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**University of Southampton**

**Faculty of Medicine, Health and Biological Sciences  
Developmental Origins of Health and Disease (DOHAD)  
Institute of Human Nutrition  
Public Health Nutrition**

**INTERACTION BETWEEN ANAEMIA AND HUMAN IMMUNO-DEFICIENCY  
VIRUS INFECTION IN AN ASYMPTOMATIC POPULATION IN SOUTH  
AFRICA**

**A thesis presented for the degree of Doctor of Philosophy (PhD)**

**By**

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UNIVERSITY OF SOUTHAMPTON

**ABSTRACT**

Faculty of Medicine Health and Biological Sciences, School of Medicine, DoHAD  
Division, Institute of Human Nutrition, Public Health Nutrition

Doctor of Philosophy

**Interaction between anaemia and human immuno-deficiency virus infection  
in an asymptomatic population in South Africa**

By Paul Armah Aryee

Anaemia is common and frequent in HIV infection and Acquired Immune Deficiency Syndrome (AIDS). This study was aimed at exploring the interactions between the effects of HIV infection and other related anaemia-causing effects (reductive adaptation, reduced energy/nutrient intake and inflammatory and/or metabolic alterations), which may be responsible for most anaemia in this population. It is postulated that these interactions can heighten the risk levels for anaemia even in an asymptomatic HIV-infected population. The study is based on a secondary analysis of data from the *Transition and Health during Urbanisation of South Africans* (THUSA) survey, a population-based, cross-sectional study carried out in the North West Province of South Africa. Out of a sample population of 1854 'apparently healthy' adults, aged  $\geq 15$  years, 216 (11.8%) were HIV sero-positive. A validated quantitative food frequency questionnaire was used to assess dietary intake and standard conditions and protocols used for anthropometric and biochemical measurements. Anaemia was defined using WHO haemoglobin (Hb) and haematocrit (Hct) definitions. Univariate ANOVA statistics showed that HIV-sero-positive subjects had lower Hb, Hct, serum iron, ferritin, total iron binding capacity (TIBC) but higher % saturation compared to their sero-negative peers. However, only the differences in Hct were significant ( $p<0.001$ ). Anaemia prevalence was generally high but was higher though not statistically significant in sero-positives than sero-negatives (51.4% cf. 45.8%,  $p=0.123$ ). Anaemia in the study population was mostly mild (about 65%), with a higher proportion of anaemia of chronic inflammation than iron deficiency anaemia. Vitamin a deficiency was significantly associated with anaemia ( $p=0.022$ ). High serum total proteins, alanine transaminase (ALT), aspartate transaminase (AST) and low albumin were significantly associated with HIV sero-positivity. Predictors of anaemia in the study population by logistic regression modelling were settlement type (aOR, 1.7;CI, 1.2-2.5; $p=0.004$ ), serum albumin (0.6;0.4-0.9; $p=0.016$ ), TIBC (1.5;1.0-2.2; $p=0.008$ ), vitamin E (0.6;0.4-0.9; $p=0.006$ ), serum gamma-glutamyl transferase (0.6;0.4-0.9; $p=0.007$ ), Direct bilirubin (0.5;0.5-1.0; $p=0.0446$ ), and abdominal skinfold (1.8;1.2-2.5; $p=0.004$ ). HIV infection was not a significant predictor of anaemia in this asymptomatic population, but the virus and related inflammatory conditions may play a crucial role in the development of anaemia. Where HIV and other inflammatory stressors are prevalent, the overall burden of anaemia could also be increased.

## **DEDICATION**

To God be ALL the GLORY!

This thesis is dedicated to my dear wife Annie, and my beloved children Huguette, Vanessa, Marcellin and Andy for their unflinching support throughout this historic academic journey.

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## LIST OF PUBLICATIONS

### Peer reviewed abstracts

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Aryee PA, Annan RA, Jackson AA, Margetts BM and Vorster E. Iron status in an asymptomatic HIV infected population in South Africa. *Annals of Nutrition and Metabolism*, 2009: **55**(*Suppl 1*):S225

Annan RA, Aryee PA, Jackson AA, Margetts BM and Vorster E. The impact of different dietary patterns on nutritional status asymptomatic people living with HIV infection. *Annals of Nutrition and Metabolism*, 2009: **55**(*Suppl 1*):S224

Annan RA, Aryee PA, Jackson AA, Margetts BM and Vorster E. A predominantly animal-based dietary pattern is a predictor of better metabolic integrity in asymptomatic people living with HIV infection in South Africa. *Annals of Nutrition and Metabolism*, 2009: **55**(*Suppl 1*):S224

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## **DECLARATION OF AUTHORSHIP**

I, Paul Armah Aryee, declare that the thesis entitled "Interaction between anaemia and Human Immuno-deficiency Virus infection in an asymptomatic population in South Africa" is wholly prepared by me whilst registered in postgraduate candidature and is based on a secondary analysis of previously collected data.

I confirm that all the analyses and work in this thesis has been done by me.

