OXIDATIVE STRESS, RENAL DYSFUNCTION AND HAEMATOLOGICAL PROFILE AMONG HAART NAÏVE HIV INFECTED GHANAIAN PATIENTS AND THOSE ON HAART

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LAWRENCE QUAYE

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST and the Regional Hospital, Bolgatanga. This work has not been submitted for any other degree.

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Lawrence Quaye

……

Dr. W.K.B.A. Owiredu

(Supervisor)

……

Dr. Nafiu Amidu

(Co-supervisor)

……

Dr. E. F. Laing (Head

of Department)

ABSTRACT

In a cross-sectional study to assess oxidative stress, renal dysfunction and haematological complications among Ghanaian HIV infected patients, 442 people living with HIV/AIDS (PLWHA) consisting of 166 patients on highly active antiretroviral therapy (HAART) and 276 HAART naïve patients from the antiretroviral (ART) clinic at the Regional Hospital, Bolgatanga were recruited for this study. Complete haemogram, immunological analysis (CD4 & CD3), biochemical analysis and weight were measured for all the patients. Females outnumbered males by 3 to 1 in the HAART naïve group and 5 to 1 in subjects on HAART. Patients on HAART were older and heavier than their naive counterparts and were on a backbone therapy of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs): 41.6% were on a combination therapy of (Combivir + Efavirenz); 41.0% (Combivir + Nevirapine); 10.8% (Stavudine + Lamivudine + Nevirapine) and 6.6% (Stavudine + Lamivudine + Efavirenz). The incidence of anaemia (Hb ≤ 10.5 = 63.0%) and lymphopoenia (16.7%) in HAART naive patients was significantly higher compared to their counterparts on HAART (46.0% and 5.3% respectively). The incidence of anaemia in HAART naïve females was 70% compared to 44% in HAART naive males (P = 0.0001). HAART naïve patients are 5 times at risk of developing microcytic hypochromic anaemia compared to those on HAART (P = 0.0002). Total lymphocyte count (TLC), haemoglobin, lymphocyte count and weight were significant predictors of CD4 counts and TLC values between 1.0 – 2.0 k μL^{-1} was a significant predictor of CD4 counts < 200 cells mm^{-3}. Vitamin C was reduced across the general study population but significantly reduced in patients on HAART in comparison to the HAART naïve group (P < 0.0001). MDA was elevated by about 10-fold across the study group. The incidence of isolated hypercholesterolaemia in HAART naïve patients and patients on HAART is 2.2% and 18.1% respectively; isolated hypertriglyceridaemia (16.3% vs. 31.9%); isolated decreased HDL-C (35.9% vs. 38.6%) and isolated increased LDL-C (1.5% vs. 3.6%). The overall incidence of hypocalcaemia was 14.1% and 41.6% in HAART naïve patients and patients on HAART respectively. The incidence of chronic kidney disease (CKD) in the study population calculated with the Cockcroft-Gault, 4vMDRD and CKD-EPI equations was 10.9%, 10.4% and 10.7% respectively. A comparison of methods saw the CKD-EPI equation yielding lower bias to the Cockcroft-Gault and 4v-MDRD equations. HAART has the capability of reducing the incidence of anaemia and lymphopoenia which are associated with disease progression and death in HIV infected patients. Total lymphocyte count, lymphocyte count, haemoglobin and weight could also serve as useful predictive tools in the management and monitoring of HIV infected patients in resource limited settings. CKD is not uncommon among HIV infected Ghanaian patients and a significant proportion (≈10%) will require antiretroviral dose adjustment either at the time of initiating therapy or sometime during on-going therapy.

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ABBREVIATIONS

3TC – Lamivudine

ABC – Abacavir

ACTG – Aids Clinical Trial Group

AIDS – Acquired Immune Deficiency Syndrome

ALP – Alkaline Phosphatase

ALT – Alanine Transaminase

ARF – Acute Renal Failure

ARV – Antiretroviral

AST – Aspartate Transaminase

AZT – Azidothymidine (Zidovudine)

BID – Direct Bilirubin

BIT – Total Bilirubin

BUN – Blood Urea Nitrogen

CDC – Center for Disease Control

CKD – Chronic Kidney Disease

CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration

CrCl – Creatinine Clearance

CRF – Circulating Recombinant

Form d4T – Stavudine ddI –

Didanosine

DNA – Deoxyribonucleic Acid

EFV – Efavirenz

EIA – Enzyme Immunoassay

ESRD – End Stage Renal Disease

FIV – Feline Immunodeficiency Virus

FTC – Emtricitabine

GFR – Glomerular Filtration Rate

GGT – Gamma Glutamyl Transferase

HAART – Highly Active Antiretroviral Therapy

HCT – Haematocrit

HDL – High Density Lipoprotein

HIV – Human Immunodeficiency Virus

HIVAN – HIV-Associated Nephropathy

HTLV – Human T-lymphotrophic Virus

IDSA – Infectious Disease Society of America

IDV – Indinavir
INF-α – Interferon alpha

K/DOQI – Kidney Disease Outcome Quality Initiative

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

MDRD – Modification of Diet in Renal Disease

MHC – Major Histocompatibility Complex

NAC – N-acetylcysteine

NFV – Nelfinavir

NKF – National Kidney Foundation

NNRTI – Non-Nucleoside Reverse Transcriptase Inhibitors

NRTI – Nucleoside Reverse Transcriptase Inhibitors

NVP – Nevirapine

PI – Protease Inhibitors

PUFA – Polyunsaturated Fatty acids
RBC – Red Blood Cell

RNA – Ribonucleic Acid

ROS – Reactive Oxygen Species

SIV – Simian Immunodeficiency Virus

SOD – Superoxide Dismutase

SQV – Saquinavir

TBA – Thiobarbituric acid

TCA – Trichloroacetic acid

TDF – Tenofovir

TG – Triglyceride

TLC – Total Lymphocyte Count

TNF-α – Tumour Necrosis Factor-alpha

UNAIDS – United Nations Programme on HIV/AIDS

VLDL – Very Low Density Lipoprotein

WBC – White Blood Cell

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and is characterized by progressive damage to the body's immune system which results in a number of opportunistic infections, immunological and haematological complications (Okolie *et al.*, 2003). The main immunological complication and hallmark of HIV infection is cellular CD4 Tlymphocyte depletion for which various mechanisms such as, HIV induced cytolysis; dysregulation of cytokines; cytotoxic T-lymphocyte responses and HIV induced autoimmune reactions (Voth *et al.*, 1988; Tersmette and Schuitemaker, 1993) which are not mutually exclusive have been suggested. Haematological complications have been documented to be the second most common cause of morbidity and mortality in HIV patients (Salond, 2005; Cosby, 2007) and are generally marked with cytopoenias such as anaemia, neutropoenia, lymphopoenia and thrombocytopenia (Moyle, 2002). The incidence and severity of the cytopoenia generally correlate to the stage of the disease with anaemia being the most commonly encountered haematologic abnormality and a significant predictor of progression to AIDS or death (Volberding, 2002; Odunukwe *et al.*, 2005).

Abnormal renal function has been identified in about 30.0% of human immunodeficiency virus (HIV) infected patients (Szczech *et al.*, 2002) with a more recent analysis of a large urban United States (US) HIV clinic showing that approximately 15.5% of the patients had chronic or end-stage renal disease (Coresh *et al.*, 2003). Chronic kidney disease (CKD) is essentially compromised kidney function that persists for more than three (3) months (Coresh *et al.*, 2007). CKD is becoming a public health problem with recently published reports emphasizing it as being under-diagnosed and under-treated (Obrador *et al.*, 1999; Nissenson *et al.*,

2001). Recognition of this chronic condition is therefore crucial to facilitate the employment of measures that can slow progression to end-stage renal disease (ESRD). However, individuals of African descent have been impacted most by renal disease as a complication of HIV infection with HIV-associated nephropathy (HIVAN) being the most commonly detected abnormality (Laradi *et al.*, 1998; Kimmel *et al.*, 2003). HIVAN has been documented in sub-Saharan Africa (Gerntholtz *et al.*, 2006; Kalayjian *et al.*, 2008) but little is known about its prevalence or impact.

The introduction of highly active antiretroviral therapy for treatment of HIV infection has generally been accepted as the gold standard in the management of HIV patients (Odunukwe *et al.*, 2005). The combination therapy involves highly active antiretroviral therapy (HAART) medications selected from nucleoside reverse transcriptase inhibitors (NRTI's), non-nucleoside reverse transcriptase inhibitors (NNRTI's), protease inhibitors (PI's) and fusion inhibitors. Through dramatic decline in viral loads and sustained increase in CD4 counts, HAART has significantly reduced morbidity and mortality in patients with advanced HIV infection (Palella *et al.*, 1998) and discontinuation of antiretroviral (ARV) treatment has been shown to increase mortality not just from HIV but also from other causes (Coresh *et al.*, 2007). Gea-Banacloche and Lane, (1999) and Odunukwe *et al.,* (2005) reported improvements in haematocrit and haemoglobin values which resulted in reduction in morbidity and mortality of HIV patients.

Some antiretroviral therapies have been identified to have clinically significant nephrotoxicity and others have been associated with acute and chronic adverse effects including gastrointestinal, metabolic and central nervous toxicities (Carpenter *et al.*, 1998). Anticipation and management of the complications of longterm antiretroviral therapy e.g. drug induced kidney damage, abnormal fat distribution, dyslipidaemia, abnormal glucose metabolism and long-standing HIV infection complications e.g. HIVAN, oxidative stress, lipid peroxidation and other co-morbidities are of increasing concern considering the potential health risks to the patient (Cohan, 2000).

Current guidelines for the management of HIV-infected patients include initial assessment to rule out chronic kidney disease and to identify patients at risk for developing kidney disease (Gupta *et al.*, 2005). Glomerular filtration rate (GFR) compared to serum creatinine or 24-hour urine creatinine measurement is considered the best overall index of kidney function in health and disease because of the attendant problems associated with creatinine measurement and 24-hour urine creatinine clearance estimation (National Kidney Foundation, 2002; Stevens *et al.*, 2006). Kidney function can be reliably estimated by calculating creatinine clearance (CrCl) or GFR through use of the Cockcroft-Gault or Modification of Diet in Renal Disease (MDRD) equations respectively (Cockcroft and Gault, 1976; Levey *et al.*, 1999). A GFR <60 mL min^{-1} 1.73 m^{-2} meets the criteria for CKD and this cutoff is supported by epidemiological data linking lower GFR to an increased frequency of hospitalization, cardiovascular events or death. Neither of the equations has been specifically validated in the HIV-infected population but they remain the most highly validated formulas available and both equations are more sensitive than measurement of serum creatinine alone (Owiredu *et al.*, 2008; Winston *et al.*, 2008).

Lipid abnormalities are known to be associated with HIV infection (Carling *et al.*, 2002; Sudano *et al.*, 2006) and HIV infected individuals receiving protease inhibitors (PI's) (Mulligan *et al.*, 2000). Chronic inflammation due to HIV infection leads to high plasma levels of inflammatory cytokines and production of reactive oxygen species (ROS) (Israel and Gougerot-Pocidalo, 1997). Increased concentration of inflammatory cytokines (α-interferon) leads to a decrease in the clearance of triglycerides in the blood leading to hypertriglyceridaemia (Carling *et al.*, 2002) and an imbalance between ROS production and its inactivation by antioxidants is capable of causing oxidative damage to major macro-molecules in cells, including lipids, proteins and nucleic acids. Oxidative attack on lipids could lead to lipid peroxidation and loss of functionality of the parent lipid compounds which are major components of cell membranes (Zoccali *et al.*, 2000) and the aetiology of arteriosclerosis is linked with the oxidation of LDL cholesterol trapped in blood vessels (Judith, 2003).

1.2 JUSTIFICATION

Nucleoside reverse transcriptase inhibitors (NRTIs) in combination with other antiretrovirals are the cornerstone of AIDS therapy, turning HIV infection into a manageable clinical entity by suppressing viral replication below the threshold of clinical detection. Despite the initial positive impact of NRTIs, therapeutic experience reveals serious side effects that appears to originate in mitochondria and which ultimately manifests as dysfunction of that organelle. As the AIDS epidemic continues and as survival with HIV infection is prolonged by treatment with HAART, long-term side effects of therapy may become increasingly common. Assessment of the impact of HAART in resolving immunological and haematological complications and anticipation of HAART associated complications therefore becomes necessary in the collective management of HIV infected patients.

1.3 AIM

To assess oxidative stress, renal dysfunction and haematological profile in HAART naïve patients and those on HAART.

1.4 SPECIFIC OBJECTIVES

To establish the continual need for HAART in the management of HIV patients in this HAART era, this study seeks to:

1. Assess the impact of HAART in resolving immunological and haematological complications in HIV patients.
2. Assess oxidative stress and the incidence of dyslipidemia.
3. Find the proportion of Ghanaian HIV-infected patients (HAART naïve and on HAART) with renal dysfunction by using renal function estimating equations.
4. Assess which of the estimating equations best defines CKD in the study population.
5. Determine the proportion of the study population who would require

antiretroviral (ART) dose adjustments based on calculated GFR.

6. To assess sex variations in the study parameters and the ability of the study parameters to predict CD4 count and serve as surrogates for CD4 in resource poor settings.

Chapter 2

LITERATURE REVIEW

2.1 BRIEF HISTORY OF HIV/AIDS

Acquired Immune Deficiency Syndrome (AIDS) was first properly defined by the Center for Disease Control (CDC) in the September 1982 edition of the morbidity and mortality weekly report (MMWR) and is now known to be caused by the human immunodeficiency virus (HIV) through blood-to-blood and sexual contact (CDC, 2003). Luc Montagnier reported the isolation of a virus called lymphadenopathy-associated virus (LAV) in May 1983 which infected and killed CD4 cells (Barre-Sinoussi *et al.*, 1983) and a year later, Robert Gallo announced the isolation of a virus called human T-lymphotrophic virus (HTLV-III) (Popovic *et al.*, 1984) but both of these viruses were later renamed HIV because they were genetically indistinguishable (Marx, 1985; Chang *et al.*, 1993).

HIV is speculated to have originated in non-human primates in sub-Saharan Africa and transferred to humans during the late 19th or early 20th century because of its close resemblance to the simian immunodeficiency virus (SIV) which is found in the sooty mangabey (white-collared monkey), indigenous to West Africa and chimpanzees in West-Central Africa (Sharp *et al.*, 2001). Exactly when the zoonosis occurred is not known but some researchers have suggested a period between 1915 and 1941 (Korber *et al.*, 2000). Several alternative hypotheses for the origin of AIDS have been proposed with the argument that HIV or AIDS does not exist or that AIDS is not caused by HIV. Some proponents believe that AIDS is caused by lifestyle including sexuality or drug use; others allege that HIV was created in a bio-weapons laboratory as an agent of genocide or accident but all these hypotheses have been contradicted by scientific studies and rejected by scientific consensus.

2.2 CLASSIFICATION OF HIV

HIV belongs to a special class of viruses called retroviruses and further classified in the subgroup of viruses called lentivirus which includes SIV and feline immunodeficiency virus (FIV) which cause diseases in monkeys and cats. Most viruses, store their genetic material on long strands of DNA but retroviruses are an exception because their genes are located on the RNA (Ribonucleic Acid). HIV mutates very readily and has many different strains. Based on genetic similarities, the numerous virus strains may be classified into types, groups and subtypes (Plantier *et al.*, 2009).

2.2.1 Types

There are two types of HIV: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood and from mother to child, and they appear to cause clinically indistinguishable AIDS. 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 (also known as white-collared monkey). HIV-2 is however less easily transmitted, and the period between initial infection and illness is longer than in HIV-1. The predominant virus worldwide is HIV-1 with HIV-2 being the relatively uncommon type, concentrated in West Africa and is rarely found elsewhere (Plantier *et al.*, 2009).

2.2.2 Human Immunodeficiency Virus – 1 (HIV-1)

HIV-1 strains can be classified into four groups: the major Group M, the outlier Group O and two new groups, N and P. These four groups may represent four separate introductions of simian immunodeficiency virus into humans.

2.2.2.1 Levels of HIV-1 classification

Group O appears to be restricted to West-Central Africa and group N - a strain discovered in 1998 in Cameroon - is extremely rare. In 2009 a new strain closely related to gorilla simian immunodeficiency virus was discovered in a Cameroonian woman and it was designated HIV-1 Group P (Plantier *et al.*, 2009). More than 90% of HIV-1 infections belong to HIV-1 Group M within which there

are known to be at least nine genetically distinct subtypes. These are subtypes A, B, C, D, F, G, H, J and K. Occasionally, when two viruses of different subtypes meet in the cell of an infected person, a mixture of their genetic material can lead to the creation of a new hybrid virus - process similar to sexual reproduction, and sometimes called viral sex (Burke, 1997). Many of these new strains do not survive for long, but those that infect more than one person are known as circulating recombinant forms (CRFs).

2.2.2.2 Distribution of subtypes and CRFs

The HIV-1 subtypes and CRFs are very unevenly distributed throughout the world, with the most widespread being subtypes A and C.

- Subtype A and CRF A/G predominate in West and Central Africa, with subtype A possibly also causing much of the Russian epidemic (Bobkov *et al.*, 2004).
- Subtype B has been the most common subtype/CRF in Europe, the Americas, Japan and Australia. Although this remains the case, other subtypes are becoming more frequent and now account for at least 25% of new HIV infections in Europe.
- Subtype C is predominant in Southern and East Africa, India and Nepal. It has caused the world's worst HIV epidemics and is responsible for around half of all infections.
- Subtype D is generally limited to East and Central Africa.
- CRF A/E is prevalent in South-East Asia but originated in Central Africa.
- Subtype F has been found in Central Africa, South America and Eastern Europe.
- Subtype G and CRF A/G have been observed in West and East Africa and Central Europe.
- Subtype H has only been found in Central Africa.
- Subtype J only in Central America; and K only in the Democratic Republic

of Congo and Cameroon.

2.2.2.3 Subtype and disease progression

A study presented in 2006 found that Ugandans infected with subtype D or recombinant strains incorporating subtype D developed AIDS sooner than those infected with subtype A, and also died sooner, if they did not receive antiretroviral treatment. The study's authors suggested that subtype D is more virulent because it is more effective at binding to immune cells (Laeyendecker, 2006). This result was supported by another study presented in 2007, which found 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, 2007). An earlier study of sex workers in Senegal, published in 1999, found that women infected with subtype C, D or G were more likely to develop AIDS within five years of infection than those infected with subtype A (Kanki *et al.*, 1999). Several studies conducted in Thailand suggest that people infected with CRF A/E progress faster to AIDS and death than those infected with subtype B, if they do not receive antiretroviral treatment (Nelson *et al.*, 2007).

2.2.2.4 Differences in transmission

It has been observed that certain subtypes and CRFs are predominantly associated with specific modes of transmission. Subtype B in particular is spread mostly by homosexual contact and intravenous drug use (essentially via blood), while subtype C and CRF A/E tend to fuel heterosexual epidemics (via a mucosal route). Whether there are biological causes for the observed differences in transmission routes still remains a subject of debate and some scientists believe such causes do exist with claims that subtype C and CRF A/E are transmitted much more efficiently during heterosexual sex than subtype B (Essex, 1996; Bhoopat *et al.*, 2001). Such theories have however not been conclusively proven (Dittmar *et al.*, 1997; Pope *et al.*, 1997) and more recent studies have looked for variation between subtypes in rates of mother-to-child transmissions (Blackard *et al.*, 2001; Yang *et al.*, 2003).

2.2.3 Human Immunodeficiency Virus – 2 (HIV-2)

HIV-2 has been isolated from a number of patients with AIDS, first in West African countries and subsequently in Western Europe, the United States, and elsewhere. Most cases have appeared in West Africa and have appeared only sporadically in other parts of the world (De Cock *et al.*, 1993). HIV-2 is believed to have been present in Africa as early as the 1960's (Miyazaki, 1995) and has greater homology to simian immunodeficiency virus (SIV) than to HIV-1.

2.2.3.1 Subtypes of HIV-2

The subtypes of HIV-2 have been designated from A through F. There is up to a 25% difference in genetic homology among these subtypes. All six subtypes can be detected by enzyme immunoassay (EIA) and Western blot assays for HIV-2 similar to those for HIV-1. Infection with HIV-2 eventually leads to AIDS and persons can 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 of HIV-2

HIV-2 is spread in a manner similar to HIV-1, though the high-risk groups are commercial sex workers and persons with other sexually transmitted diseases. The peak age of persons infected with HIV-2 appears to be higher than that of HIV-1, but there appears to be no sex difference in rates of infection (Miyazaki, 1995). HIV-2 appears to utilize 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 THE STRUCTURE OF HIV

HIV, like all viruses cannot grow or reproduce on its own. In order to make new copies of itself it must infect the cells of a living organism. Outside of a human cell, HIV exists as roughly spherical particles, called virions and the surface of each particle is studded with lots of little spikes. An HIV particle is around 100-150 billionths of a metre in diameter surrounded with a coat of fatty material known as the viral envelope or membrane. Projecting from this are around 72 little spikes,

which are formed from the proteins gp120 and gp41. Just below the viral envelope is a layer called the matrix, which is made from the protein p17. The proteins gp120 and gp41 together make up the spikes that project from HIV particles, while p17 forms the matrix and p24 forms the core which is usually bullet shaped. 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).

