System software, Unit test reagent pairs, tube of CD4/CD3 reagents and reference beads, tube of CD8/CD3 reagents and reference beads, Fixative solution (5% formaldehyde), Control beads solutions (four levels—zero, low, medium, and high) and FACSCount automated pipette. The system uses flow cytometry for the quantification of T Lymphocytes population.

When whole blood is added to the reagents, the lymphocyte surface antigens bind specifically to fluorochrome-labelled monoclonal antibodies in the reagents. After a fixative solution is added to the reagent tubes to preserve the integrity of the antibody binding, the sample is run on the instrument. The cells now come in contact with the laser light, which causes the fluorochrome-labeled cells to fluoresce. The fluorescent light provides the necessary information for the instrument to count the cells. The reagent tubes also contain a known number of fluorochrome-integrated reference beads in addition to containing the antibody reagent. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitation standard for calculating the cells. The software automatically identifies T-lymphocyte populations and calculates the absolute counts.

3.4

This system of operation is the recognized gold standard for CD4 testing, and is used to stage HIV/AIDS, guide treatment decisions for HIV-infected persons, and evaluate effectiveness of therapy.

3.4.1.2 Classification of HIV stages

Classification of CD4 count based on the Center for Disease Control (CDC) criteria was used to classify the study population into three categories : Stage 1 (CD4 ≥500 cells mm3), Stage 2 (CD4 between 200 – 499 cells mm-3) and Stage 3 (CD4 <200 cells mm3).(Control and Prevention, 2012).

3.4.2 Haematological Assays

Haematological parameters including white blood cell count (WBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), were determined by an automated blood multi-parameter haematology analyzer CELL-DYN®1800 (Abbott Laboratories Diagnostics, Abbot Park, Illinois, USA)

3.4.3 Mode of Operation of the CELL-DYN(R) 1800

The CELL-DYN(R)1800 aspirates approximately 30 µL of whole blood from an open collection tube that has been held under the sample Aspiration Probe, after which 7.5 ml of diluent is added in a pre-mixing cup to achieve a dilution ratio of 1:251. The CELLDYN(R) 1800 uses two independent measurement methods which are Electrical Impedance Method for determining white blood cells (WBC), red blood cells (RBC), and Platelets(PLT) data and Modified Methemoglobin Method for determining Haemoglobin.

.3.1 WBC measurement

WBC measurement is based on the principle of Electrical impedance. The 1:251 WBC/Hb dilution is delivered to the WBC mixing chamber where it is bubble-mixed with 1.0 mL of lyse reagent. A metered volume of the lysed sample is drawn through the aperture of the von-Behrens WBC transducer. The WBCs were then counted by impedance. As each cell is drawn through the aperture, there is a change in electrical resistance which generates an equivalent voltage pulse. The number of pulses sensed during each cycle corresponds to the number of white cells counted. The amplitude of each pulse is essentially proportional to the cell volume. If the pulse generated is above the WBC lower threshold, it is counted as a WBC. Cells that correlates to lymphocytes are included in the small cell subpopulation whiles cells correlating to granulocytes (neutrophils) are included in the large cell population. The remaining cells that correlates to monocytes, basophils, eosinophils, blasts and other precursor white cells are generally included in the mid-size cell population.

3.4.3.2 RBC and PLT Measurements

An additional 5 mL of diluent is mixed with 100 µL of the 1:251 diluted sample in the red blood cell (RBC)/platelet (PLT) mixing chamber creating a dilution ratio of 1:12801 which is analyzed to generate results for the red blood cell and platelet parameters. Electrical impedance method is used to obtain RBC/PLT data. As cells pass through the aperture of the von-Behrens RBC/PLT Transducer they are counted and sized.

Precise volume of the diluted specimen is drawn through the aperture into the counting chamber. Pulse generated above the PLT lower threshold, is counted as a PLT whereas, the pulse generated above the RBC lower threshold, is counted as an RBC.

3.4

3.4.3.3 Haemoglobin Measurement

Haemoglobin measurement is by colorimetric determination using a modified methaemoglobin method. A portion of the lysed, diluted sample from the WBC mixing chamber is used for Haemoglobin measurement. A low-energy light-emitting diode (LED) is used as the light source and shines through the Haemoglobin flow cell and a 540 nm narrow-bandwidth filter onto a photo detector. The Haemoglobin concentration is directly proportional to the absorbance of the sample.

3.4.4 HCT, MCV, MCH and MCHC Determination

3.4.4.1 Haematocrit (HCT)

Haematocrit is the portion of whole blood occupied by the red blood cells expressed as a ratio. It is calculated from the RBC count and the MCV as follows:

$$HCT = \frac{RBC \times MCV}{10}$$

3.4.4.2 Mean Cell Volume

The mean cell volume provides information on red cell size-distribution. It is the average volume of individual RBCs expressed in femtoliters (fl). It is calculated from the RBC count and HCT as follows;

$$MCV = \frac{HCT \times 10}{RBC}$$

.4.4 Mean Cell Haemoglobin (MCH)

The Mean Cell Haemglobin provides information on the average amount of haemoglobin in a red cell expressed in picograms (pg). It is calculated from the RBC and HGB as follows:

$$\text{MCH} = \frac{\text{HB}}{\text{RBC}} \times 10$$

3.4.4.5 Mean Cell Haemoglobin Concentration (MCHC)

The Mean Cell Haemoglobin Concentration (MCHC) is the ratio of the weight of Hb to the volume of the average RBC. It is calculated from the Hb and the HCT as follows:

$$MCHC = \frac{Hb}{HCT} \times 100$$

3.4.4.6 Anaemia and types of Anaemia

The World Health Organization/Aids Clinical Trial Group (WHO/ACTG) anaemia toxicity grades were used to define anaemia in study participants as Grade 1 (9.5-10.5g/dl), Grade 2 (8.0 -9.4g/dl), Grade 3 (6.5-7.9g/dl) and Grade 4(<6.5).

Classifications of types of aneamia was based on estimated values for red cell size and haemoglobin content of red cells using the mean cell volume (MCV) range of 80 – 96 fl and mean cell haemoglobin (MCH) range of 27 – 32 pg as generated from the complete blood count (CBC) analyser where low MCV (<80 fL) was indicative of microcytosis, high MCV (>96 fL) indicates macrocytosis and low MCH (<27 pg) indicates hypochromia.(WHO Case Definitions, 2007).

3.4

3.5 BIOCHEMICAL ASSAYS

All biochemical analyses were determined using Mindray® BS-120 chemistry autoanlayzer (Shenzhen Mindray Bio-Medical Electronics Companies, Shenzhen, China).

3.5.1 Principles for Biochemical Assays

3.5.1.1 Total Protein (TP)

Total protein determination in this study, was based on Biuret colour reaction first described by (Riegler, 1914). This reaction is based on based on a colour reaction of protein molecules with cupric ions. This procedure was later modified by (Weichselbaum, 1946) and (Gornall et al. , 1948) where sodium potassium tatrate was introduced to stabilise the cupric ions in the alkaline reagent. Therefore Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the resulting violet colour is proportional to the amount of proteins present when compared to a solution with known protein concentration.

$$Protein + \xrightarrow{+} Colour\ complex$$

3.5.1.2 Albumin

The method used for this assay is based on that of Doumas *et al.*, (1971) where at a Controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of the resulting colour at 630 nm is directly proportional to the albumin content.

$$BCG + Albumin \xrightarrow{\text{controlled } pH} Green\ BCG\text{-}Albumin\ complex$$

3.5.1.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase testing, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is based on the Kinetic Photometric test.

In this reaction, serum alkaline phosphatase hydrolyses colourless p-nitrophenyl phosphate (pNPP) which produces a yellow coloured p-nitrophenolate and phosphate at a pH of 10.3 at 37^{o} C. The resulting absorbance increase is measured at 405 nm.

$$\text{p-nitrophenyl phosphate} + H20 \xrightarrow{ALP} \text{p-nitrophenol} + \text{phosphate}$$

3.5.1.4 Alanine Aminotransferase (ALT)

Assaying for Alanine aminotransferase (ALT) is based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommended method (Schumann et al., 2002). This method utilizes the LDH-NADH coupled assay in which ALT catalyzes the transfer of the amino group from L-alanine to 2-oxoglutarate which results in the formation of pyruvate and L-glutamate. There is a further reduction of pyruvate and a simultaneous oxidation of NADH to NAD^{+} by the catalytic action of Lactate dehydrogenase (LDH). The ALT activity is proportionate to the resulting rate of decrease in absorbance measured at 340 nm. During the initial incubation period, there is a complete and rapid reduction of the endogenous sample pyruvate by Lactate dehydrogenase (LDH) to prevent any interference with the assay.

$$\text{L-alanine} + \text{2-oxoglutarate} \xrightarrow{ALT} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + NADH + H^{+} \xrightarrow{LDH} \text{L-Lactate} + NAD^{+}$$

3.5.1.5 Aspartate Aminotransferase (AST)

The principle of test for AST involves AST catalyzing the transamination from aspartate to 2-oxoglutarate, resulting in the formation of oxaloacetate and glutamate (IFCC, 1986). The resulting oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD^{+} in the malate dehydrogenase (MDH) catalyzed indicator reaction. AST activity is directly proportional to the resulting rate of decrease in absorbance at 340 nm.

$$\text{L-aspartate + 2-oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + L-Glutamate}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow{\text{MDH}} \text{L-malate + NAD}^{+}$$

3.5.1.6 Gamma Glutamyl Transferase (GGT)

GGT is assayed using the kinetic photometric test according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Shaw *et al*., 1983). In this reaction, serum GGT, catalyzes the transfer of the Glutamyl group from LGamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. Gamma glutamyl transferase (GGT) activity is proportional to the amount of 5-amino-2-nitrobenzoate formed which is measured kinetically at 405 nm by the increasing intensity of the resultant yellow colour formed.

$$\text{L-}\boldsymbol{\gamma - glutamyl - 3 - carboxy - 4 - nitroanilide + Glycylglycine} \xrightarrow{GGT}$$

$$\text{L-}\boldsymbol{\gamma - glutamyl - glycylglycine + 5 - amino - 2 - nitrobenzoate}$$

3.5.1.7 Blood Urea Nitrogen (BUN)

The method for BUN assay is based on the Urease/Glutamate dehydrogenase (GLDH) (Talke *et al*., 1965). In this reaction, Urea is hydrolyzed to carbon dioxide (CO2)and ammonia (NH3) in the presence of water and urease. The resulting ammonia reacts with α-ketoglutarate to form L-Glutamate and NAD^{+} in the presence of NADH and Glutamate dehydrogenase. The absorbance at 340 nm decreases, as the reaction proceeds. The amount of urea in the sample therefore is proportional to the initial rate of this change.

$$\text{Urea + } \boldsymbol{H_2O} \xrightarrow{\text{Urease}} 2NH_3 + C0_2$$

$$NH_3 + \alpha - \text{ketoglutarate} + NADH \xrightarrow{GLDH} \text{L-Glutamate} + NAD^+$$

3.5.1.8 FERRITIN

Ferritin was assayed using the fortress diagnostic ferritin test kit which is based on immunoturbidimetry. In the reaction, Latex particles are coated with anti human ferritin. Serum ferritin reacts with the coated latex particles resulting in agglutination. Turbidity formed as a result of the latex particle agglutination is proportional to the concentration of the ferritin in the serum and is measured at a wavelength of 700nm.