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## ABBREVIATIONS AND ACRONYMS

ACC/SCN	Administrative Committee on Coordination/Sub-Committee on Nutrition
ACD/ACI	Anaemia of chronic disease/Inflammation
ADA	American Dietetic Association
AIDS	Acquired immunodeficiency syndrome
ALIVE	AIDS Linked to Intravenous Experiences
ALP	Alkaline phosphatase
ALT	Alanine transaminase
APP	Acute phase protein
APR	Acute phase reaction
ARC	AIDS related complex
ART	Antiretroviral therapy
ARV	Antiretroviral drug(s)
AST	Aspartate transaminase
AZT	Azidothymidine or zidovudine
BD	Body density
BMI	Body Mass Index
BMR	Basal metabolic rate
CAM	Complimentary and/or alternative medicine
CD4	cluster of differentiation 4 (A cell surface marker)
CDC	Centres for Disease Control and Prevention
CI	Confidence interval
CRP	C-reactive protein
CTL	Cytotoxic T-lymphocytes
CVD	cardiovascular diseases
CWFS	Committee on World Food Security
DNA	Deoxy-ribonucleic acid
DR	Deming regression
EAER	Estimated average energy requirement
EDTA	Ethylenediaminetetraacetic acid
EMBASE	Excerpta Medica database
EuroSIDA	Europe-wide cohort of patients with HIV with or without AIDS
FAO	Food and Agriculture Organisation
FDA	Food and Drugs Administration
FDC	Follicular dendritic cell
FM	Fat mass
G-6-P-D	Glucose-6-phosphate-dehydrogenase
GGT	Gamma-glutamyl-transferase

HAART	Highly active antiretroviral therapy
HbSS	Sickle cell haemoglobin
HER	Human Immunodeficiency Virus Epidemiology Research
HIV	Human immunodeficiency virus
HTLV	Human T lymphotropic virus
ID	Iron deficiency
IDA	Iron deficiency anaemia
IL	Interleukin
LAV	Lymphadenopathy associated virus
LBM	Lean body mass
LDH	Lactic acid dehydrogenase
MAC	Mycobacterium avium complex
MCV	Mean corpuscular volume
MDG	Millennium Development Goals
MEDLINE	US National Library of Medicine database
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
(m)RNA	(messenger) ribonucleic acid
MTCT	Mother-to-child transmission
NAID	Nutritionally acquired immune deficiency
NCD	Non-communicable diseases
NNRTI	non-nucleoside or nucleotide reverse transcriptase
NRTI	nucleoside and nucleotide reverse transcriptase
NSI	non-syncitia-inducing
OI	Opportunistic infection(s)
OPHA	Ontario Public Health Association Food Security Work Group
PAL	Physical activity level
PCV	Packed cell volume
PI	Protease inhibitors
PLWHA	Person(s) living with HIV/AIDS
PLWHA	Person/people living with HIV/AIDS
PU for CHE	Potchefstroom University for Christian Higher Education
QFFQ	Quantitative food frequency questionnaire
QOL	Quality of life
RBC	Red blood cells
REE	Resting energy expenditure
RES	Reticulo-endothelial system
RNA	ribonucleic acid

RT	reverse transcriptase
SPSS	Statistical Package for Social Science
STI	supervised or structured treatment interruption or sexually transmitted infections
TB	Tuberculosis
TEE	Total energy expenditure
THUSA	Transition and Health during Urbanisation of South Africans
TIBC	Total iron binding capacity
TNF- $\alpha$	Tissue necrotic factor-alpha
UNAIDS	United Nations AIDS foundation
UNDP	United Nations Development Programme
UNICEF	United Nations children educational fund
UNU	United Nations University
US	United States
WFP	World Food Programme
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WIHS	Women's Interagency HIV Study
2, 3-DPG	2, 3-diphosphoglycerate

## CHAPTER ONE

### 1.0 GENERAL INTRODUCTION

The human immunodeficiency virus (HIV) is a slow acting retrovirus. Initial infection with HIV may only be associated with a mild illness, and it may take years to develop obvious manifestations of the life-threatening disease. The virus infects components of the human immune system such as CD4+ T cells, macrophages and dendritic cells. CD4 stands for cluster of differentiation 4, representing the glycoprotein expressed on the surface of helper T-cells (also called CD4+ cells), regulatory T cells, monocytes, macrophages, and dendritic cells. HIV can destroy CD4+ T cells directly as well as indirectly, but may lie relatively quiescent in macrophages for long periods with intermittent reactivation.

T cells are integral to a healthy immune system and impairment of their function increases susceptibility to secondary infection from viruses, bacteria, fungi or other parasites and reduces immune surveillance against cancer. Many of the problems faced by people infected with HIV result from failure of the immune system to protect from opportunistic infections and cancers. HIV may also attack tissues directly, such as the kidneys, the heart and the brain leading to morbidities such as renal failure, cardiomyopathy, dementia and encephalopathy. These direct effects of HIV on tissues account for some of the metabolic and physiological impairments associated with the infection, but the virus also has important effects on nutritional status, which lead directly to altered metabolic function. Together, the damaged immune system and the disordered metabolism increase the vulnerability of the infected person to life-threatening infection and ill-health. Progression of these pathologies leads to the condition known as acquired immune deficiency syndrome (AIDS).