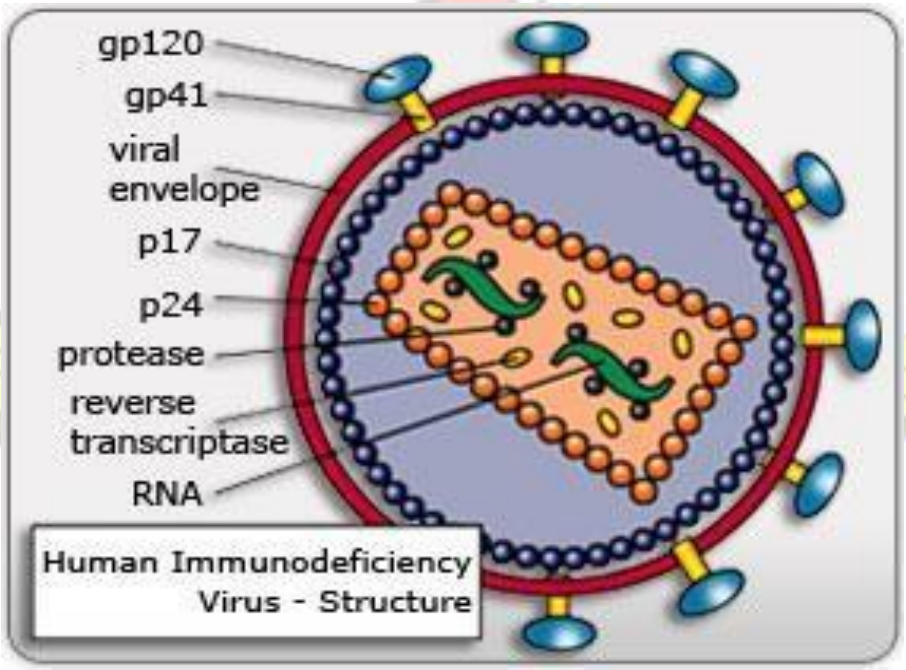


Figure 2.1 Structure of human immunodeficiency virus

2.4 HIV GENES

HIV has just nine genes - three of the genes, gag, pol and env genes contain information needed to make structural proteins for new virus particles and the other six genes, known as tat, rev, nef, vif, vpr and vpu, code for proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease (Cladera *et al.*, 2001).

2.5 HIV LIFE CYCLE

2.5.1 Entry

HIV can only replicate inside human cells and the process typically begins when a virus particle bumps into a cell that carries on its surface a special protein called CD4. The spikes on the surface of the virus particle stick to the CD4 and allow the viral envelope to fuse with the cell membrane. 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 inside the cell, 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 DNA known as provirus.

2.5.3 Transcription and Translation

HIV provirus may lie dormant within a cell for a long time but when the cell becomes activated, 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

The strands of messenger RNA produced by the cell have complete copies of HIV genetic material. These gather together with newly made HIV proteins and enzymes to form new viral particles, which are then released from the cell. The enzyme protease 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. 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 GLOBAL HIV STATISTICS

The estimated number of people living with HIV worldwide in 2008 is 33.4 million (31.1 million–35.8 million). The total number of people living with the virus in 2008 was more than 20% higher than the number in the year 2000, and the prevalence was roughly threefold higher than in 1990. An estimated 2.7 million (2.4 million–3.0 million) new HIV infections occurred in 2008 and the estimated deaths due to AIDS-related illnesses in 2008 was 2 million (1.7 million–2.4 million) (Table 2.1) approximately 10% lower compared to 2.2 million (1.9 million–2.6 million) AIDSrelated deaths in 2004. Latest epidemiological data indicates that the global spread of HIV appeared to have peaked in 1996 with 3.5 million (3.2 million – 3.8 million) new infections. The estimated number of new infections in 2008 was however approximately 30% lower than at the epidemic's peak in 1996. The estimated number of new HIV infections in children under the age of 15 in 2008 was 430,000 (240,000 – 610,000) (Table 2.1) and most of these infections are believed to have stemmed from transmission in utero, during delivery or post partum as a result of breast feeding. The number of newly infected children in 2008 is roughly 18% lower than in 2001 (UNAIDS, 2009).

2.6.1 HIV/AIDS statistics in Ghana

The first case of HIV/AIDS in Ghana was reported in 1986 (USAID, 2003) and 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 AIDSrelated deaths (USAID, 2003). The estimated number of people living with HIV in 2007 was 260,000 with an estimated prevalence rate of 1.9% and the total number of estimated AIDS-related deaths in 2007 is 21,000 (UNAIDS/WHO, 2008). After a prevalence decline to 1.7% in 2008, there was an increase in prevalence to 1.9% in 2009, with an estimated 267,069 persons living with HIV/AIDS in Ghana. An estimated 25,531 new infections was recorded in 2009 and 20,313 AIDS-related deaths (NACP, 2010).

Table 2.1 Global summary of the AIDS epidemic, December 2008

Number of people living with HIV in 2008	
Total	33.4 million [31.1 million – 35.8 million]
Adults	31.3 million [29.2 million – 33.7 million]
Women	15.7 million [14.2 million – 17.2 million]
Children under 15 years	2.1 million [1.2 million – 2.9 million]
People newly infected with HIV in 2008	
Total	2.7 million [2.4 million – 3.0 million]
Adults	2.3 million [2.0 million – 2.5 million]
Children under 15 years	430 000 [240 000 – 610 000]
AIDS-related deaths in 2008	
Total	2.0 million [1.7 million – 2.4 million]
Adults	1.7 million [1.4 million – 2.1 million]
Children under 15 years	280 000 [150 000 – 410 000]

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information. Source: (UNAIDS, 2008).

HIV prevalence rates are not uniform across the country – prevalence is high in densely populated areas, mining and border towns and towns along main transportation routes. The highest prevalence rate is found in the Eastern Region and lowest in the Upper West and Northern Regions (UNAIDS/WHO, 2008).

Heterosexual intercourse accounts for about 75 to 80 percent of all HIV/AIDS infections in Ghana. Vertical transmission from mother to child accounts for 15% and transmission through blood and blood products accounts for 5% (NACP, 2001). HIV-1 is the predominant infecting agent contributing about 94.4% of all infections; HIV-2 contributes about 0.5% and 5.1% of the cases are dual infections of HIV-1 and HIV-2 (Ghana Health Service, 2004).

2.7 ANTIRETROVIRAL DRUGS

Antiretroviral drugs (ARVs) are medications for the treatment of HIV infection and were first introduced in 1986. The combination of several of such drugs, typically three or four is termed highly active antiretroviral therapy (HAART) (British HIV Association Writing Committee, 2001; USDHHS, 2004). The United Nations General Assembly Special Session on HIV/AIDS (UNGASS) in 2001 advocated for the complementarity of HIV care and prevention urging governments to provide the highest attainable standard of care, including antiretroviral treatment to people living with HIV/AIDS (World Health Organization, 2002). HAART is therefore recommended for the treatment of all patients with AIDS but because of the complexity of selecting and following a regimen, severity of the side-effects and the importance of compliance to prevent viral resistance, emphasis of involving patients in therapy choices, analyzing risks and potential benefit is important (Dybul et al., 2002).

2.7.1 Classes of ARVs

There are different classes of antiretroviral drugs that act at different stages of the HIV life-cycle.

2.7.1.1 Nucleotide and Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Drugs in this class inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation. Examples of drugs in this class are zidovudine (AZT), stavudine (d4T), lamivudine (3TC), emtricitabine (FTC), didanosine (ddl), abacavir (ABC) and tenofovir (TDF) (Weller and Williams, 2001).

2.7.1.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Drugs in this class inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function. Examples include nevirapine (NVP) and efavirenz (EFV) (Weller and Williams, 2001).

2.7.1.3 Protease Inhibitors (PIs)

Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease which is an enzyme used by HIV to cleave nascent proteins for final assembly of new virons. Examples are saquinavir (SQV), indinavir (IDV) and nelfinavir (NFV) (de Soultrait *et al.*, 2002).

2.7.1.4 Integrase Inhibitors

Drugs in this class inhibit 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).

2.7.1.5 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. Maraviroc and enfuvirtide are the two currently available agents in this class (Kilby *et al.*, 1998).

2.7.1.6 Maturation Inhibitors

These drugs inhibit 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). Because these viral particles have a defective core, the virions released consist mainly of non-infectious particles. There are no drugs in this class currently available, though two are under investigation, bevirimat (Panacos Pharmaceuticals) and Vivecon (Kilby *et al.*, 1998).

2.8 COMBINATION THERAPY

The life cycle of HIV can be as short as about 1.5 days from viral entry into a cell, through replication, assembly, release of virions, to infection of other cells (Perelson *et al.*, 1996). HIV lacks proofreading enzymes to correct errors made when it converts its RNA into DNA via reverse transcription. The short life-cycle and high error rate causes the virus to mutate very rapidly, resulting in a high genetic variability of HIV. Combinations of antiretrovirals create multiple obstacles to HIV replication to keep the number of virions low and reduce the possibility of a superior mutation. If a mutation that conveys resistance to one of the drugs being taken arises, the other drugs continue to suppress reproduction of

that mutation. With rare exceptions, no individual antiretroviral drug has been demonstrated to suppress an HIV infection for long and as such these agents must be taken in combinations in order to have a lasting effect. As a result, the standard of care is to use combinations of antiretroviral drugs which usually comprise two nucleosideanalogue reverse transcriptase inhibitors (RTIs) and one non-nucleoside-analogue RTI or protease inhibitor (USDHHS, 2004).

Combinations of antiretrovirals are subject to positive and negative synergies, which limit the number of useful combinations. For example, Didanosine and AZT inhibit each other, so taking them together is less effective than taking either one separately. Other issues further limit some people's treatment options from antiretroviral drug combinations, including their complicated dosing schedules and often severe side-effects. In recent years, pharmaceutical companies have worked together to combine these complex regimens into simpler formulas, termed fixed-dose combinations. For instance, two pills containing two or three medications each can be taken twice daily and this greatly increases the ease with which they can be taken, which in turn increases adherence and effectiveness over the long-term. Lack of adherence is a primary cause of resistance development in medication-experienced patients. Patients able to adhere at this rate and higher can maintain one regimen for up to a decade without developing resistance. This greatly increases chances of long-term survival, as it leaves more drugs available to the patient for longer periods of time.

2.8.1 Initiation of HAART

Antiretroviral drug treatment guidelines have changed many times with a more conservative approach with a starting point somewhere between 350 and 500 CD4+ T cells mm^{-3}.The current guidelines for antiretroviral therapy (ART) from the World Health Organization (WHO) reflect the 2003 changes to the guidelines and recommend that in resource-limited settings (that is, developing nations), HIVinfected adults and adolescents should start ART when HIV infection has been confirmed and one of the following conditions is present (WHO, 2003):

- 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}.

2.8.1.1 Concerns

Antiretroviral drug regimen can be complicated, requiring patients to take several pills at various times during the day, although treatment regimens have been greatly simplified in recent years. If patients miss doses, drug resistance can develop (Dybul *et al.*, 2002). Also, providing anti-retroviral treatment is costly and resource-intensive, and the majority of the world's infected individuals cannot access treatment services.

2.8.1.2 Adverse effects

Adverse effects of antiretroviral drugs vary by drug, ethnicity, individual and interaction with other drugs, including alcohol. Hypersensitivity to some drugs may also occur in some individuals. Examples of the several common adverse effects experienced by patients taking some antiretroviral drugs are: abdominal pain, alopecia, anaemia, asthenia, diarrhoea, dizziness (vertigo), flatulence, headache, hepatitis, hyperbilirubinaemia, hypercholesterolaemia, (dyslipidemia, hyperlipidemia, high cholesterol), hyperpigmentation (of nails, palms or soles), ingrown nails, insomnia, jaundice, lipodystrophy, liver failure, 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, vomiting, xeroderma (dry skin) and xerostomia (dry mouth) (McNicholl, 2004).

2.9 HIV AND OXIDATIVE STRESS

The hallmark of HIV infection is cellular CD4 immunodeficiency for which various mechanisms which are not mutually exclusive have been suggested. These include HIV induced cytolysis, dysregulation of cytokines, cytotoxic T-lymphocyte responses and HIV induced autoimmune reactions (Tersmette and Schuitemaker, 1993). It has been established that depletion of lymphocytes, primarily of the CD4 cell subset during HIV infection might be due to programmed cell death (apoptosis) (Gougeon and Montagnier, 1993). Apoptosis is a physiological mechanism that preserves homeostasis in the turnover of normal tissue and can be initiated by several mechanisms such as defects in activation signalling, cytokine imbalance or super-antigen stimulation. The fact that many agents that induce apoptosis are either oxidants or activators of cellular oxidative metabolism suggests that generation of reactive oxygen species (ROS) could induce apoptosis. Pace and Leaf (1995) suggested that oxidative stress mediated by the generation of ROS induces apoptosis. ROS, that is, superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($H0^{\bullet}$), lipid peroxides (LOOH), nitric oxide (NO) and hypochloric acid (HOCl) are toxic and ultimately oxidize all biological matter. Oxidants produced by neutrophils and macrophages during the oxidative burst participate in the destruction of microorganisms and induce cellular injury of bystander cells (Dobmeyer *et al.*, 1995).

In HIV infection, monocytes are chronically activated resulting in the production of monokines such as tumour necrosis factor – alpha (TNF-α) which enhances ROS production of neutrophils and monocytes and has been shown to be involved in the onset of apoptosis. TNF-α and ROS can cause the release of nuclear factor κB (NF-κB) from factor IκB in the cytoplasm with NF-κB translocating to the nucleus and binding to DNA, a process which has been implicated in increased HIV transcription (Greenspan *et al.*, 1994).

2.9.1 Types of ROS

2.9.1.1 Singlet oxygen ($^1O_2$)

Singlet oxygen ($^1O_2$), which is largely involved in photochemical reactions, is very reactive, although it does not contain unpaired electrons and therefore is not a free radical. It is formed in vivo by enzymatic activation of oxygen, for example, through lipo-oxygenase activity during prostaglandin biosynthesis (Cadenas and Sies, 1984). Singlet oxygen is a very reactive ROS and induces various genotoxic, carcinogenic and mutagenic effects through its action on polyunsaturated fatty acids (PUFAs) and DNA (Di Mascio *et al.*, 1994).

2.9.1.2 Superoxide anion ($O_2^{\bullet -}$)

Superoxide is an anionic radical formed by the reduction of molecular oxygen through the acceptance of a single electron. The hydroperoxyl radical which is unstable at physiological pH dissociates to superoxide. In vivo, it is mainly produced by the electron transport chains in the mitochondria and microsomes through electron leakage - a phenomenon that increases with an increase in oxygen utilization (McCord and Omar, 1993). Superoxide radicals are also formed by metal ion-dependent oxidation of epinephrine and norepinephrine, and by the action of enzymes such as tryptophan hydroxylase, indoleamine dioxygenase, and xanthine oxygenase. Activated phagocytes also possess metabolic pathways for the production of superoxide radicals in response to bacterial infection (Curnutte and Babior, 1987).

2.9.1.3 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide and superoxide may undergo further transformations in the presence of transition metals (particularly iron and copper) (Halliwell and Gutteridge, 1990) to give rise to the highly reactive hydroxyl radicals, by the Haber-Weiss or Fenton reactions. This property, combined with the membrane permeability of hydrogen peroxide, gives superoxide and hydrogen peroxide the ability to affect the integrity of distant molecules within the cell (Cochrane, 1991).

2.9.1.4 Hydroxyl radical (HO•)

The hydroxyl radical is the most aggressive member of the ROS family and can bring about extensive damage to different types of molecules, including proteins, nucleic acids, and lipids. In DNA, the HO• can induce several effects including base and sugar modifications, cross-linking between bases, cross-linking between DNA and protein, strand breaks, and formation of adducts (Cochrane, 1991). The action of hydroxyl radicals on proteins leads to extensive protein–protein cross-linking (Stadtman, 1992). The peroxidation of polyunsaturated fatty acids (PUFAs) by hydroxyl radicals constitutes one of the most severe attacks on cellular integrity (Gutteridge, 1995).

2.9.1.5 Peroxyl radicals (HOO•)

Peroxyl radicals are believed to be produced primarily during lipid peroxidation, which is initiated by abstraction of a hydrogen atom from unsaturated lipids. Although lipid peroxidation has been found to play a useful role in some biological processes, peroxidation of membrane PUFAs may adversely affect many functionally important parameters, such as membrane fluidity, permeability, electrical potential and controlled transport of metabolites across the membrane (Halliwell, 1989).

2.9.2 Evidence for in Vivo Oxidative Stress in HIV Infection

Direct measurement of oxidative stress under clinical conditions is difficult. However, indirect evidence indicates that HIV infection is associated with increased ROS production and increased consumption of antioxidants. Concentrations of intracellular glutathione (GSH) in the peripheral blood mononuclear cells and lymphocytes of asymptomatic HIV-seropositive patients were found to be somewhat below those of healthy controls. However, intracellular GSH levels were profoundly depressed in patients with AIDS and AIDS-related complex. Zidovudine treatment of patients with AIDS produced a rise in measurable GSH levels (Buhl *et al.*, 1989). One mechanism of GSH action is through removal of intracellular H_2O_2 by providing a substrate for GSH peroxidase, the major H_2O_2 removing enzyme. It may be significant that this

enzyme is selenium dependent and that selenium levels are decreased in HIVinfected patients (Dworkin *et al.*, 1986). In addition, HIV infected patients often have lower concentrations of acid soluble thiol, an important marker of antioxidant activity in the blood (Eck *et al.*, 1989). HIV-infected patients commonly excrete higher than average quantities of malondialdehyde into their urine, reflecting increased levels of lipid peroxidation.

2.10 ANTIOXIDANTS

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and their inactivation by antioxidants. A critical balance must always be maintained between the generation of free radicals and antioxidant substances so as to prevent oxidative damage to cellular components such as deoxyribonucleic acid (DNA), proteins and lipids (Sies, 1997; Vertuani *et al.*, 2004). Halliwell and Gutteridge (1989) defined antioxidants as substances which when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of the substrate. Examples of antioxidants are: N-acetylcysteine (NAC), glutathione (GSH), selenium, glutathione peroxidase, vitamin E (tocopherol), vitamin C (ascorbic acid) and uric acid.

2.10.1 Classification of antioxidants

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, watersoluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione being mostly present within cells, while others such as uric acid are more evenly distributed. The amount of protection provided by any one antioxidant will depend on its concentration, its reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts (Vertuani *et al.*, 2004).

2.10.1.1 Ascorbic acid

Ascorbic acid is a monosaccaride antioxidant found in both animals and plants. Since one of the enzymes needed to make ascorbic acid has been lost by mutation in humans, it must be obtained from the diet and is thus considered a vitamin (Smirnoff, 2001). In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein and glutaredoxins (Meister, 1994). Ascorbic acid is a reducing agent and can reduce, thereby neutralizing reactive oxygen species such as hydrogen peroxide, hypochlorite, hydroxyl radical, peroxyl radical and singlet oxygen (Kwon, 1988; Padayatty *et al.*, 2003). It has been shown in studies in human plasma lipids that ascorbic acid is far more effective in inhibiting lipid peroxidation initiated by a peroxyl radical initiator than other plasma components such as protein thiols, urate, bilirubin and α-tocopherol by efficiently trapping peroxyl radicals in the aqueous phase before they can initiate lipid peroxidation thereby protecting bio-membranes against peroxidative damage (Frei, 1989).

2.10.1.2 Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Meister and Anderson, 1983). It is not required in diet but rather synthesized in cells from its constituent amino acids (Meister, 1988). Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister, 1994). Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Meister and Anderson, 1983).

2.10.1.3 Tocopherol (vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols which are fatsoluble vitamins with antioxidant properties (Herrera and Barbas, 2001). Of

these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form (Brigelius-Flohe and Traber, 1999). It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). This is in line with findings showing that αtocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death (Seiler *et al.*, 2008). GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

2.10.1.4 Enzyme Systems

The superoxide free radical released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases (SOD) catalyzing the first step and then catalases (CAT) and various peroxidases (glutathione peroxidase) removing hydrogen peroxide. The SODs, catalase and glutathione peroxidase constitute the major intracellular enzymic antioxidants, while the extracellular antioxidants are mainly of the preventive and scavenging types (Cui *et al.*, 2004).

2.11 COMPLICATIONS OF HIV/AIDS

2.11.1 Haematological Complications

A variety of haematological manifestations are seen at every stage of HIV infection posing a great challenge in the comprehensive management of the disease. Haematological complications have been documented to be the second most common cause of morbidity and mortality in HIV patients (Salond, 2005; Cosby, 2007) and are generally marked with cytopoenias such as anaemia, neutropoenia,

lymphopoenia and thrombocytopoenia (Moyle, 2002). The incidence and severity of the cytopoenia generally correlate to the stage of the disease with anaemia being the most commonly encountered haematologic abnormality and a significant predictor of progression to AIDS or death (Volberding, 2002; Odunukwe *et al.*, 2005). An obvious cause of anaemia in patients with HIV infection is blood loss which may be associated with conditions such as neoplastic disease e.g. Kaposi sarcoma in the gastrointestinal tract or gastrointestinal lesions that accompany opportunistic cytomegalovirus infection. Other than blood loss, the pathophysiology of HIV-associated anaemia may involve three basic mechanisms: decreased red blood cell (RBC) production, increased RBC destruction and ineffective RBC production.

2.11.1.1 Decreased RBC production

This may be a consequence of infiltration of the bone marrow by neoplasm (Sipsas *et al.*, 1999) or infection (Hambleton, 1996), use of myelosuppressive medications, HIV infection, a decreased production of endogenous erythropoietin, a blunted response to erythropoietin (Spivak *et al.*, 1989) or hypogonadism (Dobs, 1998).