3.5.1.9 TRANSFERRIN

Serum transferrin was assayed using fortress diagnostics transferring test kit based on immunoturbidimetric procedure. In this reaction, Serum transferrin reacts with anti human transferrin which results in an insoluble immune complex. The immune complexes cause an increase in light scattering due to turbidity formation. Transferrin concentration is proportional to the turbidity formed at 700nm.

3.5.2.0 ZINC

Zinc determination is based on colorimetric method (Saito *et al*., 1982) and was assayed using fortress diagnostics zinc test kit. Serum Zinc is chelated by 2-(5-Brom-2pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol to form a coloured chelate complex. The increase of absorbance is measured. Serum zinc concentration is proportional to the increasing intensity of the resulting colour formation at 560nm.

3.6 ANTHROPOMETRY

3.6.1 Body Mass Index (BMI)

Body Mass Index was determined by dividing the weight over the square height

(Weight/Height^2 ,kg/m^2) developed by the Belgian statistician Adolphe Quetelet (Nikolaos *et al*., 2010). Height was determined using a height stick (stadiometer) where Participants were asked to stand upright with shoes removed. Weight was also determined using clinical weighing scales whereby Participants were asked to stand upright on the scales with shoes removed.

3.6. 2 Mid upper arm circumference (MUAC)

MUAC was determined by locating the midpoint between the top shoulder and the elbow whiles in an upright position and measuring circumference using a measuring tape . The result was expressed in Centimeters (cm).

3.6.3 Triceps and Biceps Skin folds (TSF/BSF)

Triceps and biceps skin folds were determined using skinfold calipers (Durman and Womersley, 1974). In a upright and relaxed state, the skinfold calipers was used to measure the fold of the biceps (front of the arm) and triceps (back of the arm) accordingly.

3.6.4 Waist to Hip Ratio (WHR)

Waist to Hip ratio is the ratio of the Waist circumference to the Hip circumference. To determine the waist circumference, a measuring tape was placed in a horizontal plane around the abdomen at the level of the iliac crest at a fixed position with patient inhaling and exhaling at a normal rate. The Hip circumference was determined by placing measuring tape around the buttocks at the level between the waist and the thigh at the hip joint (Wood and Souba, 1997)

3.7. DIET FREQUENCY AND SOCIODEMOGRAPHIC FEATURES

Data on diet frequency and sociodemographic features such as age, gender and occupation were obtained through semi structured interviewer administered questionnaire.

3.8. HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) USE

HAART use was defined as a combination therapy of one or two nucleoside reverse transcriptase inhibitors (NRTIs) and one or two non-nucleoside reverse transcriptase inhibitors. Data on the HAART usage was collected from participant's medical records from the hospitals and confirmed by the participants through the questionnaires in the study.

3.9 STATISTICAL ANALYSIS

Data were entered in Microsoft excel 2007 then imported to SPSS version 20.0 and the alpha level (α) was set at 0.05. Data was analysed quantitatively using descriptive and inferential statistics. Continuous data are expressed as mean ± SEM whilst categorical data are expressed as proportions. Unpaired t-test was used to compare the means and chi square test was used to compare proportions and a p-value <0.05 was considered to be statistically significant.

3.10 ETHICAL CLEARANCE AND CONSENT

Approval for the study was given by the Administration of the hospitals involved as well as the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. All subjects gave informed consent to take part in the study after verbal and written explanation of the methods and risks involved.

CHAPTER 4

RESULTS

4.1 SOCIO-DEMOGRAPHIC FEATURES

Data gathered by the study shows that, out of the 287 HIV/AIDS participants categorized into HAART naïve and on HAART participants, 108 were highly active antiretroviral therapy (HAART) naïve participants of which 41were males and 67 were females and 179 Participants were on HAART of which 48 were males and 131 were females (Table 4.1). The ages of Participants ranged from 18 to 60 years. From the data, participants on HAART were older (41.99 ±0.75 years, P = 0.012) than their HAART naive counterparts (39.07 ± 0.84 years) (Table 4.1). Table 4.1 further shows the marital status, education level, occupation, income, residence, religion, ethnicity and duration of infection of the study participants. From the data, 50.6% (144/287) of the study population were married and 52.6% (151/287) were self employed. Higher proportion of participants on HAART were self employed (60.3%; 108/179; P <0.0001) as compared to their Naïve counterpart who were self employed (39.8%; 43/108). Moreover, most study participants had irregular income (64.1%; 184/287) and lived in rural areas (74.2%; 213/287) (Table 4.1).

Ashanti's and the Christian religion were the predominant ethnic and religious groups respectively (68.6%; 197/287, 85%; 244/287). Regarding educational levels, 49.1%(141/287) of the participants had education to the Junior High School level with a higher proportion of HAART naïve participants who had education to the Junior High school level (56.5%; 61/108, P = 0.02) as compared to their counterparts who were on HAART (44.7%; 80/179) (Table 4.1).

Table 4.1 Socio-demographic characteristics of study participants

	HIV PARTICIPANTS			
Variables	**TOTAL (287)**	**HAART NAÏVE (108)**	**On HAART (179)**	**P value**

Sex				0.065
Male	89(31.1%)	41(38%)	67(62%)	
Female	198(68.9%)	48(26.8%)	131(73.2%)	
Marital status				0.16
Divorced	70 (24.4%)	22(20.4%)	48(26.8%)	
Married	144(50.2%)	61(56.5%)	83(46.4%)	
Single	36(12.5%)	18(16.7%)	18(10.1%)	
Widowed	37(12.9%)	7(6.5%)	30(16.8%)	
Occupation				**<0.0001**
Daily laborer	88(30.7%)	54(50%)	34(19%)	
GOV'T Employee	23(8%)	9(8.3%)	14(17.8)	
Self employed	151(52.6%)	43(39.8%)	108(60.3%)	
Unemployed	17(5.9%)	2(1.9%)	15(8.4%)	
Residence				0.05
Urban	74(25.8%)	35(32.4%)	39(21.8%)	
Rural	213(74.2%)	73(67.6%)	140(78.2%)	
Income				**<0.0001**
Dependent	23(8%)	2(1.9%)	21(11.7%)	
Irregular	184(64.1%)	59(54.6%)	125(69.8%)	
Monthly	80(27.9%)	47(43.5%)	33(18.4%)	
Religion				**<0.0001**
Christian	244(85%)	78(72.3%)	166(92.7%)	
Muslim	31(10.8%)	21(19.4%)	10(5.6%)	
Traditional	12(4.2%)	9(8.3%)	3(1.7%)	
Ethnicity				0.009
Ashanti	197(68.6%)	75(69.4%)	122(68.2%)	
Dagomba	60(20.9%)	27(25%)	33(18.4%)	
Ewe	6(2.1%)	4(3.7%)	2(1.1%)	

Fante	24(8.4%)	2(1.9%)	22(12.3%)	
Educational level				**0.02**
Primary	32(11.1%)	17(15.7%)	15(8.4%)	
JHS	141(49.1%)	61(56.5%)	80(44.7%)	
SHS	34(11.9%)	8(7.4%)	26(14.5%)	
Tertiary	30(10.5%)	8(7.4%)	22(12.3%)	
Uneducated	50(17.4%)	14(13.0%)	36(20.1%)	
Duration of infection				**<0.0001**
≤ 1 year	110(38.3%)	64(59.3%)	46(25.7%)	
2-5 years	136(47.4%)	35(32.4%)	101(56.4%)	
> 5 years	41(14.3%)	9(8.3%)	32(17.9%)	
Frequency of diet	-	2.04 ± 0.70	2.11 ± 0.55	0.404
Age	-	39.07 ± 0.84	41.99 ±0.75	**0.012**

With respect to duration of infection, most HAART participants have had the HIV infection for a period of 2 to 5 years (56.4%; 101/179) whereas majority of the HAART naïve participants have had the disease for a year period or less (59.3%; 64/108). The average frequency of diet in both groups of participants was twice a day (Table 4.1). *(Note: In Table 4.1, HARRT naïve and Participants on HAART are compared using chisquare tests. P<0.05 is statistically significant).*

4.2 ANTHROPOMETRIC MEASUREMENTS

4.2.1 BODY MASS INDEX (BMI)

Our findings in the study reveals that, the mean BMI values of both groups of participants indicates overweight according to the WHO 2005 reference standards to classify nutritional status. However, Comparison of BMI values for HAART and HAART naïve

participants using unpaired t-test analysis showed no significant difference between the two groups (p=0.806) with a mean BMI of 27.76 ± 0.51 Kg/m^2 and 27.57 ± 0.49 Kg/m^2 for the HAART naïve and HAART participants respectively as shown in Table 4.2. When subjects were classified according to the World Health Organization (WHO) classification as underweight (<18.5 Kg/m^2), normal (18.5 – 24.9 Kg/m^2), overweight (25.0 – 29.9 Kg/m^2) and obese (≥30 Kg/m^2), analysis using Pearson's chi square revealed that, the proportion of HAART naïve participants who were overweight was higher (33.3%; 36/108; p=0.03) than their counterpart who were on HAART (20.7%; 37/179) as found in table 4.3. However obesity was proportionately higher in HAART participants (35.8%; 64/179; p=0.03) as compared to HAART naïve participants who were obese (35.2%; 38/108). There were no cases of underweight recorded among the HAART naïve participants but among the HAART participants, 2.2% (4/179) were underweight. Moreover**,** HAART naïve participants were found to be heavier (67.58± 0.91 kg) than their counterpart who were on HAART (64.45 ±0.93 kg) when the data was subjected to unpaired t-test analysis (p=0.025) as shown in Table 4.2. With a mean height of 1.57 ±0.013 m and 1.54 ± 0.014 m for HAART naïve and HAART participants respectively, there was no significant difference between the two groups p= 0.101) as Table 4.2 shows.