2.11.1.2 Increased RBC destruction (haemolysis)

Increased or premature RBC destruction in the spleen or the circulatory system may occur in patients with HIV infection. Haemolytic anaemia may result from RBC auto-antibodies or disseminated intravascular coagulation (Coyle, 1997). Haemolysis may also develop as a consequence of the use of various medications (Soriano *et al.*, 2002).

2.11.1.3 Ineffective RBC production

Anaemia may result from nutritional deficiencies – most commonly deficiencies in iron, folic acid or vitamin B_{12}. In patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology. Vitamin B_{12} deficiency may result from malabsorption in the ileum or from gastric pathology caused by an array of infections or other conditions that affect the gastric mucosa in HIV-infected patients (Harriman *et al.*, 1989). Some risk factors currently associated with anaemia in HIV infection are: history of clinical AIDS, CD4 cell

count <200 cells mm^{-3}, plasma viral load, sex (women), race (black), zidovudine use, increasing age, lower body mass index, history of bacterial pneumonia, oral candidiasis and history of fever (Levine *et al.*, 2001).

2.11.1.4 Thrombocytopoenia

Association of HIV infection with thrombocytopoenia was long ago realized (Sullivan *et al.*, 1997) and is caused by immune-mediated destruction of platelets in addition inadequate platelet production. In a CDC study, it was found that the overall incidence of thrombotic episodes was 2.6 per 1000 HIV-infected persons (Sullivan *et al.*, 2000).

2.11.2 Immunological Complications

Infection by HIV is characterized by several effects on the host immune system. B cells decline in number and function (Patke and Shearer, 2000), and because of the toxicity of HIV antigens, cytokine regulation is distorted causing a decrease in CD4+ T-cells (Margolick *et al.*, 1998). There is a distinct interplay between HIV and the immune defenses. Typical non-progressors (those who have been infected with HIV but do not show symptoms) display several responses that are different from those of progressors. Non-progressors show more T helper (TH1-type) cytokines like interleukin 2 (IL-2), interferon alpha (IFN-α) and an elevated response by CD4+ T-cells and cytotoxic CD8+ T-cells towards HIV is observed. Additionally, there is an increased synthesis of α-chemokines. The HIV virus counters these defenses by varying antigenic sites (Borrow *et al.*, 1997), (preventing an effective immune response and overwhelming the immune system) and by reducing major histocompatibility complex (MHC) on the surface of cells and reducing the number of CD8+ T-cells (Pantaleo *et al.*, 1997).

2.11.3 Biochemical and Metabolic Complications

HIV infection induces an acute phase response which is marked by changes in the plasma concentrations of a number of proteins. The acute phase response to infection is characterized by an increase in protein turnover with increased loss of protein (Jahoor *et al.*, 1999). Leukocyte proliferation, synthesis of cytokines, immunoglobulins and positive acute phase proteins contribute to protein turnover

(Fleck, 1989). Evidence of increased protein turnover in humans with symptomfree AIDS suggests that infection by the virus, in the absence of clinical signs and symptoms can induce changes in protein metabolism (Jahoor *et al.*, 1999).

Lipid abnormalities (dyslipidaemia) such as increased levels of serum triglycerides (TG), raised levels of very low-density lipoproteins (VLDL) and low levels of highdensity lipoprotein (HDL) are known to be associated with HIV infection (Grunfeld *et al.*, 1989). Low levels of HDL and high serum concentrations of total cholesterol (TC), LDL and TG are independent risk factors for coronary heart disease in non-HIV infected patients as well as in HIV-infected patients (Knopp, 1999; Sudano *et al.*, 2006). Furthermore, lipid abnormalities may be associated with insulin resistance and glucose intolerance (Green, 2002). The exact pathogenesis of dyslipidaemia observed in HIV infection is still unknown and appears to be due to the body's immune response to the infection mediated by various cytokines (Grunfeld and Feingold, 1996; Sudano *et al.*, 2006). A large number of cytokines including TNF-α, interleukins and interferon's increase serum triglyceride levels and decrease HDL-cholesterol (Feingold and Grunfeld, 1992). Interferon alpha is known to be elevated in AIDS and HIV positive individuals and has been shown to be positively correlated with plasma triglyceride concentrations (Grunfeld *et al.*, 1991).

2.11.4 Renal dysfunction

Abnormal renal function has been identified in less than 30% of HIV-infected patients (Szczech *et al.*, 2002) and more recent analysis of a large urban United States (US) HIV clinic showed that approximately 15.5% of patients had chronic or end-stage renal disease (Wyatt *et al.*, 2007). The underlying disturbances in renal function as well as renal tubular dysfunction, predisposes HIV-infected patient to both haemodynamic and toxic renal injury. Examples of the clinical syndromes that can result include HIV-associated glomerulopathies, various metabolic perturbations and most seriously, acute renal failure (ARF) (Rao, 1998). When compared with similarly matched subjects not infected with HIV, hospitalized HIV-infected patients are much more likely to develop ARF (Valeri and Neusy,

1991; Rao, 1996). The causes of ARF in HIV-infected patients include pre-renal azotemia, nephrotoxic and ischaemic acute tubular necrosis, crystal induced tubular injury and obstructive uropathy (Rao, 1998).

2.11.5 Liver dysfunction

Liver enzyme elevations in patients infected with HIV are common (Nunez, 2006). In some cases they are transient and of minor clinical interest, in other cases they are caused by alcohol consumption or by co-infection with other viral diseases, such as hepatitis B virus (HBV) infection or hepatitis C virus (HCV) infection. The origin of liver enzyme elevation is however not explained by an underlying liver disease or toxin and may occur either due to antiretroviral drug toxicity or HIV infection. As HIV patients benefit from their antiretroviral treatment and consequent immunological stabilization (Katzenstein *et al.*, 1996; Hammer *et al.*, 1997), continuous long-term treatment is inevitable. ART has led to a number of unexpected drug-induced toxicities: lipodystrophy, insulin resistance, dyslipidaemia and direct hepatotoxic injury (Palella *et al.*, 1998).

Transaminase elevations have been described during antiretroviral treatment by various mechanisms, leading to morbidity, mortality and drug discontinuation (Nunez *et al.*, 2006). Prevention and management of antiretroviral drug-related liver injury have emerged as major issues among HIV-infected patients in the era of combination ART (Palella *et al.*, 2006). Chronic liver disease may occur due to altered lipid patterns, insulin resistance or mitochondrial toxicity, which may contribute to the development of steatosis and steatohepatitis. In an era where the expected survival for HIV-infected persons has nearly reached that of healthy individuals (Lohse *et al.*, 2007), the right interpretation of liver enzyme elevation is crucial as it helps to define patients at risk of developing severe hepatic injury.

2.11.5.1 Mechanisms of ARV drug toxicity

NRTIs – these rarely lead to liver enzyme elevation with an onset between 3 to 13 months after treatment initiation (Sulkowski *et al.*, 2000). NRTIs have been associated with liver injury through mitochondrial dysfunction, lactic acidosis and

subsequent hepatic steatosis. Nucleoside analogues differ widely in their propensity to induce mitochondrial toxicity. Potency estimations in vitro give a descending hierarchy of their potency with zalcitabine, didanosine, stavudine, zidovudine and finally abacavir as least toxic (Verucchi *et al.*, 2003).

NNRTIs – efavirenz and nevirapine have been associated with hepatotoxicity mainly by two mechanisms. The likelihood of developing liver enzyme elevations seems to be higher with nevirapine (Ena *et al.*, 2003; Sanne *et al.*, 2005).

PIs – all PIs are metabolized in the liver by the cytochrome P450 system (Abrescia *et al.*, 2005) and can cause liver enzyme elevation. Ritonavir has the highest risk of liver toxicity but is rarely used in the clinical setting (Sulkowski *et al.*, 2000).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY SITE

This cross sectional study was conducted from September 2008 to September 2009 at the antiretroviral (ART) clinic of the Bolgatanga Regional hospital, located in Bolgatanga in the Upper East Region. The Upper East Region is located in the north-eastern corner of Ghana and covers an area of about 1,463 km² (564.9 sq mi). The population of Bolgatanga in 2002 was estimated at 964,500 with current catchment area population projected to be 1,004,244 (NDPC, 2009). The capital, Bolgatanga, is however cosmopolitan in nature with inhabitants not only hailing from northern origin but there are also many inhabitants who hail from other parts of Ghana and the sub-region. The climate is tropical with a rainy season from May to October and a long dry season with virtually no rainfall from October to April. Temperatures range between a maximum of 45°C in March / April and at least 12°C in December.

3.2 STUDY POPULATION

3.2.1 Inclusion criteria

- Patient should be confirmed to be positive for HIV (HIV-1, HIV-2 or both)
- Patient should not be pregnant at the time of sampling
- Naïve patient should not be on any medication (antibiotics, vitamin supplements and tuberculosis (TB) treatment) at the time of sampling

A total of 470 people living with HIV/AIDS were recruited from the ART clinic out of which 3 died, 5 were excluded and 20 declined participation from the study. The remaining 442 patients consisted of: 276 highly active antiretroviral therapy (HAART) naïve patients of which 76 were males and 200 were females and 166

patients on HAART comprising 28 males and 138 females were used for the study. Approval for the study was given by the Clinical Coordination and Research Development Board of the hospital. Written informed consent was obtained from each of the patients in the study group. HAART use was defined as receipt of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI).

3.3 SAMPLE COLLECTION AND PREPARATION

After overnight fasting, a total volume of 10 ml of venous blood was collected from the antecubital vein of each patient under aseptic conditions. A volume of 5 ml of the blood sample was dispensed into vacutainer plain-gel tube and allowed to clot. The sample was then centrifuged at 500 for 10 minutes and the serum separated and aliquoted into cryotubes and stored at -70^{o}C. The serum was used for biochemical analysis. The other 5 ml of blood sample was dispensed into two vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA) (2 ml and 3 ml) respectively. The 2 ml sample was used for immunological analysis (CD4/CD3 estimation) and the 3 ml sample used for haematological analysis.

3.4 ASSAY PROCEDURES

3.4.1 Immunological Analysis

Absolute cell counts of CD4 and CD3 T-lymphocytes in non-haemolyzed whole blood were estimated with a haematological autoanalyzer, BD FACSCount system (Becton Dickenson and Company, California, USA).

3.4.1.1 Principle of Operation

When whole blood is added to the reagents, fluorochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. The cells come in contact with the laser light, which causes the fluorochrome-labeled

cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells. In addition to containing the antibody reagent, the reagent tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitation standard for calculating the cells. Analysis is automatic - the software identifies T-lymphocyte populations and calculates the absolute counts.

3.5 HAEMATOLOGICAL ANALYSIS

The CELL-DYN 1800 (Abbott Diagnostics Laboratories Division, USA) is an automated, multi-parameter haematology analyzer which is designed to classify formed elements of EDTA-anticoagulated blood:

3.5.1 Sample Analysis Cycle Overview

Aspiration – the CELL-DYN 1800 aspirates approximately 30 µL of whole blood from an open collection tube and 7.5 ml of diluent is added in a pre-mixing cup to achieve a dilution ratio of 1:251.

3.5.1.1 WBC Measurement Process

The 1:251 WBC/HGB dilution is delivered to the WBC mixing chamber where it is bubble-mixed with 1.0 mL of lyse reagent. The WBC's are then counted by impedance as they pass through the aperture of the von-Behrens WBC transducer. As each cell is drawn through the aperture, a change in electrical resistance occurs generating 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 correlating to lymphocytes are included in the small cell subpopulation. Cells correlating to granulocytes (neutrophils) are included in the large cell population. The remaining cells correlating to monocytes, basophils, eosinophils, blasts and other precursor white cells are generally included in the mid-size cell population.

3.5.1.2 RBC/PLT Measurement

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 to create 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. Cells are counted and sized as they pass through the aperture of the von-Behrens RBC/PLT Transducer. A precise volume of the diluted specimen is drawn through the aperture into the counting chamber. If the pulse generated is above the PLT lower threshold, the pulse is counted as a PLT. If the pulse generated is above the RBC lower threshold, the pulse is counted as an RBC.

3.5.1.3 RBC Count

The RBC count is directly measured and the number of RBCs is expressed as follows:

$$\boldsymbol{RBC} = \frac{\boldsymbol{M}}{\boldsymbol{\mu L}} \ (\boldsymbol{millions\ per\ microliter})$$

3.5.1.4 Mean Cell Volume (MCV)

The Mean Cell Volume (MCV) is the average volume of individual RBCs. The MCV is derived from the RBC size-distribution data. MCV is reported in femtoliters (fL).

3.5.1.5 Haematocrit (HCT)

The Haematocrit is the ratio of RBCs to plasma. The HCT is calculated from the RBC count and the MCV as follows:

$$\boldsymbol{HCT} = \frac{(\boldsymbol{RBC} \times \boldsymbol{MCV})}{\boldsymbol{10}}$$

3.5.1.6 Mean Cell Haemoglobin (MCH)

The Mean Cell Haemoglobin (MCH) is the average amount of haemoglobin contained in the RBC. The MCH is calculated from the RBC and HGB as follows:

$$\boldsymbol{MCH} = \frac{\boldsymbol{HGB}}{\boldsymbol{RBC}} \times \boldsymbol{10}$$

3.5.1.7 Mean Cell Haemoglobin Concentration (MCHC)

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

$$MCHC = \frac{haemoglobin}{haematocrit} \times 100$$

3.5.1.8 PLT Count

The PLT Count is derived from the PLT histogram after the data have been analyzed by the PLT algorithm. The PLT count is expressed as follows:

$$PLT = \frac{K}{\mu L} \ (thousands\ per\ microliter)$$

3.5.2 Haemoglobin Measurement

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

3.6 BIOCHEMICAL ANALYSIS

The ATAC® 8000 Random Access Chemistry analyzer (Elan Diagnostics, Smithfield, CA, USA) was used for all biochemical analyses.

3.7 PRINCIPLES FOR BIOCHEMICAL ASSAYS

3.7.1 Albumin (ALB)

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 colour at 630 nm is directly proportional to the albumin content.

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

3.7.2 Total protein (PRO)

The present method is based on the modifications of Gornall *et al.,* (1949). Protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of proteins present when compared to a solution with known protein concentration.

$$Protein + Cu^{2+} \xrightarrow{alkali} Coloured\ complex$$

3.7.3 Alkaline Phosphatase (ALP)

The method used for this assay is based on the kinetic photometric test according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) where alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of p-nitrophenyl phosphate at a pH of 10.3 at 37°C to liberate pnitrophenol. The resulting absorbance increase is measured at 405 nm.

$$p - nitrophenyl\ phosphate + H_2O \xrightarrow{ALP} p - nitrophenol + phosphate$$

3.7.4 Alanine Aminotransferase (ALT)

The method used for this assay is based on the IFCC recommended method in 1980 (Schumann *et al.,* 2002) utilizing the LDH-NADH coupled assay where ALT catalyzes the transfer of the amino group from L-alanine to 2-oxoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD^+. The resulting rate of decrease in absorbance measured at 340 nm is directly proportional to ALT activity. Endogenous sample pyruvate is rapidly and completely reduced by lactate dehydrogenase (LDH) during the initial incubation period so that it does not interfere with the assay.

$$L - Alanine + 2 - Oxoglutarate \xrightarrow{ALT} Pyruvate + L - Glutamate$$

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

3.7.5 Aspartate Aminotransferase (AST)

The method for this assay is based on that of Karmen *et al.,* (1955) where AST catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD^+ in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$L-Aspartate + 2-Oxoglutarate \xrightarrow{AST} Oxaloacetate + L-Glutamate$$

$$Oxaloacetate + NADH \xrightarrow{MDH} L-Malate + NAD^+$$

3.7.6 Gamma Glutamyl Transferase (GGT)

The method for this assay is based on that described by Szasz, (1969). GGT in the serum sample catalyzes the transfer of the Glutamyl group from L-Gammaglutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2nitrobenzoate formed is proportional to gamma glutamyl transferase activity and may be measured kinetically at 405 nm by the increasing intensity of the yellow colour formed.

$$L-\gamma-glutamyl-3-carboxy-4-nitroanilide + Glycylglycine \xrightarrow{GGT} 5-amino-2-nitrobenzoate + \gamma-L-glutamyl-glycylglycine$$

3.7.7 Direct Bilirubin (BID)

The method for this assay is based on the acid diazo method. Sulphanilic acid reacts with sodium nitrite to produce diazotized sulphanilic acid. Conjugated bilirubin reacts with diazotized sulphanilic acid to produce a red-purple colour which is read at 550 nm.

3.7.8 Total Bilirubin (BIT)

The method for this assay is based on a modification of that of Pearlman *et al.,* (1974) in which a surfactant (Dimethysulphoxide – DMSO) is used as a stabilizer. Sodium nitrite is added to sulphanilic acid to form diazotized sulphanilic acid. Direct and indirect bilirubin couple with diazotized sulphanilic acid to produce azo-bilirubin in the presence of the surfactant. The absorbance measured at 550 nm is directly proportional to the total bilirubin concentration in the sample.

3.7.9 Blood Urea Nitrogen (BUN)

The method for this assay is a based on a modification of the Urease/Glutamate dehydrogenase (GLDH) method by Talke *et al.,* (1965). Urea is hydrolyzed to ammonia (NH_3) and carbon dioxide (CO_2) in the presence of water and urease. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH and Glutamate dehydrogenase to form L-Glutamate and NAD^+. As the reaction proceeds, the absorbance at 340 nm decreases. The initial rate of this change is proportional to the amount of urea in the sample.

$$Urea + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

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

3.7.10 Creatinine (CRE)

The method for this assay is based on the Jaffe (modified kinetic) method described by Fabiny *et al.,* (1971). Creatinine reacts with picric acid in alkaline conditions to form a colour complex which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample.

$$Creatinine + Sodium\ Picrate \xrightarrow{alkali} Creatinine - picrate\ complex$$

3.7.11 Calcium (CAL)

Calcium reacts with Arsenazo III in a slightly alkaline medium to form a purplecoloured complex which absorbs at 650 nm. The intensity of the colour is proportional to the calcium concentration.

$$Calcium + Arsenazo\ III \xrightarrow{alkaline} Calcium - Arsenazo\ complex\ medium$$

3.7.12 Total Cholesterol (TC)

The method for this assay is based on that described by Trinder, (1969). Cholesterol esterase hydrolyses esters to free cholesterol and fatty acids. The free cholesterol produced plus the preformed cholesterol are then oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The quinoneimine chromogen, with absorption maximum at 500 nm, is produced when phenol is oxidatively coupled with 4-aminophenazone in the presence of peroxidase with hydrogen peroxide. The intensity of the final red colour is directly proportional to the total cholesterol concentration.

$$\textbf{Cholesterol ester} + H_2O \xrightarrow{\textit{cholesterol esterase}} \textbf{Cholesterol} + \textbf{Fatty acids}$$

$$\textbf{Cholesterol} + O_2 \xrightarrow{\textit{cholesterol oxidase}} \textbf{Cholest} - 4 - \textbf{en} - 3 - \textbf{one} + H_2O_2$$

$$H_2O_2 + 4 - \textbf{aminophenazone} + \textbf{Phenol} \xrightarrow{\textit{peroxidase}} H_2O + \textbf{Quinoneimine}$$

3.7.13 Triglycerides (TG)

The method for this assay is based on a modified Trinder (Barham and Trinder, 1972) colour reaction to produce a fast linear endpoint reaction (McGowan *et al.*, 1983). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase. The hydrogen peroxide then reacts with 4-aminoantipyrine and 3, 5 dichloro-2hydroxybenzene (Chlorophenol) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$\textbf{Triglyceride} + H_2O \xrightarrow{\textit{lipase}} \textbf{Glycerol} + \textbf{Fatty acids}$$

$$\textbf{Glycerol} + \textbf{ATP} \xrightarrow{\textit{glycerol kinase}} \textbf{Glyerol} - 3 - \textbf{phosphate} + \textbf{ADP}$$

$$\textbf{Glycerol} - 3 - \textbf{phosphate} \xrightarrow{\textit{glycerophosphate oxidase}} \textbf{DHAP} + H_2O_2$$

$$2H_2O_2 + 4 - aminoantipyrine + chlorophenol \xrightarrow{peroxidase} Quinoneimine + 2H_2O$$

3.7.14 High Density Lipoprotein (HDL) – Cholesterol

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of Mg^{2+} ions. The cholesterol concentration in the HDL is then determined by the method described by Trinder for the assay of cholesterol.

3.7.15 Low Density Lipoprotein (LDL) – Cholesterol

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald equation (Friedewald *et al.*, 1972).

$$LDL - Cholesterol(mmol\,L^{-1}) = TC(mmol\,L^{-1}) - \frac{TG(mmol\,L^{-1})}{2.2} - HDL(mmol\,L^{-1})$$

3.7.16 Uric Acid

Uric acid is converted by oxidation by uricase to allantoin and H_2O_2, which under the catalytic influence of peroxidase, oxidizes 3, 5-dichloro-2-hydroxybenzenesulphonic acid (chlorophenol sulphonic acid) and 4-aminophenazone (4AP) to form a red-violet quinonimine compound, which is proportional to the amount of uric acid present.

$$Uric\ acid + O_2 + 2H_2O_2 \xrightarrow{uricase} Allantoin + CO_2 + H_2O_2$$

)

$$2H_2O_2 + chlorophenol\ sulphonic\ acid + 4AP \xrightarrow{peroxidase} N - (4 - antipyryl - 3 - chloro - 5 - sulphonate - p - benzo - quinoneimine$$

3.7.17 Vitamin C

Vitamin C in the serum was determined by a micro technique described by McCormick *et al.*, (1994) using Dinitrophenyhydrazine (DNPH). Ascorbic acid in

serum was oxidized by Cu (II) to form dehydroascobic acid, which reacted with acidic 2, 4-ditrophenylhydrazine to form a red dihydrazone, which was measured at 520 nm. Reagents that were used for the assay included:

- Ascorbic acid standard- 1 ml of glacial acetic acid was added to 100 mg of ascorbic acid and dissolved to 100 ml with distilled water.
- 10% Trichloroacetic acid (TCA) – 10 g of TCA and was dissolved with 100 ml of distilled water.
- Dinitrophenyhydrazine reagent – 2 g of DNPH was added to 0.25 g of thiourea and 0.03 g of $CuSO_4.5H_2O$ and this was made to 100 ml with 9M H_2SO_4. 50 ml of concentrated H_2SO_4 was further added and 150 ml of distilled water was finally added.
- 65% H_2SO_4 - 65 ml of concentrated H_2SO_4 was added to 35 ml of distilled water.

The sample was prepared by rapidly adding 1.6 ml of 10% TCA to 0.4 ml of serum. This was mixed well and allowed to stand for 5 minutes at room temperature. The mixture was then centrifuged at 500 *g* for 5 minutes. A volume of 1 ml of the aliquot was transferred to a test tube and 0.4 ml of DNPH added. This was stoppered and incubated at 37^{o}C for three hours. It was then chilled in an ice bath and 1.6ml of cold 65% H_2SO_4 was added to it. This was then allowed to stand at room temperature for 30 minutes. The standard and the test were then read against the blank at 520 nm.

$$T_{conc} = {}^{T_{abs}}/_{S_{abs}} X\, S_{conc}$$

$\mathbf{T_{conc}}$ = Concentration of the test sample

$\mathbf{T_{abs}}$ = Absorbance of the test sample

$\mathbf{S_{abs}}$ = Absorbance of the standard

$\mathbf{S_{conc}}$ = Concentration of the standard

3.7.18 Malondialdehyde (MDA)

The method used for this assay was based on that of Kamal *et al.*, (1989). A volume of 0.5 ml of serum was treated with 2.5 ml of 20% trichloroacetic acid (TCA) and then 1 ml of 0.67% TBA. The mixture was incubated at 100°C for 30 minutes. After cooling, the sample was extracted with 4 ml n-butanol and centrifuged at 500 *g* for 10 min. The absorbances of supernatant were measured at 535 nm and the results were expressed as µmol L^{-1}, using the extinction coefficient of 1.56 x 10^5 L mmol cm^{-1}.

$$\boldsymbol{Abs = C\varepsilon L}$$

Abs = absorbance of the test sample

C = concentration of the test sample

ε = extinction coefficient

L = light path (1 cm)

3.8 MEASUREMENT OF RENAL FUNCTION

This study assessed renal function in HIV patients using three renal function equations, namely the Cockcroft-Gault equation for estimating CrCl, 4v-MDRD and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). Estimated GFR's were used to stratify the study population into the five stages of CKD based on the staging system of the Kidney Disease Outcomes Quality Initiative (K/DOQI) for CKD classification (National Kidney Foundation, 2002) where: Stage 1 (Kidney damage with normal or increased GFR) = GFR ≥ 90 mL min^{-1} 1.73 m^{-2}; Stage 2 (Kidney damage with mildly decreased GFR) = 60-89 mL min^{-1} 1.73 m^{-2}; Stage 3 (Moderately decreased GFR) = 30-59 mL min^{-1} 1.73 m^{-2}; Stage 4 (Severely decreased GFR) = 15–29 mL min^{-1} 1.73 m^{-2} and Stage 5 (Kidney failure) = <15 mL min^{-1} 1.73 m^{-2}.

3.8.1 Renal function estimating equations

$$1.\ Cockcroft - Gault = \frac{(140 - age) \times weight}{72 \times SCr} \ (\times 0.85\ if\ female)$$

$$2.\ 4v - MDRD = (186 \times SCr^{-1.154} \times age^{-0.204})(\times 1.212\ if\ black)(\times 0.742\ if\ female)$$

$$3.\ CKD - EPI\ (Black)$$

$$Female: (SCr \leq 0.7) GFR = 166 \times (\frac{SCr}{0.7})^{-0.329} \times (0.993)^{age}$$

$$(SCr > 0.7) GFR = 166 \times (\frac{SCr}{0.7})^{-1.209} \times (0.993)^{age}$$

$$Male: (SCr \leq 0.9) GFR = 163 \times (\frac{SCr}{0.9})^{-0.411} \times (0.993)^{age}$$

$$(SCr > 0.9) GFR = 163 \times (\frac{SCr}{0.9})^{-1.209} \times (0.993)^{age}$$

Units of measurement: Age (years); Creatinine (mg dL^{-1}); Weight (kg); GFR (mL min^{-1} 1.73 m^{-2})

3.9 STATISTICAL ANALYSIS

Continuous data are expressed as mean ± SEM whilst categorical data are expressed as proportions. Unpaired *t*-test was used to compare the means and a pvalue <0.05 was considered to be statistically significant. Association between variables was assessed with linear regression analysis. Bland-Altman statistic was used to compare the levels of performance of the three renal function estimating equations. Statistical significance of proportions was assessed with Chi-square test. All statistical analyses were performed using MedCalc® version 10.2.0.0 (www.medcalc.be) for windows.

Chapter 4

RESULTS

4.1 HAEMATOLOGICAL PARAMETERS

4.1.1 Demographic characteristics and weight

Out of the 442 HIV/AIDS patients categorized into HAART naive and on HAART patients, majority of the HAART naive patients (88%) and those on HAART (83%) were within the age brackets of 20 – 49 years. Of the 276 HAART naive patients, 200 (72.5%) were females and 76 (27.5%) were males giving a female to male ratio of 3:1. Out of the 166 patients on HAART, 138 (83.1%) were females and 28 (16.9%) were males giving a female to male ratio of 5:1 (Table 4.1). Patients on HAART were however older (36.91 ± 0.77 years, $P \leq 0.01$) and heavier (54.92 ± 3.61 kg, $P \leq 0.05$) than their HAART naive counterparts (33.42 ± 0.88 years; 48.93 ± 1.24 kg respectively). Females on HAART were significantly older (36.10 ± 0.85 years, $P \leq 0.01$) when compared to those who were HAART naive (32.17 ± 0.89 years) but there was no significant difference in the mean ages of male patients on HAART and those who were HAART naive ($P > 0.05$) (Table 4.2). Furthermore, male patients on HAART were significantly heavier (75.50 ± 2.50 kg, $P \leq 0.01$) than their HAART naive counterparts who were also heavier (54.08 ± 2.44 kg, $P \leq 0.01$) when consequently compared with HAART naive females (47.00 ± 1.34 kg) (Table 4.2).

4.1.2 Anaemia, Packed Cell Volume (PCV) and Haemoglobin

From Table 4.2, the calculated mean PCV (35.68 ± 0.55%, $P \leq 0.001$) in patients on HAART was significantly higher than that of patients who are off HAART (32.64 ± 0.48%). Using packed cell volume (PCV) less than 30% as an indicator for anaemia, 37.6% (88/234) of the HAART naive patients were 3 times at risk of having reduced PCV compared to 15.2% (28/151) of the patients on HAART ($P < 0.0001$). HAART naïve females were also 3.7 times at risk of developing reduced PCV ($P < 0.0001$) compared to those on HAART. The odds of PCV being reduced in males on HAART compared to naïve males were not statistically significant (Table 4.4).

Table 4.1 Age and sex distribution of the study population

	HIV Patients	
	HAART Naïve	**On HAART**
Age (years)	**N = 276; (n/N)**	**N = 166; (n/N)**
0-9	10(3.6)	0(0.0)
10-19	5(1.8)	2(1.2)
20-29	78(28.3)	28(16.0)
30-39	108(39.1)	76(44.5)
40-49	57(20.7)	40(22.8)
50-59	10(3.6)	18(10.3)
60-69	5(1.8)	2(1.2)
>70	3(1.1)	0(0.0)
Sex		
Male	76(27.5)	28(16.9)
Female	200(72.5)	138(83.1)

From Table 4.3, a further classification of the study population according to the World Health Organization/Aids Clinical Trial Group (WHO/ACTG) anaemia toxicity grades gave a 63% and 46% calculated incidence of anaemia (Hb ≤10.5 g dL^{-1}) in HAART naive patients and those on HAART respectively ($X^2 = 10.68$, $P = 0.001$). Additionally, HAART naive patients are 3 times at risk of developing Grade 2 anaemia ($P = 0.0002$) and 2.4 times at risk of developing Grade 3 anaemia ($P = 0.03$) compared to the patients on HAART. The odds of developing grades 2 and 3 anaemia in HAART naive females are 3.6 and 2.4 times more respectively, when compared to their counterparts on HAART and a comparison of males on HAART to their naive counterparts in developing all four grades of anaemia did not show any significant difference ($P > 0.05$). The calculated mean haemoglobin of 11.54 ± 0.33 g dL^{-1} ($P \leq 0.05$) and 10.33 ± 0.16 g dL^{-1} ($P \leq 0.0001$) in males and females on HAART respectively were significantly higher when compared to their naive

counterparts (10.59 ± 0.29 g dL^{-1} and 9.45 ± 0.13g dL^{-1} for males and females respectively) (Table 4.2).

Table 4.2 General characteristic of the study population stratified by HAART use and gender

Parameters	HIV Patients		Male Patients		Female Patients	
	HAART naïve	**On HAART**	**HAART naïve**	**On HAART**	**HAART naïve**	**On HAART**
Age (yrs)	33.42 ± 0.88	36.91 ± 0.77**	36.68 ± 2.15	39.63 ± 1.68	32.17 ± 0.89‡	36.10 ± 0.85§§
WT (kg)	48.93 ± 1.24	54.92 ± 3.61*	54.08 ± 2.44	75.50 ± 2.50††	47.00 ± 1.34‡‡	51.18 ± 3.04$^{\#\#}$
CD4 (cell mm-^{3})	272.60 ± 13.24	315.30 ± 13.92*	251.60 ± 26.40	277.70 ± 29.85	295.50 ± 17.33	326.30 ± 15.78
CD3 (cell mm-^{3})	1243.00 ± 43.55	1216.00 ± 45.19	1251.00 ± 89.33	1148.00 ± 92.36	1276.00 ± 56.32	1238.00 ± 52.08
TWBC (x 10^{3} µL^{-1})	5.19 ± 0.18	4.39 ± 0.12**	5.53 ± 0.41	4.32 ± 0.24†	5.14 ± 0.23	4.41 ± 0.14§
TLC (x 10^{3} µL^{-1})	2.81 ± 0.09	2.73 ± 0.08	2.86± 0.21	2.48 ± 0.18	2.79 ± 0.12	2.79 ± 0.09
LC (%)	55.48 ± 1.03	62.49 ± 1.00***	53.79 ± 2.18	57.72 ± 2.57	55.97 ± 1.38	63.77 ± 1.05$^{§§§,\#}$
RBC (x 10^{6} µL^{-1})	3.55 ± 0.04	3.31 ± 0.05***	3.67 ± 0.11	3.51 ± 0.09	3.51 ± 0.05	3.25 ± 0.05$^{§§§,\#}$
HB (g dL^{-1})	9.80 ± 0.12	10.59 ± 0.15	10.59 ± 0.29	11.54 ± 0.33†	9.45 ± 0.13‡‡‡	10.33 ± 0.16$^{§§§,\#\#\#}$
PCV (%)	32.64 ± 0.48	35.68 ± 0.55***	35.19 ± 1.19	38.00 ± 0.10	31.33 ± 0.52‡‡‡	35.06 ± 0.63$^{§§§,\#\#\#}$
MCV (fL)	93.02 ± 0.94	110.50 ± 1.33***	96.32 ± 2.30	112.5 ± 2.79†††	90.99 ± 1.11‡	109.90 ± 1.51§§§
MCH (pg)	27.92 ± 0.27	32.60 ± 0.50***	29.61 ± 0.66	33.51 ± 0.81†††	27.19 ± 0.31‡‡‡	32.35 ± 0.59§§§
MCHC (g dL^{-1})	30.30 ± 0.25	29.66 ± 0.40	31.10 ± 0.65	29.95 ± 0.49	30.17 ± 0.31	29.58 ± 0.48
RDW (%)	20.88 ± 0.50	18.64 ± 0.52	21.91 ± 1.39	17.26 ± 0.59†	20.73 ± 0.59	18.98 ± 0.63§
PLT (x 10^{3} µL^{-1})	174.40 ± 7.20	148.80 ± 6.07*	178.5 ± 18.82	163.1 ± 14.09	178.10 ± 8.29	144.90 ± 6.69§§
MPV (fL)	11.25 ± 0.22	11.88 ± 0.20*	11.30 ± 0.48	11.80 ± 0.32	10.99 ± 0.27	11.90 ± 0.25§

Results are presented as means ± SEM. *P ≤ 0.05, **P ≤ 0.001, ***P ≤ 0.0001 indicates the level of significance when the HAART naïve HIV patients were compared to those on HAART (unpaired t-test); †P ≤ 0.05, ††P ≤ 0.001, †††P ≤ 0.0001 indicates the level of significance when the male HAART naïve were compared to the male on HAART; §P ≤ 0.05, §§P ≤ 0.001, §§§P ≤ 0.0001 indicates the level of significance when the female HAART naïve were compared to the female on HAART; ‡P ≤ 0.05, ‡‡P ≤ 0.01, ‡‡‡P ≤ 0.0001

indicates the level of significance when the male HAART naïve were compared to the female HAART naïve; $^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.001$, $^{\#\#\#}P \leq 0.0001$ indicates the level of significance when the male on HAART were compared to the female on HAART.

Table 4.3 Study population stratified by anaemia, type of anaemia, CD4 counts, total lymphocyte count and HAART use

	HIV Patients				Male Patients				Female Patients			
Parameters	HAART Naïve	On HAART			HAART Naïve	On HAART			HAART Naïve	On HAART		
WHO/ACTG Grade	**254**	**151**	**OR(95% CI)**	**P value**	**72**	**32**	**OR(95% CI)**	**P value**	**182**	**119**	**OR(95% CI)**	**P value**
Grade 1 (9.5-10.5)	51(20.1%)	39(25.8%)	0.7(0.5-1.2)	0.18	12(16.7%)	7(21.9%)	0.7(0.3-2.0)	0.53	39(21.4%)	32(26.9%)	0.7(0.4-1.4)	0.28
Grade 2 (8.0-9.4)	68(26.8%)	16(10.6%)	3.1(1.7-5.6)	**0.0002**	12(16.7%)	3(9.4%)	1.9(0.5-7.4)	0.34	56(30.8%)	13(10.9%)	3.6(1.9-7.0)	**0.0001**
Grade 3 (6.5-7.9)	33(13.0%)	9(6.0%)	2.4(1.1-5.1)	**0.03**	6(8.3%)	1(3.1%)	2.8(0.3-24.4)	0.35	27(14.8%)	8(6.7%)	2.4(1.1-5.5)	**0.04**
Grade 4 (<6.5)	8(3.1%)	6(4.0%)	0.8(0.3-2.3)	0.66	2(2.8%)	0(0.0%)	2.3(0.1-49.4)	0.59	6(3.3%)	6(5.0%)	0.6(0.2-2.0)	0.45
Type of Anaemia	**234**	**151**			**65**	**32**			**169**	**119**		
Microcytic Hypochromic	34(14.5%)	5(3.3%)	5.0(1.9-13.0)	**0.001**	4(6.2%)	0(0.0%)	4.7(0.2-91.1)	0.30	30(17.8%)	5(4.2%)	4.9(1.8-13.1)	**0.002**
Macrocytic Hypochromic	11(4.7%)	8(5.3%)	0.9(0.4-2.2)	0.79	2(3.1%)	1(3.1%)	1.0(0.1-11.3)	0.99	9(5.3%)	7(5.9%)	0.9(0.3-2.5)	0.84
Normocytic Hypochromic	46(19.7%)	11(7.3%)	3.1(1.6-6.2)	**0.001**	12(18.5%)	3(9.4%)	2.2(0.6-8.6)	0.25	34(20.1%)	8(6.7%)	3.5(1.6-7.9)	**0.003**
Normocytic Normochromic	50(21.4%)	11(7.3%)	3.5(1.7-6.9)	**0.001**	15(23.1%)	2(6.3%)	4.5(1.0-21.1)	**0.05**	35(20.7%)	9(7.6%)	3.2(1.5-6.9)	**0.003**
CD_4 (CDC)	**276**	**166**			**78**	**37**			**198**	**129**		
0-199	122(44.2%)	46(27.7%)	2.1(1.4-3.1)	**0.001**	39(50.0%)	14(37.8%)	1.6(0.7-3.6)	0.22	83(42.0%)	31(24.0%)	2.3(1.4-3.7)	**0.001**
200-499	112(40.6%)	94(56.6%)	0.5(0.4-0.8)	**0.001**	28(35.9%)	19(51.4%)	0.5(0.2-1.2)	0.12	84(42.4%)	76(58.9%)	0.5(0.3-0.8)	**0.004**
500+	42(15.2%)	26(15.7%)	1.0(0.6-1.6)	0.90	11(14.1%)	4(10.8%)	1.4(0.4-4.6)	0.63	31(15.7%)	22(17.1%)	0.9(0.5-1.6)	0.74

TLC	**234**	**151**			**65**	**32**			**169**	**119**		
<1.0	7(3.0%)	3(2.0%)	1.5(0.4-6.0)	0.55	4(6.2%)	1(3.1%)	2.0(0.2-18.9)	0.53	3(1.8%)	2(1.7%)	1.1(0.2-6.4)	0.95
1.0-2.0	77(32.9%)	39(25.8%)	1.4(0.9-2.2)	0.14	17(26.2%)	11(34.4%)	0.7(0.3-1.7)	0.40	60(35.5%)	28(23.5%)	1.8(1.1-3.0)	**0.03**
>2.0	150(64.1%)	109(72.2%)	0.7(0.4-1.1)	0.10	44(67.7%)	20(62.5%)	1.3(0.5-3.0)	0.61	106(62.7%)	89(74.8%)	0.6(0.1-1.0)	**0.03**

HAART = Highly Active Antiretroviral Therapy; WHO/ACTG = World Health Organization/Aids Clinical Trial Group; OR = Odds Ratio; CD = Cluster of Differentiation; TLC = Total Lymphocyte count; CI = Confidence Interval.

4.1.3 Type of anaemia

Using the mean cell volume (MCV) range of 80 – 96 fL and mean cell haemoglobin (MCH) range of 27 – 32 pg as pointers in distinguishing between the different types of anaemia where low MCV (<80 fL) is indicative of microcytosis, high MCV (>96 fL) indicates macrocytosis and low MCH (<27 pg) indicates hypochromia, HAART naive patients are 5 times at risk of developing microcytic hypochromic anaemia (P = 0.001) in comparison to their counterparts on HAART and the relative risk of developing normocytic hypochromic (P = 0.001) and normocytic normochromic anaemia (P = 0.001) is about 3 times more compared to the patients on HAART (Table 4.3). The same risk pattern, microcytic hypochromic (P = 0.002), normocytic hypochromic (P = 0.003) and normocytic normochromic (P = 0.003) is observed in a comparative analysis of females on HAART to those who are HAART naive.

A marginally significant risk of developing normocytic normochromic anaemia (P = 0.05) was seen in male patients who are HAART naive compared to those on HAART but no significant differences were observed in the relative risk of developing macrocytic hypochromic anaemia in all the study populations (P > 0.05) (Table 4.3). From Table 4.2, calculated MCV values of 110.50 ± 1.33 fL, 112.5 ± 2.79 fL, 109.90 ± 1.51 fL (P ≤ 0.001) and MCH values of 32.60 ± 0.50 pg, 33.51 ± 0.81 pg, 32.35 ± 0.59 pg (P ≤ 0.001) in patients on HAART, males on HAART and females on HAART respectively were significantly higher compared to their naive counterparts.

4.1.4 CD4 counts

In Table 4.3, the Center for Disease Control (CDC) criteria was used to classify the study population into three categories based on CD4 counts: stage 1 (CD4 ≥500 cells mm^{-3}), stage 2 (CD4 between 200 – 499 cells mm^{-3}) and stage 3 (CD4 <200 cells mm^{-3}). The risk of having CD4 count <200 cells mm^{-3} is twice in HAART naive patients than those on HAART (P = 0.001) and the chances of having CD4 count between 200 – 499 cells mm^{-3} is less likely (0.5 times) in HAART naïve patients compared to those on HAART (P = 0.001). HAART naive females are 2.3 times at risk of having CD4 count <200 cells mm^{-3} (P = 0.001) and a less likelihood (0.5 times) of having CD4 count of 200 – 499 cells mm^{-3} (P = 0.004) when compared to their counterparts on HAART. No significant difference was observed in the three categories of CD4 counts when males

on HAART were compared to HAART naive males (P > 0.05) likewise with CD4 counts >500 cells mm^{-3} when patients on HAART, males on HAART and females on HAART were compared to their naive counterparts. The mean CD4 count (315.30 ± 13.92 cells mm^{-3}, P ≤ 0.05) in patients on HAART is however greater when compared to that of patients who are HAART naive (272.60 ± 13.24 cell mm^{-3}) (Table 4.2).