Table 4.2 Anthropometric features of HIV Participants

	HIV PARTICIPANTS		
Parameters	**HAART Naïve**	**On HAART**	**p-value**
Weight (kg)	67.58± 0.91	64.45 ± 0.93	**0.025**
Height (m)	1.57 ±0.013	1.54 ± 0.014	0.101

BMI (Kg/m^2)	27.76 ± 0.51	27.57 ± 0.49	0.806
MUAC (cm)	28.13 ± 0.42	28.05 ± 0.32	0.867
TSF (mm)	20.47 ± 0.62	23.67 ± 0.77	**0.006**
BSF (mm)	16.19 ± 0.62	17.70 ± 0.67	0.125
WC (cm)	78.08 ± 0.82	80.99 ± 0.76	**0.013**
HC (cm)	94.93 ± 1.57	95.02 ± 1.10	0.961
WHR	0.90 ± 0.02	0.85 ± 0.01	**0.016**

WC:Waist Circumference, HC: Hip Circumference, BMI: Body Mass Index, WHR: Waist-to-Hip Ratio, MUAC: Mid Upper Arm Circumference, BSF:Biceps skin fold, TSF: Triceps skin Fold.

4.2.2 Mid upper arm Circumference (MUAC)

Findings from our study indicates no significant difference between the MUAC measurement of HAART naïve and HAART Participants (p=0.867) with a mean MUAC values of 28.13 ± 0.42 for HAART naïve Participants and 28.05 ± 0.32 for HAART participants as shown in table 4.2 . According to the WHO 2005 reference standard classification for nutritional status using MUAC, Participants were classified as underweight (<22.1cm for women and <22.4cm for men), normal (22.1cm - 32cm for women and 22.4cm-32cm for men), and obese (>32.0cm for all gender).

Pearson's chi square test analysis revealed that, the proportion of HAART naïve participants that are underweight are more (13.9%; 15/108; p=0.03) than their HAART patient counterpart (11.7%; 21/179). Obesity was proportionately higher in HAART participants (6.1%; 11/179) as compared to HAART naïve participants where there were no case recorded for obesity (0%). Normal MUAC values were much recorded among the

HAART naïve Participants (86.1%; 93/108; P=0.03) as compared to their counterparts on HAART(82.1%; 147/179) as shown in table 4.3.

4.2.3 Waist to Hip ratio (WHR)

Data from our study reveals that the WHR for HAART naïve participants and HAART Participants were significantly different (p= 0.0084) with a mean WHR of 0.90 ± 0.02 for the HAART naïve participants and 0.85 ± 0.01 for HAART participant group as shown in Table 4.2. When subjects were categorized as normal or obese and analyzed using Fisher's exact test, it was observed that although higher proportions of HAART naïve participants who were obese were more (28.7%; 31/108) than their HAART patient counterpart (25.7%; 46/179), there was no significant difference between the two groups (p=0.58) as shown in Table 4.3.

Table 4.3 Classifications of BMI, MUAC and WHR of study participants

	HIV PARTICIPANTS		
	HAART Naïve (n=108)	**On HAART (n=179)**	**P value**
BMI			**0.03**
Underweight(<18.5)		4 (2.2%)	
Normal(18.5-24.9)		74 (41.3%)	**<0.0001**
Overweight(25.0-29.9)	0 (0%)	37 (20.7%)	**0.002**
Obese(≥30)	34 (31.5%)	64 (35.8%)	**<0.0001**
MUAC	36 (33.3%)		**0.03**
Underweight (men:<22.4;women:<22.1)	38 (35.2%)	21 (11.7%)	**<0.0001**
Normal (Women:22.1men:22.4-32-;32)	15 (13.9%)	147 (82.1%)	**<0.0001**
Obese (>32)	93 (86.1%)	11 (6.1%)	0.58
WHR	0 (0%)	133(74.3%)	**0.04**
Normal(men:<0.9;women<0.8)	77 (71.3%)	46 (25.7%)	0.32
Obese(men:>1;women>0.85)	31 (28.7%)		

BMI:Body Mass Index(Kg/m²), WHR: Waist-to-Hip Ratio, MUAC: Mid Upper Arm Circumference(cm)

4.3 HAEMATOLOGICAL PARAMETERS

4.3.1 Haemoglobin, Haematocrit (HCT) and Anaemia

Findings from our study, revealed a significant difference ($p < 0.0001$) between the blood haemoglobin levels of HAART participants and HAART naive participants. The mean blood haemoglobin level of the HAART naïve participants was significantly lower (10.94 ± 0.20 gdl^{-1}) than that of the HAART participants group (12.33 ± 0.11 gdl^{-1}). Moreover, the calculated mean HCT in participants on HAART ($34.58 \pm 0.25\%$, $P \leq 0.001$) was significantly higher than that of participants who are HAART naive ($31.30 \pm 0.52\%$) (Table 4.4).

When Study participants were Classified based on the World Health Organization/Aids Clinical Trial Group (WHO/ACTG) anaemia toxicity grades it was observed that there was an 82.5% and 54.7% calculated incidence of anaemia (Hb $\leq$10.5 gdl^{-1}) in HAART naive participants and those on HAART respectively ($P < 0.0001$). Our findings further reveals that all anemia incidence among participants on HAART were in the Grade 1 anaemia toxicity grade (54.7%; 98/179) whereas in the HAART naïve participants, there were incidence of Grade 2 (13.9%; 15/108) and Grade 3 anemia (16.7%; 18/108). There were no incidence of Grade 4 anaemia toxicity grade in both groups of HIV participants as shown in table 4.5.

Table 4.4 Haematological Indices and CD4 count of study participants

	HIV PARTICIPANTS		
	HAART NAÏVE (n=108)	HAART (n=179)	p-value
Parameters			
Hb (g dl^{-1})	10.94 ± 0.20	12.33 ± 0.11	**<0.0001**

WBC ($\times 10^9$ L^{-1})	5.43 ± 0.33	4.64 ± 0.08	**0.003**
RBC ($\times 10^{12}$ L^{-1})	3.78 ± 0.63	3.78 ± 0.04	0.979
HCT (%)	31.30 ± 0.52	34.58 ± 0.25	**<0.0001**
MCV (fl)	83.10 ± 0.69	92.61 ± 0.71	**<0.0001**
MCH (pg)	28.97 ± 0.30	33.02 ± 0.34	**<0.0001**
MCHC (g dl^{-1})	34.85 ± 0.16	39.27 ± 1.21	0.005
ALC ($\times 10^9$ L^{-1})	1.10 ± 0.69	2.08 ± 0.06	**<0.0001**
Neutrophils ($\times 10^9$ L^{-1})	2.12 ± 0.11	1.93 ± 0.05	0.064
CD4(cell mm^{-3})	484.90 ± 32.64	523.31 ± 20.45	0.294

Hb: Haemoglobin , WBC: White blood cells, RBC: Red blood cells; HCT: Haematocrit; MCV: Mean Cell Volume, MCH: Mean Cell haemoglobin, MCHC: Mean Cell Haemoglobin Concentration, ALC: Absolute lymphocyte count

4.3.2 Red cell indices and Types of Anaemia

Analysis on our data in the study reveals a significant differences in the means of MCH (P<0.0001), MCV(P<0.0001) and MCHC(p< 0.005) using unpaired t-test analysis of both groups of HIV participants. The mean MCH of HAART naïve participants was 28.97 ± 0.30 pg and that of HAART participants was 33.02 ± 0.34pg. MCV means of HAART naïve participants and participants on HAART were 83.10 ± 0.69 fl and 92.61 ± 0.71fl respectively whereas the MCHC mean values for HAART naïve participants and Participants on HAART were 34.85 ± 0.16gdl^{-1} and 39.27 ± 1.21gdl^{-1} respectively as shown in table 4.4. However, there was no significant difference (p=0.979) between the mean RBC values for HAART naïve participants and participants on HAART with a mean values of 3.78 ± 0.63 $\times 10^{12} l^{-1}$ and 3.78 ± 0.04 $\times 10^{12} l^{-1}$ respectively.

HIV aneamic participants in the study were further classified into types of aneamia based on estimated values of red cell size and haemoglobin content of red cells using the mean cell volume (MCV) range of 80 – 96 fl and mean cell haemoglobin (MCH) range of 27 – 32 pg where low MCV (<80 fL) is indicative of microcytosis, high MCV (>96 fL) indicates macrocytosis and low MCH (<27 pg) indicates hypochromia.

Our data revealed that higher proportions of anaemia Participants on HAART were Macrocytic normochromic (40.8%; 40/98; p<0.0001) as compared to the HAART naïve participants who were Macrocytic normochromic(2.2%, 2/89) (Table 4.5). However Normocytic normochromic anaemia was seen to have a higher proportion among HAART naïve Participants (58.5%; 52/89; p<0.0001) as compared to participants on HAART who were normocytic normochromic anaemic (53.1%; 52/98). Moreover, no case of microcytic hypochromic anaemia was recorded among participants on HAART, whereas 29.2% (26/89) of the anaemic participants who are HAART naïve were microcytic hypochromic anaemic (Table 4.5).

4.3.4 White Blood Cell (WBC), Neutrophils and Absolute Lymphocyte count (ALC)

Our data from the study, revealed a significant difference (P=0.003) between the WBC count of the HAART naive participants and those on HAART. Mean values of WBC for HAART naïve participants and participants on HAART were $5.43 \pm 0.33\times10^{9}$ l^{1} and $4.64 \pm 0.08\times10^{9}$ l^{-1} respectively. The mean Absolute lymphocyte count among participants on HAART ($2.08 \pm 0.06\times10^{9}$ l^{-1}) was significantly higher (p<0.0001) than that of HAART naïve participants ($1.10 \pm 0.69\times10^{9\ -1}$). However, though the mean neutrophil count was higher in HAART naïve participants ($2.12 \pm 0.11\times10^{9}$ l^{-1}) as compared to the participants on HAART ($1.93 \pm 0.05\times10^{9}$ l^{-1}), unpaired t-test analysis of data revealed an insignificant difference (p= 0.064) between the two groups of HIV Participants as shown in table 4.4.

4.4 CD4 Count and Progression of HIV Infection

Our data reveals that, though HAART naïve participants had a lower CD4 cell count ($484.90 \pm 32.64 cellmm^{-3}$) as compared to their counterpart who were on HAART (523.31 ± 20.45 $cellmm^{-3}$), unpaired t-test analysis, revealed an insignificant difference ($p= 0.294$) between the means of the CD4 count of the two groups of HIV participants (Table 4.4). Further classification of CD4 count based on the Center for Disease Control (CDC) criteria was used to classify the study population into three categories : Stage 1 (CD4 $\geq$500 cells mm^{-3}), Stage 2 (CD4 between 200 – 499 cells mm^3) and Stage 3 (CD4 <200 cells mm^{-3}) as shown in Table 4.5. Proportions of Participants on HAART who were in stage 1(52.5%; 94/179) and stage 2 (30.7%; 55/179), were significantly higher ($p=0.024$) than HAART naïve participants who were in Stage 1 (39.8%; 43/108) and Stage 2 (30.6%; 33/108). However, there were more HAART naïve

Participants who were in Stage 3 of the disease (29.6%; 32/108) as compared to Participants on HAART in stage 3 of the disease (16.8%; 30/179) (Table 4.5).

Table 4.5 Study population stratified by anaemia, type of anaemia and CD4 counts

	HIV PARTICIPANTS		
	HAART Naïve	**On HAART**	**P value**
WHO/ACTG	**(n=108)**	**(n=179)**	**<0.0001**
Non Anaemic	19 (17.6%)	81 (45.3%)	**<0.0001**
Grade 1 Anaemia (9.5 - 10.5)	56 (51.9%)	98(54.7%)	**<0.0001**
Grade 2 Anaemia (8.0 - 9.4)	15(13.9%)	0(0%)	
Grade 3 Anaemia (6.5 - 7.9)	18(16.7%)	0(0%)	
Grade 4 Anaemia (<6.5)	0(0%)	0(0%)	
Types of Anaemia	**(n=89)**	**(n=98)**	**<0.0001**
Microcytic Normochromic	9(10.11%)	6(6.1%)	**0.001**
Microcytic Hypochromic	26(29.2%)	0(0%)	
Normocytic Normochromic	52(58.5%)	52(53.1%)	**0.001**

Macrocytic normochromic	2(2.2%)	40(40.8%)	**<0.0001**
CD4 count	**(n=108)**	**(n=179)**	**0.024**
Stage 3(<200)	32 (29.6%)	30 (16.8%)	**<0.0001**
Stage2 (200- 499)	33 (30.6%)	55 (30.7%)	**0.001**
Stage 1 (>500)	43 (39.8%)	94 (52.5%)	**<0.0001**

HAART = Highly Active Antiretroviral Therapy; WHO/ACTG = World Health Organization/Aids Clinical Trial Group; CD = Cluster of Differentiation; CDC: Center for disease control

4. 5. BIOCHEMICAL PARAMETERS OF NUTRTIONAL ASSESMENT

4.5.1 Albumin

Data obtained on serum albumin in our study revealed that there was a significant difference ($p< 0.0001$) between the albumin levels of the HAART naïve Participants compared to Participants on HAART. The mean serum albumin level of HAART naïve Participants was $36.21 \pm 0.47 gl^{-1}$ and that of participants on HAART was $41.19 \pm 0.34\ gl^{-1}$ as shown in Table 4.6. Further classification of the albumin levels using a range of $35\ 52 gl^{-1}$ as the reference range, hypoalbuminaemia was defined as albumin levels less than $35(<35 gl^{-1})$. Chi-square analysis for the comparison of the proportions of albumin levels of HAART naïve Participants and Participants on HAART showed that there was a significant higher proportions of HAART naïve participants who were hypoalbuminaemic (40.7%; 44/108; $p<0.0001$) as compared to participants on HAART who were hypoalbuminaemic (10.1%; 18/179). Most of the participants on HAART had normal albumin levels (89.9%; 161/179) as shown in table 4.7.

4.5.2 Total Protein

Our findings in the study revealed that there was a significant difference ($p<0.0001$) between the serum total protein of the HAART naïve participants and participants on HAART with a mean serum total proteins of $82.15 \pm 1.24 gl^{-1}$ and $88.23 \pm 0.74 gl^{-1}$ respectively as shown in Table 4.6. Table 4.7 shows further classification of serum protein levels using

a range of 80 -85gl^{-1} as the reference range and hypoproteinaemia defined as serum protein levels less than 80(<80gl^{-1}). Chi square analysis showed no significant difference (p=0.301) in the proportions of serum protein levels classification between HAART naïve Participants and Participants on HAART though there were no cases of hypoproteinamia in HAART naïve Participants.

4.5.3 Zinc

Findings from our study shows that participants on HAART had a significantly higher mean serum zinc levels(14.14 ± 0.23 µmol l^{-1}; p<0.0001) as compared to their counterparts who were HAART naïve (10.50 ± 0.31 µmol l^{-1}) (Table 4.6). Further classification of serum zinc levels into normal and low levels were determined using a normal range of 10.7 – 18.4 µmol l^{-1} and a low level range of less than 10.7 µmol l^{-1} (<10.7µmol l^{-1}) as shown in table 4.7. It was observed that, proportions of HAART naïve participants who had low zinc levels were significantly more (67.6%, 73/108; p<0.0001) than HAART Participants who had low serum zinc levels (15.6%; 28/179). Most Participants on HAART were in the normal range of classification of serum zinc levels

(84.4%;151/179).

4.5.4. Transferrin

The study reveals a significant difference (p<0.0001) in the mean serum transferrin levels between HAART naïve participants and participants on HAART with a mean values of 268.95 ± 3.91 $mgdl^{-1}$ and 295.67 ± 3.69 $mgdl^{-1}$ respectively (Table 4.6). Moreover, higher proportions of HAART naïve participants had low transferrin levels (38%; 41/108; P=0.04) as compared to their counterpart who were on HAART (21.8%; 39/179) when transferrin was classified using a normal range of 200 -360 $mgdl^{-1}$ and a low transferrin level defined as less than 200 $mgdl^{-1}$ as shown in table 4.7.

4.5.5 Ferritin

The study further reveals that serum ferritin levels of participants on HAART was significant higher ($140.72 \pm 3.30\ \mu gl^{-1}$; $p<0.0001$) as compared to participants who were HAART naïve ($104.21 \pm 4.85\ \mu gl^{-1}$) (Table 4.6). Further classification of serum ferritin levels as normal and low levels was done using a normal range of 20 -250 μgl^{-1} and a low range of less than 20 μgl^{-1} . Chi square analysis showed that there was a significant higher proportion of HAART naïve participants with low ferritin levels (5.6%, 6/108) as compared to their counterparts who were on HAART. Moroever, there was no case of low ferritin levels in participants on HAART (0%) as shown in table 4.7.

4.5.6 Urea

Analysis using the unpaired t-test in our study revealed that there was a significantly higher urea levels (4.19 ± 0.15 mmol l^{-1} $p<0.0001$) in HAART naïve participants as compared to participants on HAART(3.49 ± 0.08 mmol l^{-1}) although the mean values were in the normal range (2.5 -6.4 $mmoll^{-1}$) as shown in table 4.6. There was however no significant difference in the proportions of HAART naïve participants and participants on HAART who had normal and low urea levels upon further classification of urea levels with a normal range of 2.5 -6.4$mmoll^{-1}$ and a low range of less than 2.5 $mmoll^{-1}$ as shown in table 4.7.

4.5.7 Liver enzymes

The data from our study indicates no significant difference in the mean serum concentrations of Alanine aminotransferase (ALT)($p=0.098$), Aspartate aminotransferase (AST)($p=0.116$) and Alkaline phosphatase (ALP) ($p=0.947$) between participants on HAART and HAART naïve participants as shown in table 4.6.

Table 4.6 Biochemical parameters of study participants

	HIV PARTICIPANTS			
Parameters	**HAART NAÏVE**	**ON HAART**	**p-value**	
Albumin (g/l)	36.21 ± 0.47	82.15 ± 1.24	41.19 ± 0.34	**<0.0001**
TP (gl^{-1})	88.23 ± 0.74	25.71 ± 0.88		**<0.0001**
ALT (UL^{-1})	28.83 ± 1.94	34.5 ± 2.23	31.09 ± 1.00	0.098
AST (UL^{-1})	0.116	271.67 ± 12.46	272.66 ± 8.81	0.947
ALP (UL^{-1})	268.95 ± 3.91	295.67 ± 3.69		**<0.0001**
Transferrin ($mgdl^{-1}$)	104.21 ± 4.85	140.72 ± 3.30		**<0.0001**
Ferritin (μgl^{-1})	10.50 ± 0.31	14.14 ± 0.23		**<0.0001**
Zinc ($\mu moll^{-1}$)	4.19 ± 0.15	3.49 ± 0.08		**<0.0001**
Urea ($mmol\ l^{-1}$)				

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TP: Total Protein. Unpaired t-test analysis of HAART Participants compared with HAART naïve Participants

Table 4.7 Study population stratified by Biochemical parameters of Nutritional assessment

	HIV Participants		
Parameters	**HAART Naïve (n=108)**	**On HAART (n=179)**	**p value**
Total Protein			0.301
Normal	108(100%)	175 (97.8%)	**0.001**
Hypoproteinaemia	0 (0%)	4 (2.2%)	
			<0.0001
Albumin			
Normal	64 (59.3%)	161(89.9%)	**<0.0001**
Hypoalbuminaemia	44 (40.7%)	18 (10.1%)	**0.002**
Transferrin levels			**0.04**

Normal	67 (62.0%)	140 (78.2%)	**0.01**
Low	41 (38%)	39 (21.8%)	**<0.0001**
Ferritin levels			**0.03**
Normal	102 (94.4%)	179 (100%)	**<0.0001**
Low	6 (5.6%)	0 (0%)	
Zinc levels			**<0.0001**
Normal	35 (32.4%)	151 (84.4%)	**<0.0001**
Low	73 (67.6%)	28 (15.6%)	**0.001**
Urea levels			0.89
Normal	72(66.7%)	118 (67.4%)	0.18
Low	36(33.3%)	57 (32.6%)	0.26

Table 4.8. Pearson's correlation coefficients of anthropometry, CD4, haemtological and biochemical parameters of HAART naïve Participants (Upper right -hand side) and HAART Participants (Lower left-hand side)

	CD4	BMI	MUAC	WHR	Albumin	TP	ALP	ALT	AST	Urea	Tf	Frt	Zinc	Hb
CD4		**0.27****	**0.43****	-0.13	-0.19*	-0.14	-0.02	0.29**	0.29**	-0.30**	0.04	0.01	0.09	**0.23***
BMI	-0.05		0.52**	0.01	0.06	0.14	-0.08	-0.17	-0.14	-0.01	0.17	-0.07	0.03	**-0.21***
MUAC	0.00	0.29**		0.16	-0.23*	-0.24*	0.03	0.30**	0.34**	-0.24*	0.21*	0.18	0.03	-0.13
WHR	**0.16***	-0.27**	-0.07		0.24*	0.16	-0.21*	-0.16	-0.11	-0.32**	0.11	0.03	-0.10	0.02
Albumin	0.12	0.14	0.12	0.02		**0.25****	-0.46**	-0.81**	-0.74**	0.34**	-0.02	0.15	-0.05	**0.30****
TP	0.04	**0.15***	0.02	0.02	**0.53****		-0.20*	-0.49**	-0.48**	**0.23***	0.02	-0.24*	-0.11	-0.02
ALP	-0.27**	0.07	-0.09	**-0.16***	-0.07	0.25**		0.48**	0.62**	0.05	0.00	0.15	-0.01	-0.45**
ALT	0.01	0.03	-0.08	**-0.17***	-0.07	0.23**	0.29**		0.95**	-0.36**	-0.05	0.05	0.02	-0.06
AST	0.04	0.08	-0.06	-0.13	0.04	0.45**	0.33**	0.82**		-0.43**	-0.03	0.13	0.04	-0.07
Urea	-0.08	0.15*	0.01	-0.29**	0.14	**0.16***	0.17*	-0.05	0.04		-0.09	0.03	-0.10	-0.19
Tf	0.02	0.11	-0.02	0.01	-0.05	-0.02	0.02	0.10	0.12	0.03		-0.05	0.25**	0.03
Frt	0.00	0.13	0.16*	-0.10	0.06	0.07	-0.01	-0.04	-0.02	0.03	-0.01		0.05	-0.12
Zinc	-0.04	0.07	0.03	0.09	-0.04	-0.06	-0.12	-0.05	-0.03	-0.07	0.05	0.06		0.01
Hb	0.12	**-0.23****	0.08	0.12	0.25**	0.18*	-0.11	0.06	0.03	-0.04	-0.02	0.06	0.03	