4.1.5 Leucopoenia, Lymphopoenia and Neutropoenia

Using a total white blood cell count (<2.5 k μL^{-1}), lymphocyte count (<40%) and neutrophil count (<60%) as indicators of leucopoenia, lymphopoenia and neutropoenia respectively, there was no significant difference in the relative risk of developing leucopoenia in HIV patients, male patients and female patients (P > 0.05) (Table 4.4). The mean total white cell counts in patients on HAART (4.39 ± 0.12 k μL^{-1}, P ≤ 0.01), males on HAART (4.32 ± 0.24 k μL^{-1}, P ≤ 0.05) and females on HAART (4.41 ± 0.14 k μL^{-1}, P ≤ 0.05) were however significantly lower when compared to their naive counterparts (5.19 ± 0.18 k μL^{-1}; 5.33 ± 0.41 k μL^{-1} and 5.14 ± 0.23 k μL^{-1}) (Table 4.2).

The calculated incidence of lymphopoenia in HAART naive patients and those on HAART was 16.7% and 5.3% respectively and the difference in the proportion was significant (X^2 = 11.07, P = 0.001). The relative risk of developing lymphopoenia is 3.6 times in HAART naive patients (P = 0.002) and 5 times more in HAART naive females (P = 0.003) when compared to their counterparts on HAART. However, there was no significant difference in the odds of developing lymphopoenia when HAART naive males were compared to males on HAART (P > 0.05) (Table 4.4). Significant increases in the mean lymphocyte counts of patients on HAART (62.49 ± 1.00%, P ≤ 0.001) and females on HAART (63.77 ± 1.05%, P ≤ 0.001) was observed when compared to their HAART naive counterparts. A comparative analysis of the mean lymphocyte counts in males on HAART and HAART naive males showed no significant difference (P > 0.05) (Table 4.2).

There was no significant difference in the relative risk of developing neutropoenia in all three study populations (P > 0.05) (Table 4.4).

Table 4.4 Cytopoenic tendency in the study population

	HIV Patients				Male Patients				Female Patients			
	HAARTNaïve	On HAART			HAARTNaïve	On HAART			HAARTNaïve	On HAART		
Parameters	**234 (%)**	**151 (%)**	**OR(95%CI)**	**P value**	**65**	**32**	**OR(95%CI)**	**P value**	**169**	**119**	**OR(95%CI)**	**P value**
TWBC (< 2.5 k/µL)	18(7.7)	9(6.0)	1.3(0.6-3.0)	0.52	4(6.2)	1(3.1)	2.0(0.2-19.0)	0.53	14(8.3)	9(7.6)	1.1(0.5-2.6)	0.82
LYM (< 40%)	39(16.7)	8(5.3)	3.6(1.6-7.9)	**0.002**	13(20.0)	4(12.5)	1.8(0.5-5.9)	0.37	26(15.4)	4(3.4)	5.2(1.8-15.4)	**0.003**
Neut (< 60%)	225(96.2)	150(99.3)	0.2(0.02-1.30)	0.09	61(93.9)	31(96.9)	0.5(0.05-4.59)	0.53	165(97.6)	119(100.0)	0.2(0.01-2.90)	0.21
TLC (< 1.2 k/µL)	16(6.8)	6(4.0)	1.8(0.8-4.6)	0.24	6(9.2)	3(9.4)	0.9(0.2-4.2)	0.98	14(8.3)	3(2.5)	3.5(1.0-12.4)	**0.05**
PCV (< 30%)	88(37.6)	23(15.2)	3.4(2.0-5.6)	**<0.0001**	18(27.7)	4(12.5)	2.7(0.8-8.7)	0.10	70(41.4)	19(16.0)	3.7(2.1-6.6)	**<0.0001**
PLT (< 150 k/µL)	118(50.4)	78(51.7)	1.0(0.6-1.4)	0.81	32(49.2)	13(40.6)	1.4(0.6-3.3)	0.43	85(50.3)	65(54.6)	0.8(0.5-1.3)	0.47

TWBC – Total white blood cell, LYM – Lymphocyte, Neut – Neutrophil, TLC – Total lymphocyte count, PCV – Packed cell volume, PLT - Platelet

4.1.6 Thrombocytopoenia

Using a platelet count (<150 k μL^{-1}) as an indicator for thrombocytopenia in Table 4.4, there was no significant difference in the odds of developing thrombocytopenia in HIV patients, male patients and female patients ($P > 0.05$). However the calculated mean platelet counts of 148.80 ± 6.07 k μL^{-1} ($P \leq 0.05$) in patients on HAART and 144.90 ± 6.69 k μL^{-1} ($P \leq 0.01$) in females on HAART were significantly lower compared to their naive counterparts. Consequently, no significant difference was observed in the mean platelet count when males on HAART were compared to those who are HAART naive ($P > 0.05$).

4.1.7 CD4 counts and TLC

In a trend analysis of the three categories of CD4 counts and TLC in HAART naive patients (Table 4.5), when the TLC was between 1.0 – 2.0 k μL^{-1}, there was a gradual increase in the proportion of patients within the three CD4 categories from 8.3% in stage 1, 26.1% in stage 2 to a peak percentage of 46.5% within stage 3 ($P < 0.0001$). With a TLC >2.0 k μL^{-1}, a steady decline in the proportion of patients within the three categories was seen with the highest percentage of 91.7% occurring within stage 1 ($P < 0.0001$). A gradual increase in the proportion of patients within the CD4 categories was observed with a peak proportion of 4.0% in stage 3 when the TLC was <1.0 k μL^{-1} but this was not statistically significant. In patients on HAART, when the TLC was <1.0 k μL^{-1} and between 1.0 – 2.0 k μL^{-1}, there were gradual increases in the proportion of patients to the peak percentages of 7.3% ($P = 0.02$) and 41.5% ($P = 0.001$) respectively found in stage 3. However, when the TLC was >2.0 k μL^{-1}, the proportional trend decreased steadily through the CD4 categories from 96.0% ($P < 0.0001$) in stage 1 through to 51.2% in stage 3.

4.1.8 CD4 counts and anaemia

Comparing the four grades of anaemia to the three categories of CD4 for trend (Table 4.5), a gradual increase in the proportion of HAART naive patients within the three CD4 categories was observed with peak proportion of 19.1% in stage 3 developing grade 3 anaemia ($P = 0.02$) and 5.5% developing grade 4 anaemia ($P = 0.05$). About 36.6% of the patients on HAART within stage 3 CD4 count had grade 1 anaemia ($P = 0.01$).

Table 4.5 Trend analysis of the study population stratified by HAART use, TLC, anaemia and CD4 counts

		CD4 counts			
		Stage 3 0 - 199	**Stage 2 200 - 499**	**Stage 1 ≥500**	
	TLC	**101**	**96**	**36**	**P value**
	<1.0	4(4.0)	3(3.1)	0(0.0)	0.27
	1.0 - 2.0	47(46.5)	25(26.1)	3(8.3)	**<0.0001**
	>2.0	50(49.5)	68(70.8)	33(91.7)	**<0.0001**
HAART Naïve					
Grade	**Anaemia**	**110**	**104**	**39**	
1	9.5 - 10.5	20(18.2)	25(24.0)	6(15.4)	0.95
2	8.0 - 9.4	32(29.1)	31(29.8)	5(12.8)	0.12
3	6.5 - 7.9	21(19.1)	9(8.7)	3(7.7)	**0.02**
4	<6.5	6(5.5)	2(1.9)	0(0.0)	**0.05**
	TLC	**41**	**79**	**25**	
	<1.0	3(7.3)	0(0.0)	0(0.0)	**0.02**
	1.0 - 2.0	17(41.5)	20(25.3)	1(4.0)	**0.001**

	>2.0	21(51.2)	59(74.7)	24(96.0)	**<0.0001**
On HAART					
Grade	**Anaemia**	**41**	**79**	**25**	
1	9.5 - 10.5	15(36.6)	20(25.3)	2(8.0)	**0.01**
2	8.0 - 9.4	4(9.8)	7(8.9)	4(16.0)	0.50
3	6.5 - 7.9	5(12.2)	3(3.8)	1(4.0)	0.12
4	<6.5	2(4.9)	4(5.1)	0(0.0)	0.40

CD = Cluster of Differentiation; HAART = Highly Active Antiretroviral; TLC = Total Lymphocyte Count

4.1.9 Predictive parameters for CD4 counts

Figure 4.1 shows linear regression graphs of some selected haematological parameters and weight in HAART naive patients against CD4 counts in order to test for their ability to predict CD4 counts. For every cell μL^{-1} increase in TLC ($r^2 = 0.07$, $P < 0.0001$), a percent increase in lymphocyte count ($r^2 = 0.12$, $P < 0.0001$), a g dL^{-1} increase in haemoglobin ($r^2 = 0.10$, $P < 0.0001$) and a kg increase in weight ($r^2 = 0.04$, $P = 0.004$), there was a mean increase of 0.002, 0.025, 0.003 and 0.009 cells mm^{-3} in the CD4 count. Platelet count showed a negative relationship with CD4 count ($\beta = -0.057$, $r^2 = 0.013$, $P = 0.08$) and TWBC showed no significant association with CD4 ($\beta = 0.000$, $r^2 = 0.00$, $P =$

0.34).

A related regression analysis conducted in patients on HAART with the same study predictors showed that, for every cell μL^{-1} increases in TWBC ($r^2 = 0.13$, $P < 0.0001$) and TLC ($r^2 = 0.18$, $P < 0.0001$), a percent rise in lymphocyte count ($r^2 = 0.08$, $P = 0.001$), a g dL^{-1} rise in haemoglobin ($r^2 = 0.06$, $P = 0.003$) and a kg increase weight ($r^2 = 0.05$, $P = 0.03$), there was a mean increase of 0.003, 0.002, 0.019, 0.002, and 0.012 cells mm^{-3} in the CD4 count was observed. There was no significant linear relationship between platelet count and CD4 count ($\beta = 0.015$, $r^2 = 0.00$, $P = 0.67$) (Figure 4.2).

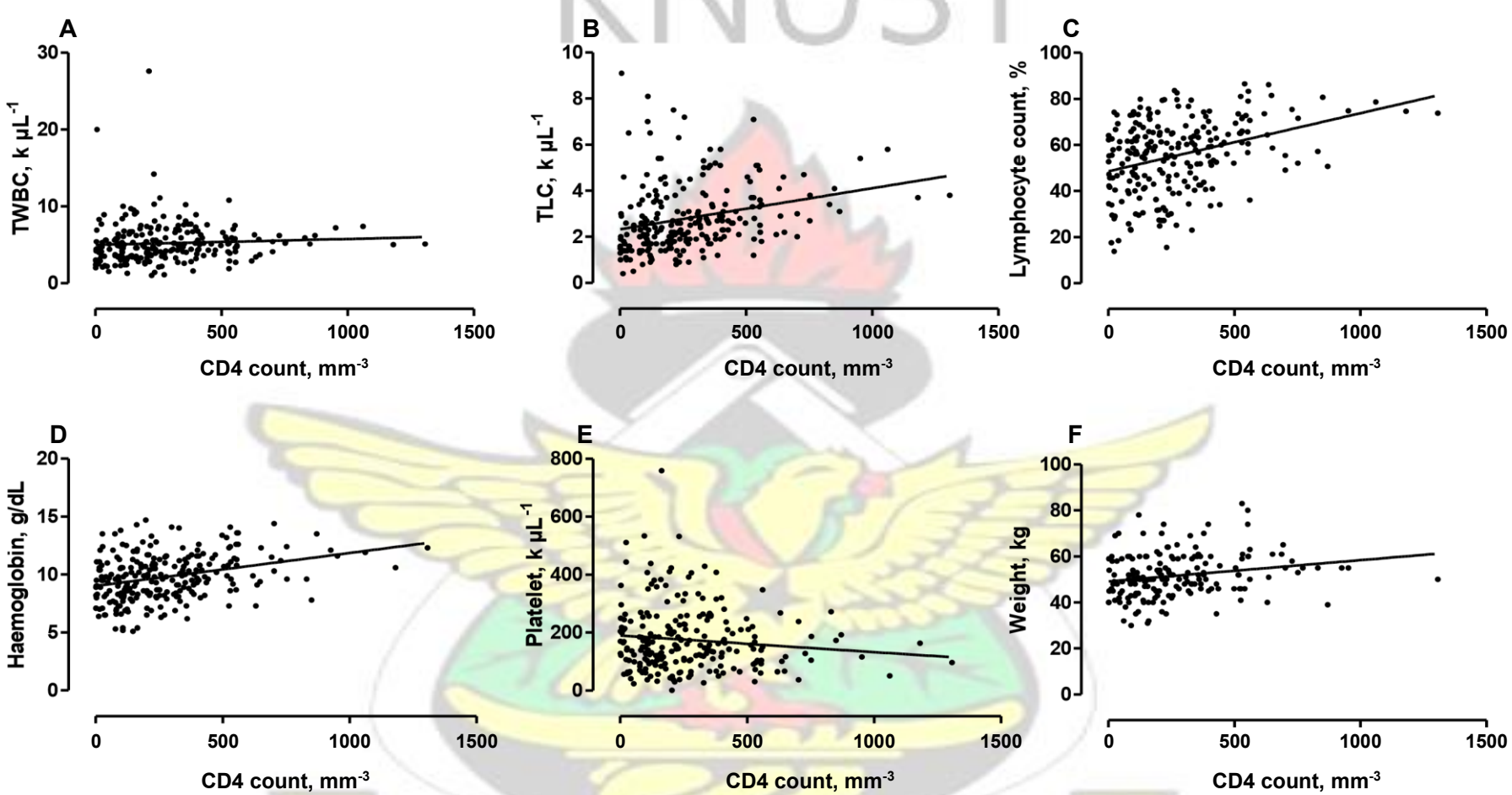
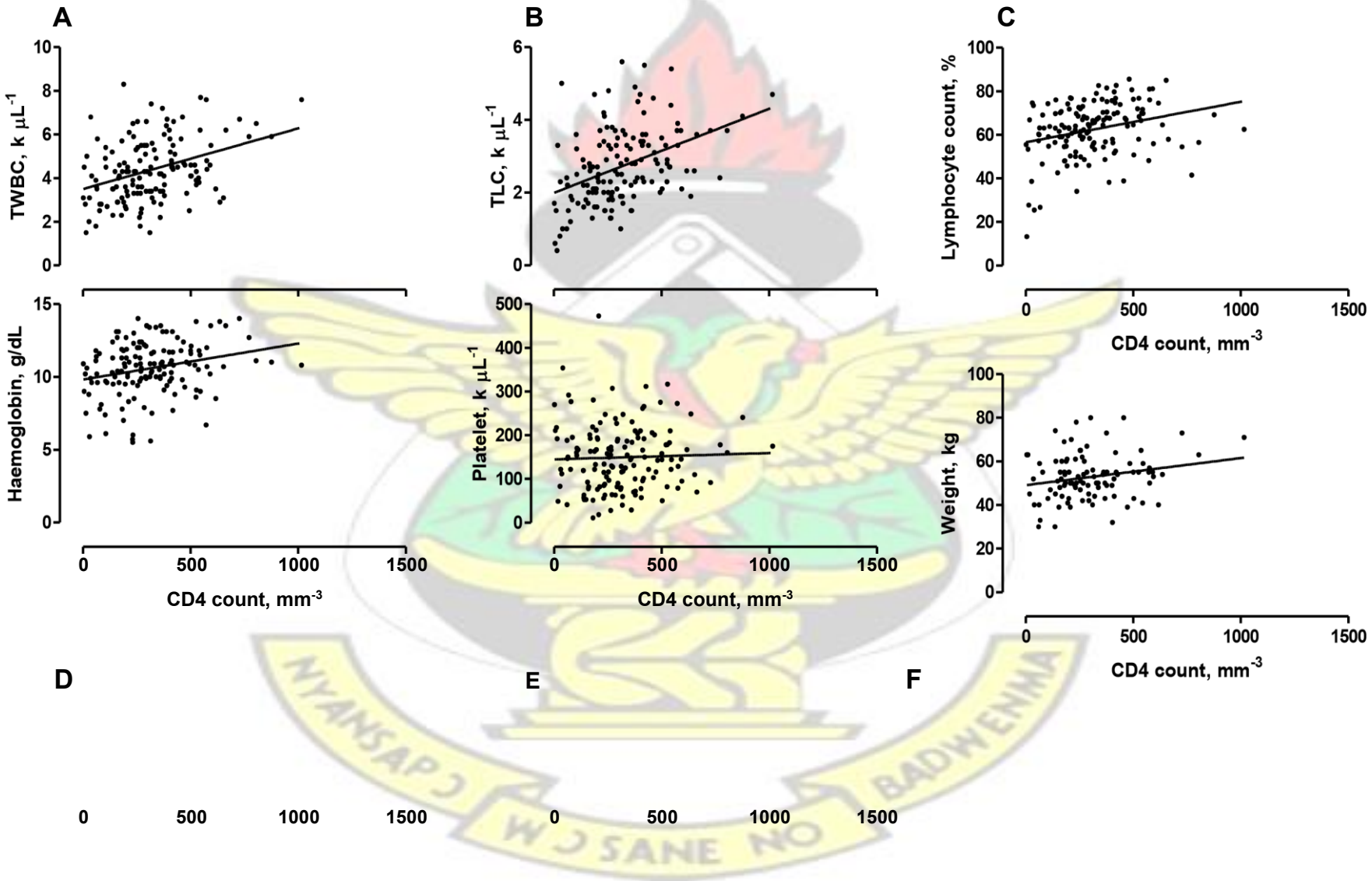


Figure 4.1 Regression line graphs between total white blood cell (TWBC), total lymphocyte count (TLC), Lymphocyte count, Haemoglobin, Platelet, Weight and CD4 counts of HAART naïve subjects.

Results



CD4 count, mm^{-3} CD4 count, mm^{-3}

Figure 4.2 Regression line graphs between total white blood count (TWBC), total lymphocyte count (TLC), Lymphocyte count, Haemoglobin, Platelet, Weight and CD4 counts in subjects on HAART.

4.2 BIOCHEMICAL PARAMETERS

4.2.1 Renal Function

The mean concentration of the measured analytes, blood urea nitrogen (BUN), creatinine and albumin when compared in all the study populations showed no statistical significant differences (Table 4.6). Patients on HAART were further stratified by type of drug combination which mostly comprised of two nucleoside reverse transcriptase inhibitors (NRTI's) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). 41.6% (69/166) were on a combination therapy of Combivir + Efavirenz; 41.0% (68/166) on Combivir + Nevirapine; 10.8% (18/166) on Stavudine + Lamivudine + Nevirapine and 6.6% (11/166) on Stavudine + Lamivudine + Efavirenz (Table 4.6). Combivir is a combined drug of Zidovudine (AZT) and Lamivudine (3TC).

4.2.2 Staging and Incidence of CKD

Table 4.7 shows the mean estimated GFR's of the study population as determined with the three estimating equations presented as means ± SEM. The study population was further staged into the five stages of the CKD classification system adapted from the National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (K/DOQI) advisory board based on estimated GFR. The percentage proportions of individuals classified into the various stages of CKD by the predictive equations were very close between 4v-MDRD and CKD-EPI. The Cockcroft-Gault equation generally generated lower proportions compared to the other equations. Interestingly, no subject on HAART was grouped into stage 5 and no male subject (naïve or on HAART) was grouped into stage 5. A marked decline in GFR (a level of less than 60 mL min^{-1} $1.73m^{-2}$ for ≥ 3months) is considered as evidence of CKD although substantial kidney damage can exist without a decrease in GFR. Based on such evidence, the calculated incidence of CKD (stages 3, 4 and 5) in the study population for Cockcroft-Gault, 4v-MDRD and CKD-EPI equations respectively is 8.3%, 9.8% and
9.4% in HAART naïve patients; 15.1%, 12.0% and 12.0% patients on HAART; 7.7%,

11.5% and 11.5% in HAART naïve males; 10.8%, 8.1% and 8.1% in males on HAART; 9.0%, 8.1% and 7.5% in HAART naïve females and 16.3%, 13.9% and 13.9% in females on HAART.

Table 4.6 Renal function parameters of the study population

Parameters	HIV PATIENTS		MALE PATIENTS		FEMALE PATIENTS	
	HAART NAÏVE	ON HAART	HAART NAÏVE	ON HAART	HAART NAÏVE	ON HAART
ANALYTES						
BUN (mmol L^{-1})	5.17 ± 0.25	4.76 ± 0.35	5.19 ± 0.43	5.88 ± 1.44	5.13 ± 0.31	4.42 ± 0.14
Creatinine (µmol L^{-1})	78.13 ± 5.62	72.72 ± 2.77	91.41 ± 14.97	82.35 ± 6.17	70.33 ± 5.14	69.76 ± 3.06
Albumin (g L^{-1})	36.58 ± 2.54	38.36 ± 0.54	33.58 ± 1.27	36.35 ± 1.26	37.99 ± 3.48	38.98 ± 0.59
HAART Use		**166 (n/N %)**		**37**		**129**
CBV + EFV	-	69 (41.6)	-	23	-	46
CBV + NVP	-	68 (41.0)	-	8	-	60
d4T + 3TC + NVP	-	18 (10.8)	-	1	-	17
d4T + 3TC + EFV	-	11 (6.6)	-	5	-	6

Continuous data are presented as mean ± SEM and categorical data presented as proportions. Comparison of the means (unpaired ttest) showed no statistically significant differences (P > 0.05). CBV: Combivir, EFV: Efavirenz, NVP: Nevirapine, d4T: Stavudine, 3TC: Lamivudine. BUN: Blood Urea Nitrogen, HAART: Highly Active Antiretroviral Therapy.