** Correlation is significant at the level 0.05, ** Correlation is significant at the level 0.001, *** Correlation is significant at the level 0.0001. Hb: Haemoglobin , CD: Cluster of differentiation, BMI: body mass index, WHR:Wiast to hip ratio, MUAC: Mid upper circumference, TP:total protein, ALP:Alkaline phosphatase ALT:Alanine aminotransferase, AST: Aspartate aminotransferase, Tf:Transferrin, Frt: Ferritin.*

4.6 HAART USE AND NUTRITIONAL STATUS

Data represented in Table 4.9 shows the relationship between HAART use and nutritional assessment among participants on HAART. Our findings show that 31.9 % (57/179) of HAART Participants were on a combination of Zidovudine(AZT), lamivudine(3TC) and nevirapine (NVP) whereas 27.9%(50/179) were on a combination therapy of Stavudine(d4t), lamivudine(3TC) and nevirapine (NVP). 25.7%(46/179) were on a combination therapy of zidovudine(AZT), lamivudine(3TC) and Efavirenz(EFV) and 14.5%(26/179) were on a combination therapy of Zidovudine(AZT) and lamivudine(3TC).

Using BMI as a nutritional marker, significant higher proportions of obesity were observed in Participants taking a combination therapy of AZT-3TC-NVP (45.6%; 26/57; p=0.03) as compared to the other combination therapy. Moreover, using WHR as marker for nutritional status, participants on a combination therapy of Zidovudine(AZT) and lamivudine(3TC) had a higher proportion of obesity. No significant difference was observed in the proportions of HAART use Mid upper arm circumference as an indicator of nutritional status(p=0.4) (Table 4.9).

There was no significant difference in the proportions of HAART use and biochemical parameters of nutritional status as well as CD4 and anaemia as shown in table 4.9.

Table 4.9 HAART use and Nutritional status of study participants

	HAART PARTICIPANTS (n=179)				
	AZT-3TC (n=26)	**AZT-3TCEFV (n=46)**	**AZT-3TC-NVP (n=57)**	**d4t-3TC-NVP (n=50)**	**P value**
BMI					**0.03**
Underweight	0 (0%)	0 (0%)	4 (7.0%)	0 (0.0%)	
Normal	13 (50%)	21(45.7%)	19(33.3%)	21 (42.0%)	
Overweight	3 (11.5%)	10(21.7%)	8 (14.0%)	16(32.0%)	
Obese	10(38.5%)	15(32.6%)	26 (45.6%)	13 (26.0%)	
MUAC					0.4
Underweight	3 (11.5%)	2 (6.5%)	1 (1.8%)	4 (8.0%)	
Normal	19(73.1%)	38(72.6%)	47(82.5%)	43 (86.0%)	
Obese	4 (15.4%)	5 (10.9%)	9 (15.8%)	3 (6.0%)	
WHR					**<0.0001**
Normal	7 (26.9%)	36(78.3%)	50(87.8%)	40 (80.0%)	
Obese	19(73.1%)	10(21.7%)	7 (12.3%)	10 (20.0%)	
Total Protien					0.13
Normal	26 (100%)	43(93.5%)	56 (98.2%)	50 (100%)	
Hypoproteinaemia	0 (0%)	3 (6.5%)	1 (1.8%)	0(0.0%)	
Albumin Levels					0.95
Normal	23(88.5%)	41(89.1%)	51 (89.5%)	46 (92.0%)	
Hypoalbuminaemia	3 (11.5%)	5 (10.9%)	6(10.5%)	4 (8.0%)	
Transferrin levels					0.84
Normal	19(73.1%)	36(78.3%)	44 (77.2%)	41 (82.0%)	
Low	7 (26.9%)	10(21.7%)	13 (22.8%)	9 (18.0%)	
Ferritin levels					
Normal	26 (100%)	46 (100%)	57 (100%)	50 (100%)	
Low	0 (0%)	0 (0%)	0 (0%)	0 (0%)	

Zinc					0.13
Normal	23(88.5%)	41(89.1%)	50 (87.7%)	37 (74.0%)	
Low	3 (11.5%)	5 (10.9%)	7 (12.3%)	13 (26.0%)	
Urea					0.63
Normal	17(65.4%)	31(67.4%)	35 (62.5%)	35 (74.5%)	
Low	9 (34.6%)	15(32.6%)	21 (37.5%)	12(35.5%)	
WHO/ACTG					0.64
Non Anaemic	10(38.5%)	19(41.3%)	26 (45.6%)	26 (52.0%)	
Grade 1 Anaemia	16(61.5%)	27(58.7%)	31 (54.4%)	24 (48.0%)	

Zidovudine(AZT); Lamivudine(3TC); Nevirapine (NVP); Stavudine(d4t); Efavirenz(EFV)

4.7 INCOME AND ANTHROPMETRIC FEATURES OF NUTRITIONAL STATUS

Our findings as presented in Table 4.10 illustrates the nutritional status of both groups of HIV participants in the study stratified by income. From the results obtained, it indicates that, Participants who had irregular income in both HAART naïve and participants on HAART had a higher proportions of obesity as compared to those who earned a regular income as well as dependent using BMI as an indicator though this was not significant in Participants on HAART. However no significant difference in proportions among study participants were observed using WHR as an indicator as shown in table 4.10. Higher proportions of obesity were also among irregular money earners using MUAC as an indicator. However no significant proportions were observed among Participants on HAART.

Table 4.10 Income and Anthropometric features of nutritional assessment among study participants.

	HIV Participants						
	HAART Naïve (n=108)				On HAART (n=179)		
	INCOME				INCOME		
Parameters	Dependent (n=2)	Irregular (n=57)	Regular (n=47)	P value	Dependent (n=21)	Irregular (n=125)	Regular (n=33)	P value
BMI				**<0.0001**				0.26
Underweight	0 (0%)	0 (0%)	0 (0%)		0 (0%)	2 (1.6%)	2(6.1%)	
Normal	0 (0%)	6 (10.2%)	28 (59.6%)		9 (42.9%)	49 (39.2%)	16(48.5%)	
Overweight	2 (100%)	17(28.8%)	17(36.2%)		6 (28.6%)	23 (18.4%)	8 (24.2%)	
Obese	0 (0%)	36(61.0%)	2 (4.3%)		6 (28.6%)	51 (40.8%)	7 (21.2%)	
MUAC				**0.03**				0.24
Underweight	0 (0%)	0 (0%)	0 (0%)		0 (0%)	11 (8.8%)	0 (0%)	
Normal	2 (100%)	46(78.0%)	45(95.7%)		18 (85.7%)	99 (79.2%)	30(90.9%)	
Obese	0 (0.0%)	13(22.0%)	2 (4.3%)		3 (14.3%)	15 (12.0%)	3 (9.1%)	
WHR				0.41				0.69
Normal	2 (100%)	44(74.6%)	31 (66.0%)		14 (66.7%)	94 (75.2%)	25(75.8%)	
Obese	0 (0%)	15(25.4%)	16 (34.0%)		7 (33.3%)	31 (24.8%)	8 (24.2%)	

4.8 DURATION OF INFECTION AND NUTRITIONAL STATUS

Our findings illustrated in table 4.11 shows the relationship of duration of HIV infection and nutritional status of HAART naïve participants and participants on HAART. Duration of infection was classified into less than a year (<1 year), 2-5 years and more than 5 years (>5 years) of HIV infection. It is observed in the study, that majority of HAART naïve Participants were less than a year old for the duration of the HIV infection (59.3%; 64/108) whereas majority of the Participants on HAART were within 2-5 years duration of infection (56.4%;101/179).

Using BMI as a marker of nutritional status, significantly higher proportions (51.4%; 18/108) of obesity was recorded among those with 2-5 years of HIV infection in HAART naive Participants whereas in Participants on HAART, obesity was proportionately higher in Participants with more than 5 years of infection (50%; 16/32) as shown in table 4.11.There was no significant difference in the proportions of duration of HIV infection and MUAC classifications in both HAART naïve Participants and Participants on HAART(P=0.06 and 0.49 respectively). In Anaemia classifications among HAART naïve Participants, Grade 3 anaemia was higher in proportion in Participants with less than a year old of HIV infection (28.1%; 18/108) whereas there was no recorded case of Grade 3 anaemia in the other classification of duration of HIV infection in HAART naïve Participants.In relation to progression of disease, higher proportions of Participants in stage 3 (CD4 count <200) of the HIV infection were less than one year old of HIV infection duration in both category of the study participants as shown in table 4.11.

However there were no significant difference in the proportions of duration of HIV infection and transferrin, ferritin and zinc level classifications.