Table 4.7 CKD staging of the study population with the renal function equations

			Mean GFR	Stage 1 (≥90)	Stage 2 (60-89)	Stage 3 (30-59)	Stage 4 (15-29)	Stage 5 (<15)
HIV PATIENTS	HAART Naïve (276)	CG	90.59 ± 14.38	251.00 ± 41.53 (25.3%)	77.05 ± 1.34 (12.3%)	46.69 ± 2.11 (5.4%)	17.48 ± 1.48 (2.2%)	9.73 ± 5.00 (0.7%)
		4v-MDRD	306.50 ± 39.53	381.20 ± 49.90 (64.9%)	74.71 ± 1.68 (9.4%)	45.73 ± 2.53 (5.1%)	22.29 ± 1.46 (4.3%)	6.95 ± 0.00 (0.4%)
		CKD-EPI	137.20 ± 4.35	159.20 ± 4.04 (66.7%)	77.46 ± 1.81 (8.0%)	45.09 ± 1.98 (4.7%)	20.14 ± 1.29 (4.0%)	9.77 ± 3.33 (0.7%)
	On HAART (166)	CG	93.79 ± 15.50	269.80 ± 48.06 (25.3%)	76.49 ± 1.39 (18.1%)	43.95 ± 1.41 (15.1%)	0.00 ± 0.00 (0.0%)	0.00 ± 0.00 (0.0%)
		4v-MDRD	215.00 ± 28.45	291.00 ± 41.03 (62.6%)	76.10 ± 1.51 (19.9%)	49.61 ± 1.38 (11.4%)	29.57 ± 0.00 (0.6%)	0.00 ± 0.00 (0.0%)
		CKD-EPI	120.00 ± 4.36	145.10 ± 4.51 (65.1%)	75.34 ± 1.39 (17.5%)	50.06 ± 1.38 (11.4%)	28.61 ± 0.00 (0.6%)	0.00 ± 0.00 (0.0%)
MALE PATIENTS	HAART Naïve (78)	CG	85.68 ± 18.98	216.90 ± 44.37 (26.9%)	70.91 ± 3.24 (11.5%)	53.89 ± 0.26 (2.6%)	24.23 ± 3.09 (5.1%)	0.00 ± 0.00 (0.0%)
		4v-MDRD	249.90 ± 37.45	306.00 ± 45.07 (62.8%)	82.69 ± 2.57 (6.4%)	43.27 ± 3.51 (6.4%)	25.61 ± 2.26 (5.1%)	0.00 ± 0.00 (0.0%)
		CKD-EPI	136.60 ± 8.29	160.5 ± 7.53 (62.8%)	83.31 ± 2.65 (6.4%)	44.63 ± 3.04 (7.7%)	24.61 ± 1.84 (3.8%)	0.00 ± 0.00 (0.0%)
	On HAART (37)	CG	102.10 ± 41.32	371.9 ± 163.40 (21.6%)	78.40 ± 3.12 (21.6%)	43.90 ± 5.16 (10.8%)	0.00 ± 0.00 (0.0%)	0.00 ± 0.00 (0.0%)
		4v-MDRD	221.50 ± 66.99	303.2 ± 99.99 (64.9%)	77.56 ± 2.32 (27.0%)	57.48 ± 0.39 (5.4%)	29.57 ± 0.00 (2.7%)	0.00 ± 0.00 (0.0%)
		CKD-EPI	120.20 ± 9.64	145.0 ± 11.08 (67.6%)	75.58 ± 2.04 (24.3%)	57.22 ± 0.30 (5.4%)	28.61 ± 0.00 (2.7%)	0.00 ± 0.00 (0.0%)
FEMALE PATIENTS	HAART Naïve (198)	CG	97.30 ± 15.07	253.20 ± 40.31 (31.1%)	75.66 ± 1.37 (17.2%)	44.63 ± 2.75 (5.6%)	18.81 ± 2.66 (2.5%)	9.73 ± 5.01 (1.0%)
		4v-MDRD	332.30 ± 45.67	393.40 ± 54.56 (80.3%)	75.68 ± 1.94 (9.6%)	46.88 ± 3.35 (3.5%)	19.40 ± 1.60 (4.1%)	6.95 ± 0.00 (0.5%)
		CKD-EPI	143.00 ± 4.56	160.20 ± 4.22 (81.8%)	77.35 ± 2.05 (8.6%)	47.01 ± 3.74 (3.5%)	19.75 ± 0.92 (3.5%)	8.05 ± 0.00 (0.5%)
	On HAART (129)	CG	90.47 ± 15.77	245.80 ± 46.05 (26.4%)	75.79 ± 1.55 (17.1%)	42.13 ± 1.21 (16.3%)	0.00 ± 0.00 (0.0%)	0.00 ± 0.00 (0.0%)
		4v-MDRD	202.20 ± 29.96	273.30 ± 43.27 (62.0%)	75.47 ± 1.94 (17.8%)	48.37 ± 1.33 (13.9%)	0.00 ± 0.00 (0.0%)	0.00 ± 0.00 (0.0%)

CKD-EPI	117.80 ± 4.75	143.0 ± 4.711 (64.3%)	75.24 ± 1.83 (15.5%)	48.91 ± 1.37 (13.9%)	0.00 ± 0.00 (0.0%)	0.00 ± 0.00 (0.0%)

Results are presented as Mean ± SEM. CG: Cockcroft – Gault, 4v-MDRD: Four-variable Modification of Diet in Renal Disease, CKD-EQI: Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation, HAART: Highly active antiretroviral therapy, GFR: Glomerular filtration rate.

4.2.3 Study parameters as predictor variables

Table 4.8 shows summarized beta (β) and "r squared (r^2)" values of multiple linear regression analysis of some study parameters with the exception of CD4, for which variability changes are known to have varied effects on serum creatinine concentration and GFR estimated with the three renal function equations. Serum creatinine showed a consistent reciprocal relationship to estimated GFR with r^2 values observed in CKD-EPI equation > 4v-MDRD > Cockcroft-Gault equations respectively in all the study populations. CD4 was the next predictor variable which showed a consistent positive relationship with estimated GFR. Generally, r^2 values in the Cockcroft-Gault equation > 4v-MDRD > CKD-EPI equations respectively with exceptions in HIV patients on HAART (r^2 in 4v-MDRD = CKD-EPI); HAART naïve males (r^2 in CKD-EPI > 4v-MDRD) and females on HAART (r^2 in Cockcroft-Gault and 4v-MDRD = 0.00%; CKD-EPI = 1.00%).

Weight was the next predictor in succession and it also showed a positive relationship to GFR with the exception of observed negative associations in the 4v-MDRD and CKD-EPI equations for females on HAART. Strong r^2 values were observed in the Cockcroft-Gault equation compared to the 4v-MDRD and CKD-EPI equations with an exception in females on HAART where the r^2 in Cockcroft-Gault = 0.00% and 1.00% in 4v-MDRD and CKD-EPI equations respectively. Age was the next in succession showing a generally negative or reciprocal relationship to estimated GFR with exceptions of positive associations in males on HAART and estimated GFR by Cockcroft-Gault in HAART naïve males. There was no consistency in the r^2 values calculated for the three equations in all the study populations. Serum albumin concentration was the last predictor in succession and it showed reciprocal relationship in the HAART naïve study population (naïve males and females) and positive linear relationship in the subject population on HAART (males and females on HAART).

Table 4.8 Beta (β) and "r" squared (r^2) values from regression analysis of some study parameters against estimating equations.

			Age		Weight		CD_4		Creatinine		Albumin	
HIV PATIENTS												
		CG		0.00	7.49 ± 2.44	0.07	0.18 ± 0.06	0.04	-48.37 ± 15.27	0.04		0.00
	HAART Naïve	4v-MDRD	-15.35 ± 3.26	0.09	5.66 ± 3.58	0.02	0.35 ± 0.17	0.02	-193.70 ± 40.95	0.09	-0.73 ± 0.98	0.01
		CKD-EPI	-2.37 ± 0.34	0.17	0.85 ± 0.52	0.02	0.03 ± 0.02	0.01	-50.49 ± 3.35	0.50	-0.34 ± 0.14	0.05
MALE PATIENTS												
		CG	-0.42 ± 1.69	0.00	4.18 ± 2.27	0.04	0.14 ± 0.09	0.02	-174.60 ± 30.62	0.17	1.68 ± 2.06	0.00
	On HAART	4v-MDRD	-1.35 ± 3.10	0.00	2.63 ± 4.32	0.00	0.18 ± 0.16	0.01	-413.70 ± 52.14	0.29	2.78 ± 3.78	0.00
		CKD-EPI	-0.70 ± 0.47	0.02	0.10 ± 0.61	0.00	0.03 ± 0.02	0.01	-102.00 ± 4.75	0.75	0.46 ± 0.58	0.00
		CG	1.26 ± 1.31	0.42	7.183 ± 2.89	0.15	0.08 ± 0.09	0.02	-62.60 ± 23.29	0.11	-0.02 ± 1.22	0.00
	HAART Naïve	4v-MDRD	-6.33 ± 2.49	0.10	7.258 ± 4.87	0.06	0.16 ± 0.17	0.01	-203.20 ± 41.05	0.29	-3.75 ± 5.35	0.02
		CKD-EPI	-2.18 ± 0.51	0.23	1.106 ± 1.07	0.03	0.04 ± 0.04	0.02	-69.77 ± 6.00	0.69	-1.28 ± 1.18	0.04
FEMALE PATIENTS												
		CG	4.65 ± 4.43	0.03	15.65 ± 6.17	0.26	0.51 ± 0.23	0.13	-210.20 ± 70.57	0.20	4.41 ± 5.07	0.02
	On HAART	4v-MDRD	11.54 ± 7.02	0.07	24.10 ± 10.71	0.22	0.81 ± 0.36	0.12	-401.40 ± 108.60	0.28	9.14 ± 8.15	0.03
		CKD-EPI	0.59 ± 1.05	0.01	2.12 ± 1.52	0.1	0.07 ± 0.05	0.04	-90.40 ± 10.29	0.69	0.86 ± 1.19	0.02
		CG	-1.46 ± 1.52	0.01	8.06 ± 2.45	0.09	0.22 ± 0.07	0.05	-44.70 ± 16.48	0.04	-0.16 ± 0.58	0.00

HAART Naïve	4v-MDRD	-20.71 ± 4.37	0.11	8.03 ± 4.09	0.03	0.41 ± 0.20	0.02	-195.60 ± 48.87	0.08	-0.67 ± 1.05	0.00
	CKD-EPI	-2.61 ± 0.42	0.17	1.02 ± 0.56	0.03	0.03 ± 0.02	0.01	-46.99 ± 3.77	0.45	-0.31 ± 0.14	0.05
	CG	-2.15 ± 1.74	0.01	0.66 ± 2.34	0.00	0.04 ± 0.09	0.00	-175.40 ± 33.23	0.19	0.98 ± 2.16	0.00
On HAART	4v-MDRD	-4.84 ± 3.29	0.02	-3.99 ± 4.64	0.01	0.02 ± 0.17	0.00	-415.10 ± 58.91	0.29	1.04 ± 4.11	0.00
	CKD-EPI	-0.97 ± 0.52	0.03	-0.59 ± 0.67	0.01	0.03 ± 0.03	0.01	-110.10 ± 4.65	0.83	0.67 ± 0.65	0.01

Equations	**β**	**r^2**	**β**	**r^2**	**β**	**r^2**	**β**	**r^2**	**β**	**r^2**
	-0.07 ± 1.24								-0.16 ± 0.59	

CG – Cockcroft-Gault; 4v-MDRD – four-variable Modification of Diet in Renal Disease; CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration Equation. β – Beta (slope); HAART – Highly Active Antiretroviral Therapy.

Therefore in grading the predictor variables with regard to strength of association and percentage variability with estimated GFR in the study population, the outcome is creatinine > CD4 > weight > age > albumin.

4.2.4 Comparison of methods (Bland-Altman Analysis)

Graphical comparisons of the performance of the three estimating equations were assessed by the Bland-Altman method of comparison where the difference between measurements of the estimating equations is plotted as a function of the average of the two measurements. Bias was assessed as the mean difference, with positive values indicating an underestimation of GFR. Limits of agreement were computed as the mean bias plus or minus 1.96 times its standard deviation (SD). From figures 4.3, 4.4 and 4.5, the 4v-MDRD *vs.* CKD-EPI comparison consistently yielded lower bias than the CKD-EPI *vs.* Cockcroft-Gault comparison which also gave a lower bias in relation to the 4v-MDRD *vs.* Cockcroft-Gault comparison. Figures A, B and C indicate comparison of the estimating equations in HAART naïve patients, naïve males and naïve females and A_1, B_1 and C_1 indicates comparison of the equations in patients on HAART, males on HAART and females on HAART.

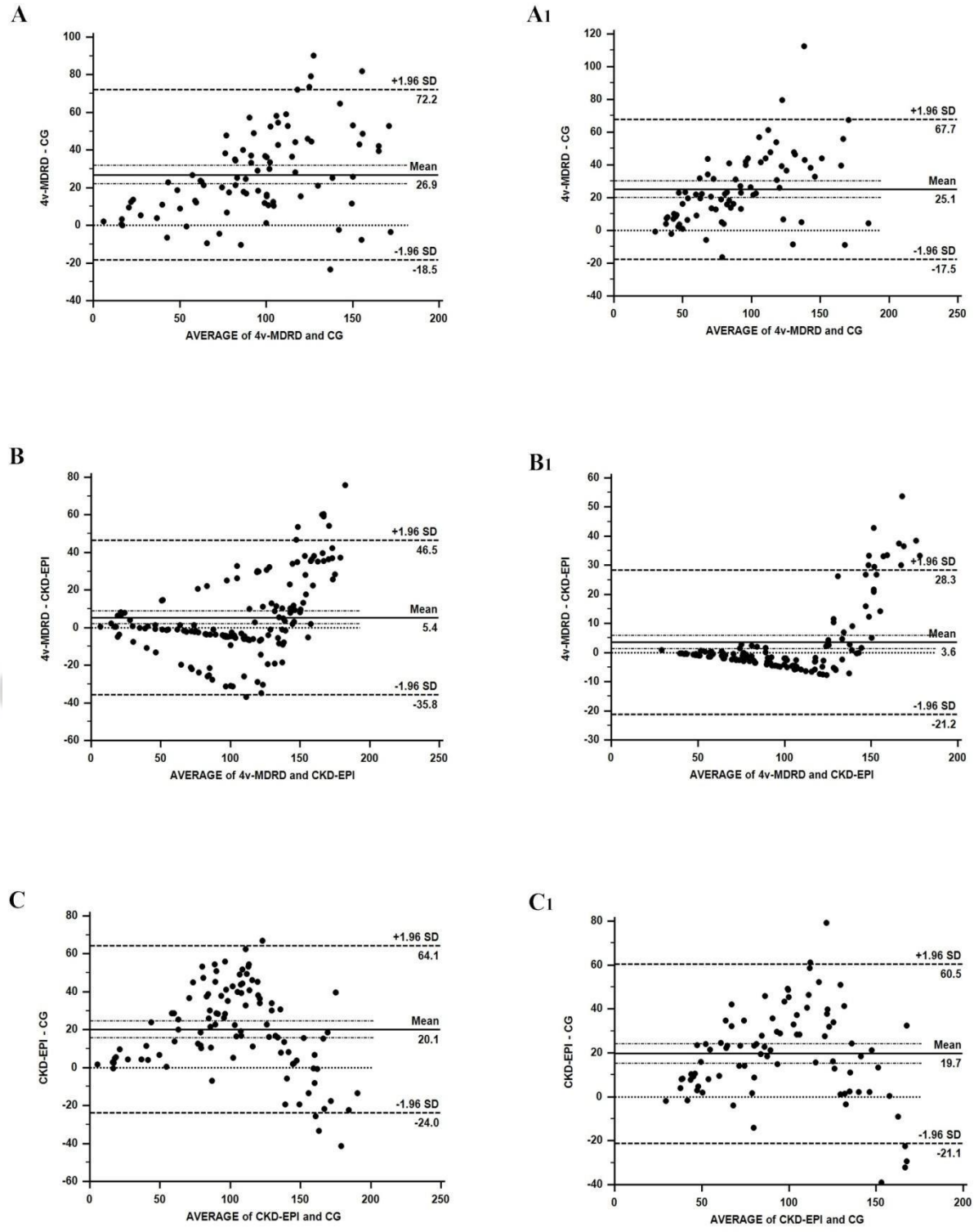


Figure 4.3 Bland-Altman comparison of the estimating equations in HIV patients

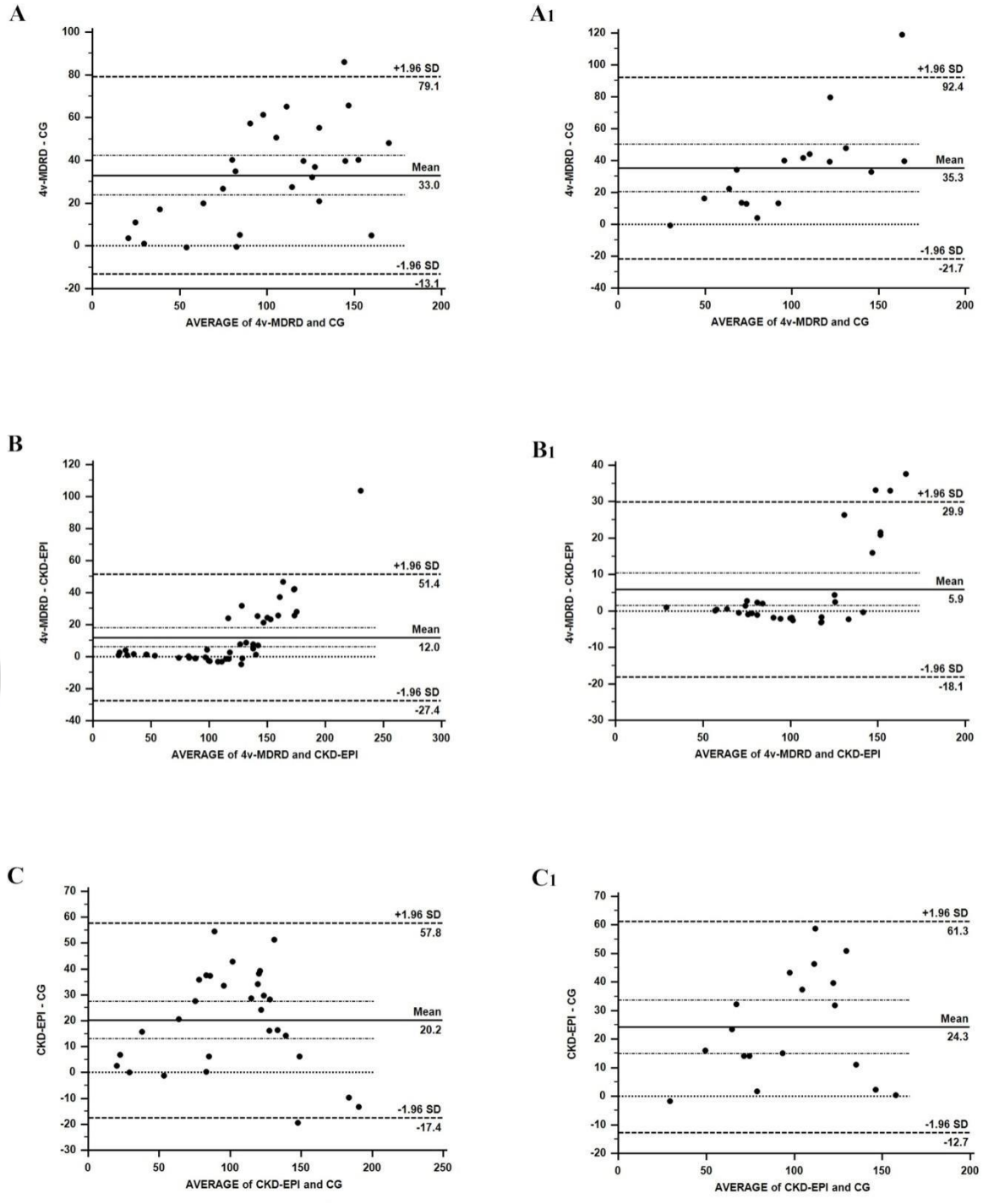


Figure 4.4 Bland-Altman comparison of the estimating equations in male patients

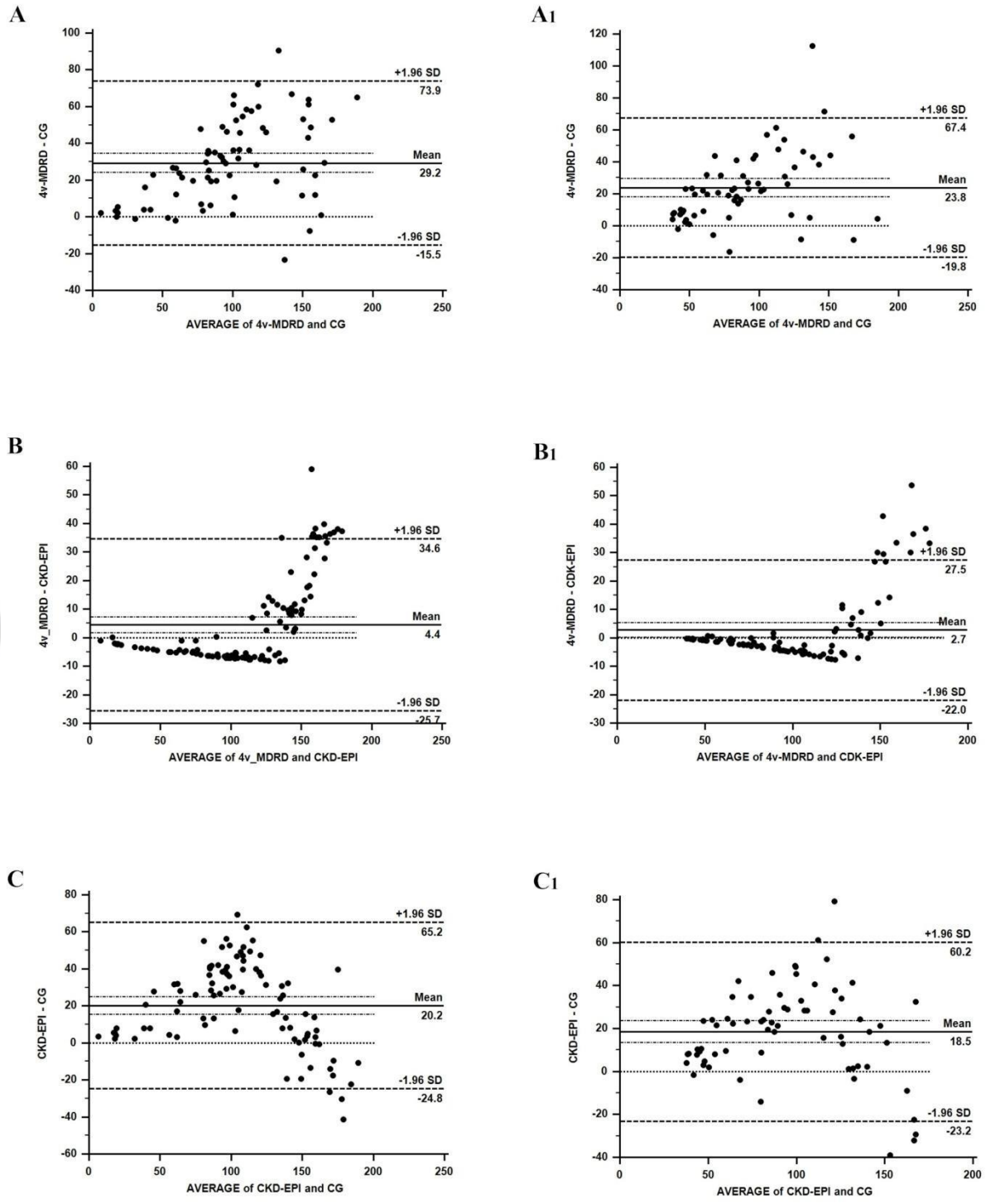


Figure 4.5 Bland-Altman comparison of the estimating equations in female patients

4.3 OXIDATIVE STRESS AND DYSLIPIDAEMIA

4.3.1 Liver Function Tests

The mean concentrations of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were above the upper limit of the reference ranges with that of alanine aminotransferase (ALT) falling within the normal range. No significant differences were observed across the general study population for AST and GGT. The mean concentrations of ALP in patients on HAART, males on HAART and females on HAART were significantly higher ($P < 0.001$, $P < 0.05$ and $P < 0.001$ respectively) than in their naïve counterparts and the mean ALT concentration in patients on HAART was significantly higher ($P < 0.05$) than in their naïve counterparts though within normal limits (Table 4.9).

The mean concentrations of total bilirubin (BIT) and direct bilirubin (BID) was well within the normal limits and showed no significant differences across the study population. Apart from the mean serum albumin concentration of HAART naïve males which fell below the normal limits, all other serum albumin values were within normal limits and showed no significant difference across the general study population. Mean serum protein concentration was above the upper limit of normal range and the concentration in study participants and females on HAART was significantly higher ($P < 0.05$) compared to their naïve counterparts with that of HAART naïve males and males on HAART showing no statistical significance (Table 4.9).

Table 4.9 Demographic and biochemical characteristics of the study population stratified by HAART use and gender.

Parameters		HIV PATIENTS		MALE PATIENTS		FEMALE PATIENTS	
	RANGES	HAART NAÏVE	ON HAART	HAART NAÏVE	ON HAART	HAART NAÏVE	ON HAART
LFT							
AST (U L^{-1})	10 – 40	41.54 ± 3.25	44.35 ± 2.31	49.74 ± 10.62	51.32 ± 6.13	37.95 ± 2.19	42.21 ± 2.35
ALT (U L^{-1})	5 – 40	19.02 ± 1.11	23.57 ± 1.85*	21.14 ± 1.88	26.40 ± 4.79	18.27 ± 1.37	22.70 ± 1.92
ALP (U L^{-1})	30 – 140	150.10 ± 8.76	181.60 ± 8.10**	147.80 ± 18.57	224.40 ± 22.95†	130.80 ± 11.14	168.40 ± 7.67§§
GGT (U L^{-1})	0 – 50	61.61 ± 8.49	63.76 ± 4.52	78.65 ± 16.33	83.70 ± 14.44	55.40 ± 9.99	57.61 ± 3.81
BIT (µmol L^{-1})	1.7 - 20	15.77 ± 1.96	14.55 ± 0.51	18.94 ± 7.25	13.52 ± 0.95	14.66 ± 0.62	14.87 ± 0.60
BID (µmol L^{-1})	1.7 – 5.1	4.45 ± 0.74	4.62 ± 0.29	6.10 ± 2.58	4.51 ± 0.59	3.79 ± 0.41	4.65 ± 0.34
Protein (g L^{-1})	60 – 80	92.78 ± 1.29	98.64 ± 2.10*	93.97 ± 2.61	94.36 ± 4.08	92.53 ± 1.49	99.96 ± 2.44§
Albumin (g L^{-1})	35 - 50	36.58 ± 2.54	38.36 ± 0.54	33.58 ± 1.27	36.35 ± 1.26	37.99 ± 3.48	38.98 ± 0.59
LIPID PROFILE							
Cholesterol (mmol L^{-1})	3.1 – 5.2	3.18 ± 0.09	4.00 ± 0.08***	2.96 ± 0.15	3.82 ± 0.19†††	3.24 ± 0.10	4.06 ± 0.09§§§
Triglyceride (mmol L^{-1})	0.3 – 1.8	1.69 ± 0.10	1.80 ± 0.08	1.49 ± 0.08	1.60 ± 0.16	1.74 ± 0.13	1.86 ± 0.09
HDL (mmol L^{-1})	< 1.1	0.77 ± 0.03	1.42 ± 0.07***	0.75 ± 0.07	1.42 ± 0.15†††	0.76 ± 0.04	1.42 ± 0.07§§§
LDL-C (mmol L^{-1})	2.6 – 4.2	1.03 ± 0.12	1.79 ± 0.11***	0.82 ± 0.12	1.74 ± 0.24†††	0.97 ± 0.09	1.83 ± 0.12§§§
OXIDATIVE STRESS							
Calcium (mmol L^{-1})	2.05 – 2.55	2.15 ± 0.09	1.86 ± 0.04**	2.16 ± 0.09	1.87 ± 0.08†	2.15 ± 0.13	1.85 ± 0.05§
Uric Acid (µmol L^{-1})	240 – 480	395.00 ± 12.87	435.20 ± 16.33	465.90 ± 32.06	473.30 ± 31.31	370.40 ± 12.78‡‡	423.50 ± 19.02§
Vitamin C (mg dL^{-1})	0.4 – 1.5	0.11 ± 0.00	0.03 ± 0.00***	0.11 ± 0.01	0.04 ± 0.00†††	0.11 ± 0.00	0.03 ± 0.00§§§
MDA (µmol L^{-1})	< 0.7	7.51 ± 0.22	7.75 ± 0.21	7.48 ± 0.44	7.49 ± 0.44	7.52 ± 0.27	7.83 ± 0.24

Results are presented as means ± SEM. *P ≤ 0.05, **P ≤ 0.001, ***P ≤ 0.0001 indicates the level of significance when the HAART naïve HIVpatients were compared to those on HAART (unpaired t-test); †P ≤ 0.05, ††P ≤ 0.01, †††P ≤ 0.001 indicates the level of significance when

the male HAART naïve were compared to the male on HAART; $^{§}P \leq 0.05$, $^{§§}P \leq 0.01$, $^{§§§}P \leq 0.001$ indicates the level of significance when the female HAART naïve were compared to the female on HAART; $^{‡}P \leq 0.05$, $^{‡‡}P \leq 0.01$, $^{‡‡‡}P \leq 0.001$ indicates the level of significance when the male HAART naïve were compared to the female HAART naïve. GGT: Gamma Glutamyl Transferase, CHOL: Cholesterol, TRG: Triglyceride, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, MDA: Malondialdehyde

4.3.2 Lipid profile

The mean serum concentrations of total cholesterol (TC), high density lipoproteincholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were within the normal ranges but significantly higher ($P < 0.0001$) in patients on HAART, males on HAART and females on HAART compared to naïve subjects. No significant differences were observed in a comparison of the mean serum triglyceride (TG) concentrations in all the study populations (Table 4.9).

4.3.3 Calcium and Uric acid

In Table 4.9, the mean serum calcium concentration was significantly reduced in patients on HAART ($P < 0.001$), males on HAART ($P < 0.05$) and females on HAART ($P < 0.05$) in comparison to their HAART naïve counterparts. The difference in the calculated incidence of hypocalcaemia in HAART naïve and patients on HAART (14.1% *vs.* 41.6%), HAART naïve males and males on HAART (10.3% *vs.* 48.7%) and HAART naïve females to females on HAART (15.7% *vs.* 39.5%) respectively was statistically significant (Table 4.10). Serum uric acid concentration was also within the normal limits but the mean value in females on HAART was significantly higher ($P < 0.05$) than in naïve females. A comparison of uric acid concentration in males on HAART and patients on HAART to their naïve counterparts showed no statistical significance ($P > 0.05$) (Table 4.9).

4.3.4 Vitamin C and MDA

Mean vitamin C concentrations across the study population was below the lower limit of the reference range with concentrations in HAART naïve patients, naïve males and naïve females being significantly higher ($P < 0.0001$ respectively) than in patients on HAART. The mean concentration of malondialdehyde (MDA) across the study population was about 10-fold higher than the reference limit but there was no statistically significant difference ($P > 0.05$) between HAART naïve patients and patients on HAART (Table4.9).

4.3.5 Dyslipidaemia

Table 4.10 presents a general overview of the proportions of the study population with dyslipidemia. Patients on HAART are ten times at risk of developing elevated TC levels (χ^2 = 35.00; P < 0.0001) and two and half times at risk of developing elevated TG levels (χ^2 = 14.70; P < 0.0001) when compared to their HAART naïve colleagues. In males on HAART, the risk of developing elevated levels of TC is 38 times more than in their naïve counterparts (χ^2 = 15.70; P < 0.0001) and the relative risk for elevated LDL-C was 11 times more than in naïve males (χ^2 = 4.30; P = 0.04). The relative risk of developing elevated TC and TG levels in females on HAART was 7 (χ^2 = 21.20; P < 0.0001) and 2.4 (χ^2 = 11.70; P = 0.001) times respectively more than in HAART naïve females.

In a comparative Chi-square analysis to determine the statistical significance of developing hypertriglyceridaemia to hypercholesterolaemia or vice versa in the study population, TG levels were significantly elevated in HAART naïve males (11.5% *vs.* 0.0%, P = 0.003); HAART naïve females (18.2% *vs.* 3.0%, P < 0.0001) and HAART naïve patients (16.3% *vs.* 2.2%, P < 0.0001). The same trend was observed in patients on HAART (31.9% *vs.* 18.1%, P < 0.005) and females on HAART (34.9% *vs.* 17.8%, P = 0.003) but that in males on HAART showed no statistically significant difference (21.6% *vs.* 18.9%, P = 1.00).

4.3.6 Incidence of dyslipidaemia

The estimated incidence of dyslipidaemia in the general study population (HAART naïve patients and those on HAART) is as follows: isolated hypercholesterolaemia (2.2% *vs.* 18.1%); isolated hypertriglyceridaemia (16.3% *vs.* 31.9%); isolated decreased high-density lipoprotein (35.9% *vs.* 38.6%) and isolated increased low-density lipoprotein (1.5% *vs.* 3.6%) (Table 4.10).

Table 4.10 Analysis of dyslipidaemia and hypocalcaemia in the study population.

	HIV Patients				
	HAART Naïve	On HAART			
Variable	**276 (%)**	**166 (%)**	χ^2	**OR(95% CI)**	**P value**
TC (>5.2 mmol L^{-1})	6 (2.2)	30 (18.1)	35.00	9.9(4.0 - 24.4)	**<0.0001**
TRG (>1.8 mmol L^{-1})	45 (16.3)	53 (31.9)	14.70	2.4(1.5 - 3.8)	**<0.0001**
HDL-C (<1.0 mmol L^{-1})	99 (35.9)	64 (38.6)	0.32	1.1(0.8 - 1.7)	0.57
LDL-C (>4.1 mmol L^{-1})	4 (1.5)	6 (3.6)	2.19	2.5(0.7 - 9.2)	0.14
Calcium (<2.1mmolL^{-1})	39 (14.1)	69 (41.6)	42.26	4.3(2.7 – 6.8)	**<0.0001**
	Male Patients				
	HAART Naïve	On HAART			
Variable	**78 (%)**	**37 (%)**	χ^2	**OR(95% CI)**	**P value**
TC (>5.2 mmol L^{-1})	0 (0.0)	7 (18.9)	15.70	38.6(2.1 - 697.3)	**<0.0001**
TRG (>1.8 mmol L^{-1})	9 (11.5)	8 (21.6)	2.03	2.1(0.7 - 6.0)	0.15
HDL-C (<1.0 mmol L^{-1})	27 (34.6)	12 (32.4)	0.05	0.9(0.4 - 2.1)	0.82
LDL-C (>4.1 mmol L^{-1})	0 (0.0)	2 (5.4)	4.30	11.1(0.5 - 236.5)	**0.04**
Calcium (<2.1mmolL^{-1})	8 (10.3)	18 (48.7)	21.4	8.3(3.1 – 21.9)	**<0.0001**
	Female Patients				
	HAART Naïve	On HAART			
Variable	**198 (%)**	**129 (%)**	χ^2	**OR(95% CI)**	**P value**
TC (>5.2 mmol L^{-1})	6 (3.0)	23 (17.8)	21.20	6.9(2.7 - 17.59)	**<0.0001**
TRG (>1.8 mmol L^{-1})	36 (18.2)	45 (34.9)	11.70	2.4(1.4 - 4.0)	**0.001**
HDL-C (<1.0 mmol L^{-1})	72 (36.4)	52 (40.3)	0.52	1.2(0.7 - 1.9)	0.47

LDL-C (>4.1 mmol L^{-1})	4 (2.0)	4 (3.1)	0.38	1.5(0.4 - 6.3)	0.54
Calcium (<2.1mmolL^{-1})	31 (15.7)	51 (39.5)	23.70	3.5(2.1 – 5.9)	**<0.0001**

TC: Total Cholesterol, TRG: Triglyceride, LDL-C: Low Density Lipoprotein Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, HAART: Highly Active Antiretroviral Treatment, OR: Odds Ratio, CI: Confidence Interval, χ^2: Chi-square.

Chapter 5

DISCUSSION

5.1 DEMOGRAPHICS AND HIV INFECTION

In this study, about 86% (88% of HAART naive patients and 83% of patients on HAART) of the study population fell within the 20 – 49 years age brackets which is known to be the sexually active age group with highest peak percentage observed within the 30 – 39 age groups for both sexes. Amornkul *et al.,* (2009) reported a 25 – 29 years peak age for females and 30 – 34 years peak age for males. This means that heterosexual contact may probably contribute to a greater proportion of HIV infections in our study group and this finding is buttressed by the Ghana Aids Commission report (Ghana AIDS Commission, 2001) which states that heterosexual intercourse accounts for 75 to 80 percent of all HIV/AIDS infections. Amballi *et al.,* (2007) in their study on people living with HIV/AIDS reported a percentage of 75 within this age bracket. Females formed about 76% of the study population and a further finding of female to male ratio of 3:1 in HAART naive patients and 5:1 in patients on HAART strongly suggests that females are at a greater risk of getting infected with HIV than men and this could happen at an early stage in their lives probably early to mid thirties considering the significant differences in the mean ages of HAART naive males to HAART naive females.

The World Health Organization (WHO) reported that HIV/AIDS affects females most severely in sub-Saharan Africa and women of reproductive age make up almost 57% of adults living with HIV, accounting for up to 80% of HIV infected women in the world (Dabis and Ekpini, 2002; WHO, 2004). The significant

difference in age between patients on HAART and HAART naive patients further supports the proposition that HIV/AIDS infection may be acquired at an early age and most probably during the reproductive stage of life. The strict eligibility criteria for qualifying for HAART (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, 2006) may also explain why most of the patients on HAART appear to be older than their naive counterparts in that there could be a time lag between the time a patient tests positive for HIV and the time treatment is initiated and treatment once started is continued throughout the life of the patient.

Woods *et al.,* (2002) reported inadequate nutrient intake among a large proportion of HIV patients and Oguntibeju *et al.,* (2006) reported that there is evidence to show that nutritional intervention assists in maintaining and optimizing nutritional status and immune function, prevents the development of nutritional deficiency, loss of weight and lean body mass, promotes response to medical treatment and increases longevity in HIV patients. The improvement in the weight of patients on HAART, males on HAART and females on HAART over their naive counterparts observed in this study could be a direct consequence of strict adherence to counseling which insists on good nutritional status alongside drug therapy. Bouic *et al.,* (2001) and Oguntibeju *et al.,* (2006) reported that nutritional supplementation can decrease viral load and through this intervention therefore, HAART might improve upon the quality of life of an HIV patient.

5.1.1 Anaemia and HAART

The calculated incidence of anaemia from this study was 63.0% in HAART naive patients and 46.0% in patients on HAART using the upper limit of the WHO/ACTG grade 1 toxicity range. HAART naive patients have a high risk of developing reduced PCV compared to their counterparts on HAART. Furthermore, HAART naive patients and HAART naive females have a higher risk of developing moderate to severe anaemia compared to their counterparts on HAART and the calculated mean haemoglobin values in males and females on HAART were significantly higher compared to their naive counterparts. As related in the studies conducted by Moore *et al.,* (1998), Mocroft *et al.,* (1999) and Levine *et*

al., (2001), the improvement in haemoglobin levels could be due to increase in the proportion of erythrocytes in relation to total blood volume as a result of elevated PCV. The reduced incidence of anaemia, the reduced risk of developing moderate to severe anaemia, the overall improvement in PCV and haemoglobin concentration when on HAART in this study confirms the effectiveness of HAART in improving the quality of life of HIV patients.

Belperio and Rhew (2004) and Odunukwe *et al.,* (2005) reported improved haematocrit values, increased haemoglobin concentration and decreased prevalence of anaemia in their study which is in agreement with our studies and Abrams *et al.,* (2000) reported that small increases in haemoglobin level (up to 2 g dL^{-1}) were associated with a beneficial effect on total quality of life. The calculated incidence of anaemia by sex was 50.0% in females on HAART and 70.0% in HAART naive females compared to 34.0% and 44.0% in males on and off HAART respectively. The significant difference in proportion of HAART naive females to HAART naive males with anaemia shows that HIV infected females in this study are more at risk of developing anaemia compared to infected males. Levine *et al.,* (2001) reported a similar finding in their study and attributed it to sex and race whereas Volberding *et al.,* (2004) attributed it to menstrual blood loss in women and to the drain on iron stores that occur with pregnancy and delivery. This finding is however in sharp contrast to the findings of a study conducted by Omoregie *et al.,* (2009) which reported high prevalence of anaemia in HAART naive males compared to their female counterparts. The absence of a significant difference in the proportion of females on HAART to males on HAART further buttresses the ability of HAART to improve upon the severity of anaemia in females.

5.1.2 Type of anaemia and HAART

The relatively high risk of developing microcytic hypochromic anaemia found in HAART naive patients and females off HAART compared to those on HAART in this study reflects the overall nutritional deficiencies (malnutrition and

malabsorption) associated with HIV patients. Blood loss and drains on iron stores that occur with pregnancy and delivery in HIV infected women could be an added consequence of anaemia in females especially since microcytic anaemia is associated with iron deficiency. The high risk of developing the other types of anaemia (normocytic hypochromic, normocytic normochromic and macrocytic hypochromic) in HAART naive patients in this study can be attributed to the multifactorial aetiology of anaemia as related in the study conducted by Volberding *et al.,* (2004) where causes of anaemia were associated to blood loss or decreased red blood cell (RBC) production, increased RBC destruction and ineffective RBC production.

The odds of developing macrocytic hypochromic anaemia was the same in all the study populations and did not differ significantly (Table 4.3) but the average MCV for patients on HAART, males on HAART and females on HAART (Table 4.2) were significantly higher compared to their naive counterparts. Moyle (2002) reported that elevated MCV (macrocytosis) is typically associated with vitamin B_{12} or folate deficiency and in the setting of HIV treatment reflects the use of zidovudine (AZT) or stavudine (d4T). The elevated MCV observed in patients on HAART in this study could therefore be attributed to drug usage since most of them had a combination therapy of either AZT or d4T with lamivudine (3TC) but other factors will come into play considering the fact that HAART naive patients had a similar likelihood of developing macrocytosis compared to the patients on HAART. Burkes *et al.,* (1987) first reported low levels of vitamin B_{12} in HIV positive patients and Beach *et al.,* (1988) as well as Boudes *et al.,* (1990) described folate deficiency in HIV infected patients. Conversely, Hepburn *et al.,* (2004) reported that HAART may increase serum vitamin B_{12} levels and patients with low serum vitamin B_{12} in their study did not display characteristic findings of vitamin B_{12} deficiency, namely macrocytic anaemia and neuropathy. Remacha *et al.,* (1999) suggested that low serum vitamin B_{12} levels are reflective of low levels of vitamin B_{12} transport proteins (transcobalamin I or haptocorrin) which are produced by neutrophils and

not a tissue deficiency of vitamin B_{12}. A high percentage of neutropoenia was observed in the study population with the percentages in patients, males and females on HAART being slightly higher than their naive counterparts although the difference was not statistically significant. Low levels of transport proteins associated with neutropoenia could therefore be indirectly implicated in elevated MCV observed in both HAART naive patients and those on HAART (AZT induced). Serum vitamin B_{12} level in the study population was not examined in this study.