Table 4.11 Duration of infection and nutritional status among study participants

	HIV Participants							
	HAART Naïve (n=108)				on HAART (n=179)			
	<1yr (n=64)	**2-5yrs (n=35)**	**>5yrs (n=9)**	**P value**	**<1yr (n=46)**	**2-5yrs (n=101)**	**>5yrs (n=32)**	**P value**
BMI				**<0.0001**				**0.03**
Underweight	0(0%)	0(0%)	0(0%)		0(0%)	4(4%)	0(0%)	
Normal	21(32.8%)	4(11.4%)	9(100%)		19(41.3%)	40(39.6%)	15(46.9%)	
Overweight	23(35.9%)	13(37.1%)	0(0%)		15(32.6%)	21(20.8%)	1(3.1%)	

Obese	20(31.2%)	18(51.4%)	0(0%)		12(26.1%)	36(35.6%)	16(50%)	
MUAC				0.06				0.49
Underweight	0(0%)	0(0%)	0(0%)		3(6.5%)	5(5.0%)	3(9.4%)	
Normal	51(79.7%)	33(94.3%)	9(100%)		37(80.4%)	82(81.2%)	28(87.5%)	
Obese	13(20.3%)	2(5.7%)	0(0%)		6(13.0%)	14(13.9%)	1(3.1%)	
WHR				**0.002**				0.06
Normal	51(79.7%)	24(68.6%)	2(22.2%)		33(71.7%)	81(80.2%)	19(59.4%)	
Obese	13(20.3%)	11(31.4%)	7(77.8%)		13(28.3%)	20(19.8%)	13(40.6%)	
WHO/ACTG				**<0.0001**				**0.003**
Non Anaemic	4(6.2%)	15(42.9%)	0(0%)		21(45.7%)	54(53.5%)	6(18.8%)	
Grade 1 Anaemia	38(59.4%)	18(51.4%)	0(0%)		25(54.3%)	47(46.5%)	26(81.2%)	
Grade 2 Anaemia	4(6.3%)	2(5.7%)	9(100%)		0(0%)	0(0%)	0(0%)	
Grade 3 Anaemia	18(28.1%)	0(0%)	0(0%)		0(0%)	0(0%)	0(0%)	
CD4 count				**<0.0001**				**<0.0001**
<200	24(37.5%)	8(22.9%)	0(0%)		14(30.4%)	8(7.9%)	8(25.0%)	
200- 499	17(26.6%)	7(20.0%)	9(100%)		19(41.3%)	31(30.7%)	5(15.6%)	
>500	23(35.9%)	20(57.1%)	0(0%)		13(28.3%)	62(61.4%)	19(59.4%)	
Total Protein								0.26
Normal	64(100%)	35(100%)	9(100%)		46(100%)	97(96.0%)	32(100%)	
Hypoproteinaemia	0(0%)	0(0%)	0(0%)		0(0%)	4(4%)	0(0%)	
Albumin Levels				**<0.0001**				0.86
Normal	24(37.5%)	33(94.3%)	7(77.8%)		42(91.3%)	91(90%)	28(87.5%)	
Hypoalbuminaemia	40(62.5%)	2(5.7%)	2(22.2%)		4(8.7%)	10(9.9%)	4(12.5%)	
Transferrin levels				0.06				0.95
Normal	34(53.1%)	27(77.1%)	6(66.7%)		36(78.3%)	79(78.2%)	25(78.1%)	
Low	30(46.9%)	8(22.9%)	3(33.3%)		10(21.7%)	22(21.8%)	7(21.9%)	
Ferritin levels				0.75				

Normal	60(93.8%)	33(94.3%)	9(100%)		46(100%)	101(100%)	32(100%)	
Low	4(6.2%)	2(5.7%)	0(0%)		0(0%)	0(0%)	0(0%)	
Zinc levels				0.78				0.93
Normal	21(32.8%)	12(34.3%)	2(22.2%)		38(82.6%)	86(85.1%)	27(84.4%)	
Low	43(67.2%)	23(65.7%)	7(77.8%)		8(17.4%)	15(14.9%)	5(15.6%)	
Urea levels				**<0.0001**				0.77
Normal	51(79.7%)	19(54.3%)	2(22.2%)		33(31.7%)	64(66.0%)	21(65.6%)	
Low	13(20.3%)	16(45.7%)	7(77.8%)		13(28.3%)	33(34.0%)	11(34.4%)	

4.9 CORRELATIONS OF ANTHROPMETRIC, CD4, HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF STUDY PARTICIPANTS

Pearsons correlation of anthropometric, CD4, haematologic and biochemical features indicates a significant positive correlation between BMI, MUAC and CD4 in HAART naïve participants. Our study shows that BMI had a significant negative correlation with haemoglobin in both groups of study participants. However no significant correlation was observed between BMI and CD4 in HAART participants as shown in table 4.8.The study further showed a significant positive correlation between WHR with albumin in HAART naïve participants as shown in table 4.8.

Data from our study indicates that haemoglobin significantly correlated positively with total protein in HAART participants. There was no significant correlation between haemoglobin and the other biochemical parameters in HAART participants. However in HAART naïve participants, there was a significant positive correlation between Haemoglobin and CD4 and albumin and a significant negative correlation between Haemoglobin and Alkaline phosphatase levels. Haemoglobin correlated negatively with BMI in both category of Participants (Table 4.8).

It was also observed in the study that CD4 did not have any significant correlation with Haemoglobin in participants on HAART (Table 4.8).

Pearson's correlation method of analysis, did not reveal any significant correlation between albumin levels, CD4 and other biochemical parameters in our study with the exception of Total protein where there was significant positive correlation in participants on HAART. Significant positive correlation was however observed between albumin and haemoglobin in participants on HAART as shown in Table 4.8. Serum protein significantly correlated positively with Albumin in HAART naïve participants as shown in Table 4.8.

CHAPTER FIVE

DISCUSSION Nutritional status of HIV/AIDS patients, plays a significant role in the survival of infected persons as well as the progression of the disease (Laeyendecker *et al.*, 2006). This study sought to compare and assess the nutritional status among HAART naïve participants and participant on HAART as a means of advocating for comprehensive nutritional assessment and interventions before and after initiation of HAART.

5.1 SOCIO DEMOGRAPHIC CHARACTERISTICS

Evidence from our study shows that, females formed about 68.9% (178/287) of the study population and a further finding of female to male ratio of 3:1 in participants on HAART and 2:1 in HAART naïve participants (Table 4.1) strongly suggests that females are at a higher risk of getting infected with HIV than men. The finding in our study of more females infected than men is related to the WHO report that indicates that HIV/AIDS affects females most commonly in sub-Saharan Africa accounting for up to 60% of HIV infected people in the world (Dabis and Ekpini, 2002; HIV/AIDS, 2004).

Moreover, in this study, participants on HAART were significantly older than participants who were HAART naive and most HAART participants fell in the 2-5 years of duration of HIV infection whereas most HAART naïve Participants were less than a year old (<1 year) in duration of HIV infection (Table 4.1). This significant difference in age between these two groups of HIV participants and the longer duration of infection in HAART participants

further supports the suggestion that HIV/AIDS infection is likely to be acquired at an early age and most probably during the reproductive stage of life.

The strict eligibility criteria for qualifying for HAART (That is; WHO recommendation of CD4 <350 cells mm^{-3}, WHO stages I, II and III HIV disease with CD4 <200 cells mm^{-3}, WHO stage IV HIV disease (clinical AIDS) irrespective of CD4, WHO stage II HIV disease with TLC <1200 cells mm^{-3}) (WHO Case Definitions, 2007) could also be a reason why most of the Participants on HAART appear to be older and have a longer duration of infection than their HAART naive counterparts because there could be a time lag between the time a patient tests positive for HIV and the time of initiation of treatment and since treatment once started is continued throughout the life of the patient. Age and gender were not confounding factors in the study since similar results of the study were obtained after adjusting for age and gender.

This study further shows that a higher proportions of the general study participants (50.6%) were married (Table 4.1) . This was also evident in each category of the HIV participants in the study, in that, within each group of HAART participants and participants on HAART, higher proportions of married people were recorded as compared to those who were divorced, single and widowed (Table 4.1). The findings of this study corroborate a report by the international Family planning perspectives (Clark *et al.*, 2006) which states that, married couples are at a higher risk of contracting the HIV infection due to frequent unprotected sexual intercourse among married people. Therefore there is a higher likelihood of transmission from an infected spouse to the other spouse (Clark *et al.*, 2006).

Moreover, the study suggests that prevalence of HIV infection is higher in rural areas as compared to urban localities in both category of study participants (Table 4.1). It further suggests that higher proportions of study participants are self employed and also earn irregular income. This is also evident in each category of study participants. This finding is in line with the report by the Ghana AIDS commission (Ghana AIDS Commission, 2006) that indicates that, rural residents account for a larger share of PLWHAs and this may be due to fact that a larger share of Ghana's population (55%) live in the rural settlement (Ghana AIDS Commission, 2006). Job losses due to frequent ill health and the stigmitisation associated with HIV contraction, may lead to Participants abandoning cooperate work and seeking their personal source of livelihood. This may account for the higher proportions of study participants being self employed and as well earning irregular income. Moreover, the catchment area of the two (2) study sites may also contribute to this findings.These factors may contribute positively or negatively to the nutritional status depending on how the individual copes with these challenges (Ghana AIDS Commission, 2006).

This studies further indicates that higher proportions of study participants had JHS as the highest form of education (Table 4.1). This finding also in line with the Ghana AIDS commission report in 2006 and the International food policy research institute report (IFPRI) in 2001, which indicates that larger share of HIV prevalence was among individuals with primary and secondary education. This may be due to the high youthful population of Ghana thereby increasing the risk of people in this category of getting the disease. More so, most HIV Participants stop schooling at an early age due to stigmitisation

and ill health leading to self employment and the possible irregular income associated with it which may directly or indirectly influence their nutritional status

(Ghana AIDS Commission, 2006).

Though there was no significant difference in the frequency of diet in both category of Participants (Table 4.1), the study shows that the average frequency of diet of the study population was twice a day and this may be attributed to the economic status or loss of appetite which may be associated with the HIV infection. This finding depicts the middle income status of the country and its determination of nutritional status (Ghana AIDS Commission, 2006).

5.2 ANTHROPOMETRY

The anthropometric nutritional status according the World Health Organisation is determined primarily by the body mass index (BMI). However, this study utilized three methods in defining nutritional status of the participants. Methods used were the Body mass index (BMI), the mid upper arm circumference (MUAC) and the Waist to hip ratio (WHR). These three anthropometric features provides a wide view of obesity and weight loss caused by fat or fat loss. BMI alone is known to be influenced by muscle mass, age, gender and ethnicity and may not be an efficient tool in assessing overweight or obesity due to body fat composition.(Torheim *et al.*, 2003). However, WHR is known to provide substantive information on central adiposity causing obesity and overweight(Torheim *et al.*, 2003). Therefore a combination of these measures of anthropometry could give a wider view of obesity, overweight or weight loss caused by muscle mass, age and gender for comprehensive assessment and hence the need to employ these methods in our study.

The study found out that, there were no significant difference in the mean BMI and

MUAC between Participants on HAART and HAART naive Participants although HAART naïve Participants appeared to have a higher mean BMI as compared to their counterparts who were naïve (Table 4.2).

BMI among study participants was generally overweight whereas the MUAC among participants were generally normal. However, HAART naïve had a significant higher WHR than Participants on HAART. Further classification of the anthropometric features of nutritional status in this study, showed that, there were higher proportions of HAART naïve Participants who were overweight as compared to their counterpart on HAART whereas Obesity was much higher in participants on HAART as compared to their counterparts who were naïve (Table 4.3). These findings seems to contradict several findings which suggests an underweight (lower BMI and WHR) in HIV Participants especially Participants on HAART (Teodor *et al.*, 2003; Obi *et al.*, 2010).

However a more recent findings have suggested obesity and overweight using BMI and WHR as an indicator among HIV participants similar to the general population (CrumCianflone *et al.*, 2008). Since most of the HAART naïve participants were less than a year for the duration of infection, the high BMI and WHR in HAART naïve participants may be not be directly associated with the HIV infection but rather the obesity and overweight may have been experienced prior to the disease (Crum-Cianflone *et al.*, 2008). Increase and easy access to nutritional counseling in ART clinics especially at a very early stage of the disease, across the country in Ghana may also account for the overweight and obesity in HIV naïve participants. Moreover, research indicates that obesity in HIV participants in Ghana may also be due to the high starch contents of Ghanaian foods such as cassava, yam, potatoes and plantain which are consumed largely by Ghanaians

associated with sedentary lifestyle (Biritwum *et al.*, 2005). This finding is in line with our study which found out that major foods grown and consumed by study participants are cassava, yam, plantain and cocoyam and may also account for the overweight and obesity recorded.

Obesity in participants on HAART where most of them were in the 2-5 and greater than 5 years duration of HIV infection may be due to the lipodystrophic action of the drugs being taken together with nutritional counselling as seen in this study. HAART participants on a combination therapy of AZT-3TC as well as those on AZT-3TC-NVP were found to have a higher proportions of obesity. Lipodystrophy together with quality nutritional counseling may lead to substantive increase in BMI and WHR levels in participants on HAART therefore increasing survival rate. However excess increment in these parameters could put participants at risk of cardiovascular diseases (Ghana AIDS Commission, 2006; Crum-Cianflone *et al.*, 2008; Obi *et al.*, 2010). The study further shows that higher proportions of obesity were associated with irregular income earners (Table 4.10). However, the amount of money received and the sedentary lifestyle of the participants were not determined by the study. Moreover, the catchment area of our study reveals that the major occupations are fishing and farming. Meanwhile, a possible explanation to this occurrence may be due to the fact that irregular income earned does not necessarily mean economic instability. It may therefore be implied that these irregular income earners who are mostly farmers and fishermen, may be economic stable and therefore increasing the possibility of good nutritional practices and associated sedentary lifestyle could be a possible reason for the overweight and obesity in HIV Participants especially HAART Participants.

Triceps and biceps skin folds as a measure of subcutaneous fat, was higher in Participants on HAART as compared to Participants who were naïve (Table 4.2). However no significant difference was observed in the mean values of the biceps skin fold between the two categories of study participants. This may be an additional suggestion for the lipodystrophic actions of the drugs being taken by Participants on HAART causing higher values of the skin folds as a measure of subcutaneous fat (Sutinen *et al.*, 2005).

5.3 HAEMATOLOGICAL PARAMETERS

From the study, the calculated incidence of anaemia was 82.5% in HAART naive Participants and 54.7% in participants on HAART according to the WHO/ACTG classification of Anaemia (Table 4.5). All cases of Aneamia in participants on HAART were Grade 1 anaemia however, 13.9% and 16.7% incidence of Grade 2 and Grade 3 anemia respectively were observed in HAART naïve participants. Haematocrit values and total lymphocyte count were significantly higher in participants on HAART as compared to HAART naïve participants. WBC counts were however, higher in HAART naïve Participants as compared to their counterparts on HAART (Table 4.4).

These findings in the study, corroborates with several studies conducted that shows that increased haemoglobin concentration , increased haematocrit values, and decreased prevalence of anaemia are mostly associated with Participants on HAART (Moore *et al.*, 1998; Mocroft *et al.*, 1999; Owiredu *et al.*, 2011). This may indirectly indicate the effectiveness of HAART in reducing HIV associated anemia. HAART may improve from anemia through several mechanisms, including a reduction in diarrheal disease and an improvement in micronutrient absorption and metabolism. HAART use is further known to reduce opportunistic infections and associated inflammation that may suppress

erythropoiesis. The overall improvement in Haemoglobin concentration in Participants on HAART confirms the effectiveness of HAART in improving quality of life of HIV participants.(Moore *et al*., 1998; Volberding *et al.*, 2004)

The significant higher values of WBC and neutrophils in HAART naïve participants as compared to participants on HAART may be due to the broad myelosuppressive effects of the HAART regimen leading to a possible lower values in neutrophils and WBC. This phenomenon is mostly associated with AZT usage (Sharma and Garg, 2010).

With further classification of type of anaemia, the study found that microcytic hypochromic anaemia was associated with HAART naïve participants since no case was recorded among HAART participants (Table 4.5). This finding in the study corroborates with a similar study done in Ghana which indicates that HAART naïve participants are at a higher risk of developing microcytic hypochromic anaemia as compared to participants on HAART (Owiredu *et al.*, 2011). This result is highly predictive of nutritional deficiencies such as malnutrition and malabsorption especially among HAART naïve Participants. Microcytic aneamias may be mostly associated with iron deficiency which could lead to under nutrition in HAART naïve participants.

Moreover, participants on HAART had a higher proportion of Macrocytic normochromic aneamia as compared to their counterparts who were HAART naïve (Table 4.5). This occurrence is in relation to a study conducted which indicates a higher prevalence of macrocytic anaemia in HIV Participants on HAART (Romanelli *et al.*, 2002). This incidence is probably due to effect of zidovudine (AZT) as most of the Participants on HAART in the study were on a combination therapy of AZT and lamivudine (3TC).

Though the mechanism of Zidovudine- induced macrocytic anaemia is not fully comprehended, however it is known to interfere with DNA production which may lead to megaloblastic changes. Moreover, higher prevalence of macrocytic anaemia in HIV Participants on HAART may also be due to nutritional Vitamin B12 deficiency or folate deficiency especially among people who have an irregular income as indicated in this study where higher proportions of HAART participants were seen to have irregular income (Table 4.10). Inadequate income may lead to poor diversity of diet and could lead to Vitamin B12 and folate deficiency. Additionally, a study conducted also indicates that low serum vitamin B12 levels are reflective of low levels of transcobalamin I or haptocorrin which are vitamin B12 transport proteins produced by neutrophils and not necessarily a tissue deficiency of vitamin B12(Remacha *et al.,* 2003). This finding is in relation to this study, where participants on HAART had a significantly lower neutrophil count as compared to participants who were naïve. This may explain the reason for higher proportion of macrocytic anaemia in participants on HAART although Vitamin B12 and folate were not measured in this study.

The higher proportions of normocytic normochromic and microcytic normochromic anaemias in both category of study participants may be due to the complex and multifactorial nature of the aetiology of anaemia. This finding is consistent with the study conducted by (Volberding *et al.*, 2004) where causes of anaemia were known to be associated with blood loss or changes in erythropoietin production, insufficient dietery intake of nutrients such as folate and iron, diarrhea and severe infection.

The study further found a significant positive correlation between haemoglobin concentration and albumin levels in both category of study participants. Haemoglobin

concentration was also found to correlate positively with CD4 cell count in both category of participants, however, it was significant in Naïve participants and not in participants on HAART (Table 4.8). Haemoglobin had a significant negative correlation with BMI in both category of study participants and no significant correlation with WHR and MUAC (Table 4.8).

A study conducted indicates a significant correlation between haemoglobin levels and albumin in HIV participants in the view that both heamoglobin and albumin were found to be reduced before initiation of HAART and a subsequent increase in both haemoglobin and albumin after initiation of HAART (Chauhan *et al.*, 2011). This finding is consistent with this study where HAART naïve participants were found to have a higher proportion of hypoalbuminaemia and anaemia. participants on HAART evidently had a significant higher haemoglobin concentration accompanied by lower proportion of hypoalbuminaemia.

This finding suggests that higher haemoglobin concentration with a correlated high albumin levels are associated with HAART use whereas low haemoglobin concentration and hypoalbuminaemia are associated with HAART naïve participants. HAART use is known to decrease hypoalbuminemia through several mechanisms. This could be due to better body mass index and increased absorption of nutrients. High haemoglobin and albumin levels associated with HAART use may also be due to the reduction in the tumor necrosis factor alpha level following HAART use which might result in reduced cachexia in HIV infected individual, leading to better dietary patterns, and hence a possible increase in the albumin level.

Correlation between CD4 and haemoglobin levels found in our study corroborates with several studies that suggests that haemoglobin levels could increase the risk of AIDS (Hoover *et al.*, 1992; Rabeneck *et al.*, 1997). Several studies have also suggested that important information can be provided by haemoglobin levels (Turner *et al.*, 1996; Spino *et al.*, 1997).

Moreover, our findings suggest a negative correlation between haemoglobin concentration and BMI in both category with no signifcant correlation between haemoglobin and WHR of study participants (Table 4.8). This findings contradicts a study conducted in the United States of America that indicates a positive correlation between haemoglobin concentration and BMI (Ausk and Ioannou, 2008). However, our findings corroborates with a study conducted in China by (Qin *et al.*, 2013) which shows that overweight/obesity and central obesity were inversely associated with anemia or low haemoglobin concentration. Overweight and obesity with decrease haemoglobin concentration is a complex mechanism which has been reported differently in various research findings. However, because obesity is characterized by chronic low-grade inflammation, it may be associated with the features of anemia of inflammation. The possible explanation to this mechanism may be multfactorial in nature which may not have been captured in this study therefore the true meaning of this mechanism calls for further studies with multiple iron and inflammatory markers.

5.4 BIOCHEMICAL PARAMETERS

From our study, the incidence of hypoalbuminaemia was 40.7% in HAART naïve participants and 10.1% in participants in HAART (Table 4.7). This finding in our study is in agreement with a recent cross sectional and longitudinal study done by (Sicotte *et al.*,

2015) which suggests that hypoalbuminaemia is less prevalent in Participants on HAART as compared to participants who are HAART naïve. This study shows that the improvement of albumin levels in participants on HAART could indicate the lessening of HIV associated inflammatory process as a result of the anti viral replication activity of HAART. The study further suggested that chronic malnutrition without HAART use could worsen the hypoalbuminaemic prevalence. Albumin is known to be associated with nutritional status and hypoalbuminaemia is associated with undernourishment. Our current study, identifies higher proportions of hypoalbminaemia in HAART naïve Participants especially those who are less than a year of duration of infection possibly due to ongoing inflammatory process accompanied by poor dietary pattern prior to the diagnosis of the disease. This study further identifies a positive correlation between Waist to Hip ratio and Albumin levels in HAART naïve participants.

These finding suggest a relationship between obesity and high albumin levels which is in agreement with a study conducted by (Dusingize *et al.*, 2012) which suggests that obesity is strongly accompanied by high albumin levels in HIV Participants. Though in this study, there was a positive correlation between waist to hip ratio as a measure of obesity and albumin levels, hypoalbuminaemia still remains prevalent despite high prevalence of obesity in HAART naïve Participants. Negative correlations were also observed between CD4 and Albumin levels in HAART naïve Participants and no significance in participants on HAART. Positive correlation between albumin and total protein in both category of participants is a possible indication that albumin forms a greater portion of protein levels in the body. There seems to be a complex interaction between albumin and severity of

disease as well as nutritional status in HIV participants as suggested by several studies (Seres, 2005; Mildvan *et al.*, 2007; Sicotte *et al.*, 2015).