5.1.3 Immunological status and HAART

Depletion of lymphocytes, primarily of the CD4 cell subset subsequent to cellular CD4 immunodeficiency has been noted as the hallmark of HIV infection (Gil *et al.*, 2003) with leucopoenia and lymphopoenia being documented in different proportions in HIV patients (Amballi *et al.*, 2007). In testing the ability to readily identify the presence of lymphopoenia by using the differential lymphocyte count and absolute lymphocyte count in this study, it was observed that the differential lymphocyte count gave strong indications of lymphopoenia than the absolute counts when HAART naive patients were compared to those on HAART. The absolute lymphocyte counts gave a marginally significant indication of lymphopoenia when HAART naive females were compared to their counterparts on HAART whilst the differential count gave a stronger indication. On a gender comparison therefore, HIV infected females will probably have a faster progression in the depletion of lymphocytes than infected males. A further observation of significant increments in the mean differential lymphocyte counts in patients on HAART and females on HAART over their naive counterparts proves that the observed lymphopoenia may have been corrected and improved upon by an intervention (HAART usage). The mean absolute counts gave no such findings in all the study populations. Differential lymphocyte counts may therefore serve as a useful tool in indicating lymphopoenia and improvements in lymphocyte counts in HIV infected patients than absolute counts. Significant reductions in the total

white blood cell counts when the study population on HAART were compared to their naive counterparts could be due to the broad myelosuppressive effects of the drug regimen mostly associated with AZT usage.

The mean CD4 count in patients on HAART was higher compared to their naive counterparts and it was consistently observed that HAART naive patients and females are at greater risk of having CD4 <200 cells mm^{-3} in comparison to their counterparts on HAART who are better placed to have greater improvements in their CD4 counts to levels within 200 – 499 cells mm^{-3}. It was further observed that CD4 counts <200 cells mm^{-3} was strongly associated with severe anaemia (Grades 3 & 4) in HAART naive patients and could lead to rapid disease progression and decreased survival as related in the study conducted by Obirikorang and Yeboah (2009) and Curkendall *et al.,* (2007). When on HAART however, a high proportion of the patients with CD4 <200 cells mm^{-3} had a better chance of falling within the Grade 1 (9.5–10.5 g dL^{-1}) anaemia toxicity range. The significant finding of the ability of HAART to improve upon CD4 counts and anaemia in HIV infected patients in this study proves the ability of HAART to reduce morbidity and mortality in HIV infection as related in the study of Gea-Banacloche & Lane, (1999). The ability of HAART to improve upon CD4 counts and anaemia observed in this study can be attributed to the effectiveness of HAART in reducing viral replication and viral load as related in the studies of Belperio & Rhew (2004) and Odunukwe *et al.,* (2005).

5.1.4 Thrombocytopenia and HAART

Although the relative risk of developing thrombocytopenia in all the study populations was the same, the mean platelet counts of patients on HAART and females on HAART was significantly lower compared to their naive counterparts meaning that we are likely to find lower platelet counts in patients and females on HAART than in HIV infected patients. HAART though effective in improving on haematocrit and decreasing the prevalence of anaemia could lead to a decrease in

platelet counts. This finding is in sharp contrast to that of Attili *et al.,* (2008) where thrombocytopenic incidence of 4.8% in HIV patients was reported and their platelet counts increased after antiretroviral therapy. An incidence of about 50% was calculated in both HAART naive and patients on HAART which almost agrees with the findings of the study conducted by Pechere *et al.,* (1993) who reported a thrombocytopenic incidence of 40%.

5.1.5 Parameters that can predict CD4

The ability of TLC, lymphocyte count, haemoglobin and weight to predict CD4 count in both HAART naive patients and those on HAART suggests that these parameters which are relatively inexpensive and easily available compared to techniques for assaying CD4 and viral load could serve as accurate tools that can be used for monitoring the patients' immune status during therapy in addition to determining when patients should start antiretroviral therapy. Mwamburi *et al.,* (2005) reported a similar finding and suggested modification of the models to suit specific needs for use in underserved areas.

5.1.6 TLC and CD4

The value of TLC as a surrogate for CD4 in monitoring HIV disease in the absence of viral loads and CD4 counts has been argued (Kumarasamy *et al.*, 2002; Crowe *et al.*, 2003) but current WHO guidelines only commit to using TLC in conjunction with clinical data as a criterion to initiate HAART in resource poor settings (WHO, 2006). A trend analysis to find the ability of TLC to predict CD4 <200 cells mm^{-3} was conducted and it was observed that with a TLC of 1.0–2.0 k μL^{-1}, a greater proportion of the HAART naive patients had CD4 counts <200 cells mm^{-3} and TLC >2.0 k μL^{-1} was associated with a peak proportion of patients with CD4 ≥500 cells mm^{-3}. The same trend was observed in patients on HAART. A range of TLC cutoffs have been used and reported as predictors of CD4 <200 cells mm^{-3} and these range from 1.0 k μL^{-1} with a specificity of 98% and a sensitivity of 53% to 1.4 k μL^{-1} with a sensitivity of 73% and a specificity of 88% (Kumarasamy *et al.*, 2002; Schreibman

and Friedland, 2004). This study shows that a TLC range of 1.0–2.0 k μL^{-1} could predict CD4 <200 cells mm^{-3} but this will serve a better purpose in the management and monitoring of HIV patients if a calibration cut-off could be established in our settings. The calibration cut-off in addition to clinical data (haemoglobin, weight, lymphocyte count) could serve as useful models in resource poor setting.

5.2 RENAL FUNCTION AND CHRONIC KIDNEY DISEASE (CKD)

5.2.1 Incidence of CKD

The Infectious Diseases Society of America (IDSA) recommends the simplified MDRD equation for staging CKD and the Cockcroft-Gault equation for dosage modification (Gupta *et al.*, 2005). Currently, the only validated renal function equation in Ghana is among CKD patients where Cockcroft-Gault and fourvariable Modification of Diet in Renal Disease (4v-MDRD) equations were recommended out of the six equations validated (Owiredu *et al.*, 2008). The calculated incidence of CKD (GFR <60 mL min^{-1} 1.73m^{-2}; stages 3, 4 & 5) in male and female patients (HAART naïve + on HAART) was 9.8% and 11% respectively giving an overall average incidence of 10.4% using the 4v-MDRD equation. In a related cross-study in an antiretroviral (ARV) naïve Kenyan population, WoolsKaloustian *et al.*, (2007) reported a renal insufficiency (CrCl <60 mL min^{-1} and <50 mL min^{-1}) incidence of 11.5% and 4.8% respectively. This significant finding suggests that CKD is a growing health concern in this study population and it is certainly under-diagnosed and under-treated considering the fact that apart from results of full serum chemistries, none of the study participants had a documented creatinine clearance on which antiretroviral (ART) dosing was based. The provision of absolute creatinine values rather than calculated creatinine clearance values by most laboratories for patients is not in line with recommended guidelines of the IDSA which recommends that therapy should be dependent on level of creatinine clearance. Renal insufficiency in this study as estimated by the Cockcroft-Gault equation in male and female (on HAART + HAART naïve) patients was 8.4% and

13.5% respectively giving an overall average of 10.9%. A significant proportion of this study population may therefore have required dosage adjustment at the time of initiation of therapy or sometime during ongoing therapy.

5.2.2 Dose adjustment

The IDSA ART dosing recommendations suggests that appropriate reduction of dosing for ARTs that are primarily eliminated by the kidneys is warranted. Dose adjustment for NNRTIs in patients with CKD is not required because NNRTIs in general are much more tightly bound to plasma proteins and are primarily metabolized by the liver (Gupta *et al.*, 2005). They also have high molecular weights and are excreted into the urine in low amounts. Nevirapine, however, does not fully share the drug properties of the other NNRTI's because of its relatively low molecular weight and protein-binding fraction (Gupta *et al.,* 2005). It has therefore been suggested that dialysis may remove substantial amounts of the drug and that it should be administered after dialysis. NRTI's on the other hand are primarily excreted by the kidneys, therefore reduced dosage is required in patients with impaired renal function, especially for a drug like stavudine, which requires further reduction because its pharmacokinetics is influenced by weight (Gupta *et al.*, 2005).

Furthermore, because NRTIs are neither tightly bound to protein nor have a high molecular weight, they may be easily removed by dialysis. The drug regimen for the study population includes the standard combination therapy of 2 NRTIs and 1 NNRTI. The NRTI's include stavudine (d4T), lamivudine (3TC) and combivir (combined drug of Zidovudine (AZT) + 3TC) and the NNRTI's includes efavirenz (EFV) and Nevirapine (NVP). The study population on d4T and 3TC who present with renal insufficiency will require dose adjustments as per the IDSA recommendations with combivir being administered as separate component medications in patients with creatinine clearance <50 mL min^{-1}.

5.2.3 Method comparison

A comparison of the performance of the equations in estimating GFR in the study population showed the 4v-MDRD *vs.* CKD-EPI comparison giving consistently lower bias than the CKD-EPI *vs.* Cockcroft-Gault comparison which also gave a lower bias in relation to the 4v-MDRD *vs.* Cockcroft-Gault comparison. The lower bias observed in the 4v-MDRD *vs.* CKD-EPI should reduce the rate of false-positive diagnoses of stage 3 CKD (estimated GFR <60 mL min^{-1} 1.73m^{-2}) in patients without CKD and no markers of kidney damage. The ability of the CKD-EPI equation to compare well with the 4v-MDRD equation, the recommended equation for staging CKD (Gupta *et al.*, 2005), simply means that the CKD-EPI equation although not validated in this study population could be used interchangeably with the 4v-MDRD equation for effective staging of CKD in HIV-infected patients. Cockcroft-Gault equation on the other hand underestimates GFR compared to the 4v-MDRD and CKD-EPI equations and could lead to falsely low GFR's which could lead to insufficient drug dosing in patients without CKD. This observation is in agreement with the previous finding among CKD patients (Owiredu *et al.*, 2008).

5.2.4 Study predictors

In the linear regression model to assess the ability of creatinine, weight, age, CD4 and albumin to predict GFR in all the estimating equations, creatinine and age showed reciprocal relationships to GFR. Endogenous creatinine is solely excreted by the kidneys and a fractional reduction in urine creatinine excretion will lead to an increase in serum concentrations of creatinine hence reduced GFR being associated with high serum creatinine concentration. Older age is an independent predictor of lower GFR and mainly reflects the relationship between age and muscle mass (Heymsfield *et al.*, 1983). Lower muscle mass in older persons cause lower urine creatinine excretion because of the lower serum creatinine concentration hence the negative relationship. Weight and CD4 gave positive linear relationship with GFR estimated with the equations. Increase in body weight

is associated with increase in muscle mass with proportional increase in creatinine production which is excreted with a fractional increase in GFR. Cockcroft-Gault equation incorporates creatinine (inverse relationship), weight (direct relationship) and age (natural scale) in estimating GFR whilst CKD-EPI incorporates log serum creatinine (modeled as a 2-slope linear spline with sex specific knots at 0.7 mg dL^{-1} for women and 0.9 mg dL^{-1} in men) and age (on a natural scale) (Levey *et al.*, 2009) and 4v-MDRD incorporates log serum creatinine (without a spline) and age (on log scale) (Levey *et al.*, 2009).

The composition and variables in the estimating equations may explain some of the differences in their ability to estimate GFR. Weight was an independent predictor of estimated GFR in the Cockcroft-Gault equation than in the 4v-MDRD and CKD-EPI equations and this could be attributed to weight in a direct relationship to GFR in the Cockcroft-Gault equation compared to the latter equations which have been adjusted for body surface area. Serum creatinine is a strong predictor of GFR in the CKD-EPI equation and CD4 which is not included in any of the estimating equations was a strong predictor of GFR in the CockcroftGault equation. The Cockcroft-Gault equation, which is recommended for dose modification, may therefore serve as an invaluable tool in the HIV-infected population if an adjustment could be made for the inclusion of CD4. The association of serum creatinine and the CKD-EPI equation may give an added reason why the CKD-EPI equation would be a better tool in staging CKD in HIVinfected patients. Coresh *et al.* (2007) reported a CKD prevalence of about 50% in the elderly population and the significant finding of increases in age and weight in patients on HAART and females on HAART compared to their naïve counterparts calls for a critical assessment of GFR in this study group for effective dosage adjustment in the presence of CKD.

5.3 DYSLIPIDAEMIA

Dyslipidemia, simply defined as "abnormal lipid levels", has been associated with HIV infection independent of antiretroviral therapy (ART) (Riddler *et al.*, 2003), but ART can also contribute to dyslipidemia with episodes being more common and more severe in patients receiving ART than in patients not on therapy (Sullivan and Nelson, 1997; Segerer *et al.*, 1999). Abnormal lipid levels were observed in the HAART naïve study population and those on HAART and a comparison of the proportion of occurrences showed a high risk of developing hypercholesterolaemia and hypertriglyceridaemia in patients on HAART which is consistent with the findings of Segerer *et al.,* (1999).

A further finding of significantly elevated TG levels and TC levels in the HAART naïve subgroup in this study is consistent with the report of Grunfeld *et al.,* (1991) who reported that HIV infection was associated with elevated triglyceride levels that worsened with progression of HIV related disease. The same trend was observed in patients on HAART and females on HAART with an exception in males on HAART who showed no significant difference in their TG and TC levels. Exactly how HIV and antiretroviral therapy causes dyslipidaemia has not been determined, but Carling *et al.,* (2002) suggested a decreased clearance of triglycerides in the presence of increased concentration of cytokines e.g. αinterferon as the cause of hypertriglyceridaemia. Chisolm *et al.,* (1999) suggested a metabolic basis linked with cholestasis as the cause for the appearance of abnormal plasma lipoproteins and Ljubuncic *et al.,* (2000) reported that experimental cholestatic liver disease is associated with increased lipid peroxidation.

5.3.1 Reactive Oxygen Species (ROS)

Chronic inflammation due to HIV infection leads to high plasma levels of inflammatory cytokines and production of reactive oxygen species (ROS) (Israel and Gougerot-Pocidalo, 1997). An imbalance between ROS production and its

inactivation by antioxidants is capable of causing oxidative damage to major macro-molecules in cells, including lipids, proteins and nucleic acids. Zoccali *et al.*, (2000) reported that such extensive oxidative attack on major macro-molecules could lead to loss of functionality of the parent compounds which are major components in cell metabolism and cell membranes. ROS can directly attack the mitochondrial membrane, blocking oxidative phosphorylation thereby leading to marked decrease in intracellular adenosine triphosphate (ATP). Decreased ATP results in a block of messenger-ribonucleic acid (m-RNA) and impaired protein synthesis. Lack of protein moiety of lipoproteins ensues resulting in the absence of the carrier protein to which triglycerides must be attached thus explaining the accumulation of triglycerides (Glaser and Mager, 1972).

Oxidative stress in the study population was assessed by assaying for malondialdehyde (MDA), an end-product of lipid peroxidation and vitamin C, an antioxidant in serum. MDA was elevated by about 10-fold above the upper limit of the normal range in all the study groups and vitamin C concentration was reduced in all the study groups but significantly reduced in patients on HAART compared to the naïve subgroup. The significantly reduced vitamin C concentration in patients on HAART in comparison to their naïve counterparts could lead to a greater imbalance of ROS hence a concomitant increase in oxidative stress and mitochondrial toxicity leading to impaired carrier-protein synthesis and reduced clearance of triglycerides resulting in the observed significant increases in TG concentrations in patients on HAART. Hypertriglyceridaemia could also be as a consequence of the direct activity of reactive metabolites (ROS) on the carrierprotein leading to loss of functional ability of the parent carrier-protein.

5.3.2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

NRTIs have been associated with hepatic mitochondrial toxicity and lactic acidosis with the latter providing proof of mitochondrial toxicity as diminished oxidative phosphorylation and diminished acid clearance are required for acidosis to occur.

Acidosis is therefore typically associated with liver dysfunction as the liver is the primary site of lactate clearance and also dysfunction in other tissues (Moyle, 2001). The observed dyslipidaemia in patients on HAART could further be exacerbated by the treatment regimen in view of the fact that they are mainly on a combination therapy of NRTIs and NNRTIs.

5.4 CHOLESTASIS

Cholestasis is functionally defined as decreased or absent bile flow from the liver into the intestine and as such disturbances in this function strongly impacts on various aspects of lipid metabolism in the body. The molecular and cellular processes that form bile first involves transport events in liver cells and energy is required to drive the ATP-dependent transponders involved (Velayudham and Farrell, 2003). Cholestasis thoroughly deranges the whole body sterol balance thus increases in plasma free cholesterol observed in cholestasis is accompanied by an equimolar elevation of plasma phospholipid (Miller, 1990). Therefore, liver dysfunction resulting from the activities of pro-oxidants, inflammatory cytokines, toxic metabolites of drugs and direct action of drugs could lead to mitochondrial toxicity and blockage of oxidative phosphorylation thereby decreasing intracellular ATP required to drive the events of bile formation. There was a general observation of a tendency to cholestasis across the study population as evidenced by increases in the mean concentrations of ALP and GGT and this could further worsen the high incidence of dyslipidaemia observed in patients on HAART.

5.5 HYPOCALCAEMIA

A significant reduction in mean calcium concentration (hypocalcaemia) was observed in patients on HAART compared to their naïve counterparts with an estimated incidence of hypocalcaemia being 41.6% and 14.1% respectively. Serum calcium exists in three forms: free or ionized calcium, calcium complexed to anions

and calcium bound to proteins (Liamis *et al.*, 2009). Approximately 80% of the protein-bound calcium fraction is associated with albumin (Boden and Kaplan, 1990) and as such a patient with abnormally high serum albumin will have proportionally elevated serum calcium whereas the reported serum calcium in a patient with low serum albumin will be reported as less than the true value (Liamis *et al.*, 2009). The mean serum albumin concentration in patients on HAART, males on HAART and females on HAART was within the normal range and as such could not be a possible cause of the observed hypocalcaemia in the subgroup.

Calcitriol is an essential hormone for the absorption of calcium and phosphate from the intestine and is synthesized from vitamin D (cholecalciferol) through 25hydroxylation of cholecalciferol in the liver and then 1-hyroxylation of the 25hydroxycholecalciferol in the kidney to give 1, 25-hydroxycholecalciferol which is the active form. Impaired renal function leads to a decrease in the conversion of 25hydroxycholecalciferol to its active form thereby leading to loss of calcium in the intestine (Haddad *et al.*, 1993). In estimating the incidence of impaired renal function (GFR <60 ml min^{-1} 1.73 m^{-2}), 11% of the patients on HAART had abnormal renal function compared to 9.8% in naïve patients and this coupled with the presence of extensive liver injury could lead to a resultant decrease in calcitriol levels hence reduced uptake of calcium from the intestine leading to a reduced serum calcium concentration. Also, enhanced activation of the tumor necrosis factor (TNF) system and numerous cytokines are known to induce differentiation of bone marrow precursors into osteoclasts which would favour bone resorption and development of osteoporosis thereby leading to calcium release into the system (Mondy and Tebas, 2001). Most of such calcium is excreted in urine because of attendant renal impairment which reduces the ability of the kidneys to reabsorb calcium. Alkaline phosphatase (ALP) is used as a marker for osteoporosis (Mondy and Tebas, 2001) and a significant finding of increased ALP in patients on HAART, males on HAART and females on HAART compared to their naïve counterparts provides evidence of on-going osteoporosis which subsequently leads to calcium

release and calcium loss in the kidneys hence a significant finding of reduced calcium concentrations in patients on HAART.

Chapter 6

CONCLUSIONS

This study has proven therefore that HAART has the capability of reducing morbidity and mortality associated with HIV infection through dramatic improvements in haematocrit and haemoglobin values, positive impact on lymphocyte counts and improvement in CD4 counts to values ≥200 cells mm^{-3}. Also females in their reproductive age are at a greater risk of getting infected, developing anaemia and experiencing a faster depletion of CD4 lymphocytes and as such, all necessary efforts should be taken to scale up therapy in this HAART era to curb the social burden due to HIV infection. TLC, Lymphocyte count, haemoglobin and weight could predict CD4 counts and may serve as useful tools in the monitoring and management of HIV patients in resource poor settings considering the fact that they are easier and cheaper to perform than techniques for assaying CD4 counts and viral load.

Results also provide evidence of the presence of CKD in HIV infected patients in Ghana with an average prevalence of 10%. A significant proportion of these patients will require dose adjustment either at the time of initiation of therapy or sometime during ongoing therapy (as realized with patients on HAART in this study). The CKD-EPI equation could serve as the best equation for staging of CKD in this study population considering its low bias in comparison to the 4v-MDRD equation and its strong association with serum creatinine concentration.

The results of this study also provides evidence of dyslipidaemia and hypocalcaemia which can further be exacerbated by antiretroviral combination drug regimen. Such complications and other metabolic abnormalities may predispose HIV infected patients and particularly those on ART to cardiovascular disease. HAART naïve patients and patients on HAART should therefore be screened for lipid disorders and hypocalcaemia given the prevalence, potential for morbidity and possible long-term cardiovascular risk.

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