However, these findings suggest that although hypolbuminaemia is associated with undernourishment which is prevalent in HAART naïve participants, deficiency in albumin levels could also be due to ongoing inflammatory process which is less in participants on HAART due to the antiviral replication activity of HAART together with good nutritional counseling. Therefore HAART use accompanied by good dietary pattern could be a better intervention for HIV participants. The significant positive correlation between albumin and urea levels in both category of participants as well as Protein and urea, is an indication of the fact that urea is the product of protein catabolism in nutritional assessment.

Findings in this study also indicates a high incidence of hypozincaemia in HAART naïve patient. (67.6%) as compared to Participants on HAART (15.6%) (Table 4.7). A study conducted by Mocchegiani et al., 1995, has shown an increase in plasma zinc levels in participants on HAART as compared to HIV participants who not on ART treatment (Mocchegiani *et al.*, 1995). Other findings contrary to our findings indicates that HAART use was associated with zinc deficiency probably due to the increased utilisation of zinc in Participants receiving zidovudine(AZT) treatment (Odeh, 1992; Baum *et al.*, 2000).

Even though plasma zinc is an acute phase reactant which is most likely to change in response to metabolic alterations, plasma zinc levels react to dietary intake in a rapid and measurable manner. Several studies suggests a strong correlation of plasma zinc levels with dietary intake of zinc (Beach *et al.*, 1992; Baum *et al.*, 2000). However, HAART use and good nutritional practices has been found to be associated with high zinc levels

(Baum *et al.*, 2000). Our findings suggest that, hypozincaemia in HARRT naïve Participants may be due to low dietary intake associated with active viral replication since higher proportions of HAART naïve participants are in stage 3 of the HIV disease though no significant correlation existed between zinc and CD4 count.

Zinc is known to promote multimerization and enzymatic activity enahancement of viral integrase (Lee *et al.*, 1997). Moreover, HIV transactivating protein (Tat) is known to have a unique cysteine-rich region with zinc binding properties (Huang and Wang, 1996) and has a high binding affinity for zinc (Frankel *et al.*, 1988). Therefore, zinc utilization by HIV for gene expression, multimerization and integration describes HIV as a zincdependent virus, which may explain in part the low plasma zinc levels frequently observed in HIV infected Participants especially among those who are not on HAART treatment (Darlix *et al.*, 1995; Mocchegiani *et al.*, 1995; Berthoux *et al.*, 1997). However, with HAART Participants, HAART use decreases the over utilization of zinc by HIV virus through the anti replicative properties of anti retroviral drugs especially zidovudine (AZT). Zinc in combination with other micronutrients is known to considerably improve gastrointestinal disturbances and infections leading to decrease cachexia and this may result in better dietary patterns and weight gain which in turn may increase survival during treatment (Friis, 2005). Though BMI and WHR had no correlation with zinc levels in the study but obesity was generally evident in the mean values of BMI and WHR in the study. Therefore this study suggest that adequate dietary intake of zinc or zinc supplement together with HAART use will improve the nutritional status of HIV participants irrespective of the duration of infection. Moreover a correlation of zinc and transferrin in

this study suggests a combination of haematinics in HAART use for optimal activities of zinc.

This study has further illustrated the higher proportions of low transferrin levels in HAART naïve Participants as compared to Participants on HAART. However,

proportions of low ferritin levels were very low with no recorded case in Participants on HAART (Table 4.7). This finding in the study contradicts a study done in South Africa in which there were high transferrin levels and low ferritn levels among HIV infected women (Walsh *et al.*, 2010). This study further stands in contradiction to a study conducted in Indonesia in which high ferritin and low transferrin levels were seen in HAART naïve Participants whereas Participants on HAART had low ferritin and transferrin concentration though it was not statistically significant (Wisaksana *et al.*, 2011). Wisakana et al., 2011 further indicated a negative correlation between ferritin levels and CD4 in HAART naïve patient and no correlation in Participants on HAART. These finding by Wisakana et al., 2011 stands in contradiction with our current findings where no significant correlation existed in ferritin levels and CD4.

Serum ferritin levels are usually known to be in a state of equilibrium with body iron stores and measurements of serum ferritin may best reveal an early negative iron balance, while high serum ferritin may indicate significant iron overload or levels. However, serum ferritin levels may also increase in chronic diseases unrelated to iron metabolism such as inflammatory diseases. Therefore it is suggested in this study that the higher proportion of ferritin levels in the normal range and lower proportions of low ferritin levels may be due to iron redistribution in the case of acute phase response by the body during HIV infection though no significant correlation existed between CD4 and ferritin levels. The HIV virus

itself may increase ferritin levels as HIV nef protein directly downregulates the protein HFE which is responsible for hemochromatosis and as such causes iron accumulation leading to high ferritin levels in both HAART participants and HAART naïve participants (Drakesmith *et al.,* 2005). However, the higher proportion of microcytic anaemia in HAART naïve Participants and no case recorded in HAART participants suggest that the higher proportion of normal ferritin levels in both category of Participants may be mostly due to the HIV infection itself and ferritin levels alone may not reflect an accurate assessment of iron storage in the HIV infected Participants. However, this study does not rule out the possibility of good iron storage due to adequate iron intake among participants especially those on HAART which was not determined in the study in both category of participants.

Moroever, serum transferrin may decrease in chronic illnesses associated with low serum iron concentrations and this may account for the high proportion of transferrin deficiency in HAART naïve participants together with a higher proportions of microcytic anaemia (Monteiro *et al.*, 2000).

The study has shown no significant difference in the mean values of ALT and AST levels though both parameters were in the normal range. However, mean ALP levels were elevated in both groups of HIV participants though the difference in both participants were not statistically significant. This finding is in contradiction with a study conducted in India by (Kamble *et al.,* 2013) that indicated high levels of liver enzymes in HIV

Participants. Kamble *et al*., 2013 attributed elevations of liver enzymes to cholestatic disease due to granulomatous inflammation caused by mycobacterium tuberculosis and fungal infections which may be common in HIV infection.

However in our current study, the elevated mean values of ALP in both category of participants may most likely be due to bone disorders and not necessarily liver abnormalities due to the normal mean values of ALT, AST and total protein levels. Moreover, Owiredu et al; 2011, reports of significant high values of ALP values especially among participants on HAART with an associated decrease in calcium levels due to on-going osteoporosis in HIV infected participants. This may as well be a possible explanation to the findings of this study though calcium was not measured in the study. Evidence indicates that HIV itself could cause problems with bone mineralization (Bruera *et al.*, 2003; Glesby, 2003; Thomas and Doherty, 2003). However, other studies suggest the role of ART especially Efavirenz use in increasing ALP level due to increase vitamin D catabolismless but less clarity lies in the relative contribution of ART to these problems (Viganò and Mora, 2004; Welz *et al.*, 2010). Some studies have compared HIV-infected men and women with and without lipodystrophy and have reported an association between bone loss and visceral fat or lipodystrophy (Huang *et al.*, 2001; Brown *et al.*, 2004) which is much evident in our study. Additionally, increase in growth of tissues in response to the increase catabolism associated with HIV infection may account for this occurrence of elevated ALP. Moreover, the fact remains unclear whether bone problems are due to metabolic disorders resulting primarily from HIV infection, use of antiretroviral agent or a combination of both(Brown *et al.*, 2004; Viganò and Mora, 2004). Nonetheless, bone loss is a potentially serious problem that may affect the longterm health and quality of life of PLWHA, and assessment of bone health during nutritional assessment should become part of the comprehensive clinical care of PLWHA.

Liver enzymes, though not a direct assessment of nutritional status, could provide necessary information on the general metabolism and functions of the liver and the body where most micronutrients are metabolised and hence its necessity in nutritional assessment.

CHAPTER 6

CONCLUSION AND RECOMMENDATION Comprehensive management of persons infected with HIV has been shown to reduce mortality in addition to improving their quality of life of the infected. Nutritional assessment in the HAART era is known to be a crucial part in the care of HIV

Participants.

This study has therefore confirmed that HAART has a crucial role to play in improving the nutritional status during HIV infection. It is known to reduce morbidity and mortality associated with HIV infection through significant improvements in haematocrit and haemoglobin levels and thus reducing the prevalence of nutritional anaemias as well as anaemia of chronic diseases during HIV infection. HAART use has also been shown in this study to improve CD4 counts to values ≥200 cells mm^{-3} although HAART use may also be associated with side effects such as low neutrophils and white blood cell count. Results in the study also confirms the effective role of HAART use to reduce other nutritional deficiencies such as hypozincaemia, hypoalbuminaemia and iron deficiencies. HAART naïve Participants are susceptible to exoerience these nutritional deficiencies and hence the need for comprehensive intervention.

Contrary to popular studies conducted on HIV Participants where wasting and underweight were associated with the infection, this study has indicated shown that being underweight or wasted is uncommon among HIV Participants in our study population. Obesity is an emerging epidemic among HIV Participants especially those on HAART. However, the study has shown that the obesity is associated with duration of infection,

HAART use and economic stability of the individual. However, body wasting could occur in HIV participants who had gained weight prior to the HIV infection without HAART use as the duration of infection increases. Lipodystrophy associated with

HAART use especially Zidovudine could lead to obesity in HAART participants. Therefore to strengthen the benefits of ART in developing countries like Ghana, the evaluation of nutritional assessment and intervention is essential to improve and maintain the nutritional and clinical status of participants. The study further shows the unreliable nature of using

anthropometry alone in determining nutritional status in an era of obesity and overweight in HIV participants. Nutritional studies must be conducted in line with other biochemical and haematological findings.

Results also provide evidence that ferritin and transferrin levels may not be a direct measure of iron deficiency especially in HIV Participants due to chronic inflammation and infections associated with the disease. Therefore C-reactive proteins, iron turn-over and other inflammatory cytokines are necessary in addition to iron determining parameters to distinguish anemia of chronic disease and anemia caused by iron deficiency as well as to determine the accurate iron stores of HIV Participants.

The study has further shown that assessment of liver function and lipid profile is very crucial in determining the aetiology of nutritional deficiencies and disorders. Further liver enzymes and lipid assessment is necessary to determine the side effects of HAART drugs as well as disorders due to HIV infection.

6.1 LIMITATIONS AND RECOMMENDATION

This study was a comparative cross sectional study and therefore could not assess the dietary intake of the study participants and their possible impact on nutritional status over a period of time. Other micronutrients such as selenium, copper, vitamin A, B12, C were not assessed in this study. Their influence on the burden of nutritional deficiencies in this setting can therefore become subject for further scientific investigation.

Quantification of income of study participants was not done in this study due to lack of information by study participants. However, further investigations could find better means

of quantifying income of participants. Income is known to influence dietary diversity and hence improving nutritional status of HIV infected individuals.

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