HIV/AIDS is considered to have emerged from sub-Saharan Africa during the mid-twentieth century (Gao et al., 1999). Widespread awareness of HIV followed a brief report in 1981 of a rare pneumonia caused by *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) as well as other unusual infections in 5 young homosexual men in Los Angeles (Centres for Disease Control and Prevention (CDC), 1981; Curan et al. 1992; World Health Organisation (WHO), 1994). The realisation that a significant epidemic was developing grew as case reports mounted and similar immune deficiency syndromes were described in New York, California (CDC, 1981; CDC, 1982), and elsewhere among homosexual men, intravenous drug users, Haitians (CDC, 1982), haemophiliacs (CDC, 1982), recipients of blood transfusions, infants (CDC, 1982), female sexual partners of infected men (Masur et al., 1982; CDC, 1983), prisoners (CDC, 1983), and black Africans (Clumeck et al, 1983; Piot et al, 1984; Van de Perre et

al. 1984). As researchers began to describe the epidemiology and risk factors in a systematic way, many theories emerged regarding the cause of the 'mysterious' disease. An infectious agent was postulated, and, in 1983, scientists led by Luc Montagnier at the Pasteur Institute in France first described the virus that causes AIDS, lymphadenopathy-associated virus - LAV (Barre-Sinoussi et al., 1983). Confirmation came a year later from a team led by Robert Gallo of the United States, but they renamed it human T lymphotropic virus type III (HTLV-III) (Gallo et al., 1984; Levy et al., 1984). After much debate, the virus was renamed human immunodeficiency virus, or HIV (Coffin et al., 1986).

It is estimated that at the end of 2008 there were 40 million people living with HIV, and during the year there had been 3 million deaths from HIV with 5 million new cases (UNAIDS, 2008). In countries most heavily affected by HIV, such as in sub-Saharan Africa, life expectancy has been reduced by more than 20 years, economic growth slowed, and household poverty deepened (UNAIDS/WHO, 2008). In stricken countries, HIV may have caused a greater loss of productivity than any other disease, and is likely to push an additional 6 million households into poverty (UNAIDS/WHO, 2008). In this sense, the emergence of HIV/AIDS has inflicted the "single greatest reversal in human development" in modern history (UNDP, 2005).

HIV/AIDS has always been associated with the wasting syndrome, which remains a major manifestation of AIDS (Suttmann et al, 1995). However, wasting may not be a sufficient identification of poor nutrition, especially if micronutrient status is compromised. Nutrient status can be severely compromised in people with HIV/AIDS and low serum levels of a number of micronutrients have been reported and associated with disease progression (Beach et al. 1992; Baum et al, 1991; Baum et al, 1995; Tang and Smit, 1998; Friis and Michaelsen, 1998; Friis, 2005; Tang et al., 2005). However, some caution is required in interpreting serum levels in HIV/AIDS because of the effects of ongoing low-grade chronic inflammation (Thurnham and Northrop-Clewes, 2007).

The introduction and availability of potent antiretrovirals (ARV) for the treatment of HIV/AIDS has contained the condition to an extent. Attention has been directed to identifying factors that may be indicative of an increased risk of progression of clinical disease in both the short and the longer term. The main indicators appear to be measures related directly to the virus itself or its impact, viral load and CD4 count, or factors related to the altered metabolic function of the host, relative weight, anaemia or maybe plasma albumin concentration (Morcroft et al., 1999; Shah et al., 2007).

Anaemia has emerged as a potentially important prognostic factor and has frequently been associated with HIV infection and its progress, but the causal relationship remains unclear. The virus might have a direct effect on red cell precursors, but anaemia also commonly accompanies acute infection and low grade chronic inflammation, is often the result of poor nutritional status, might be related to medications given to treat the virus, or any combination of these and other factors.

With a rising trend in the prevalence of HIV/AIDS, there is a growing consensus that the pandemic could constitute an important cause of anaemia (Volberg, 2000). In those areas where anaemia is already a significant problem, HIV/AIDS could further aggravate this burden, but the relationship is neither simple nor clear and it is difficult to assess which interventions might be effective (Adetifa and Okomo, 2009). In sub-Saharan Africa, where the problems of both HIV/AIDS and anaemia have considerable impact and appear most intractable the evidence is least clear. Those who are most sexually active are young adults and this makes them most likely to be exposed to the risk of infection. They are also the most productive members of the workforce. Hence, HIV/AIDS impact directly on the production, availability, stability and access to food.

By reducing local or domestic food and nutrition security HIV will increase the likelihood of poor nutrition (Gillespie and Kadiyala, 2005). HIV and malnutrition will interact negatively to have an adverse effect on the immune system (ACC/SCN, 2001). In a cyclical relationship HIV increases susceptibility to secondary infections, thereby compromising nutritional status, whilst malnutrition itself weakens the immune system, thereby further exacerbating the effects of HIV (Semba and Tang, 1999, Calder and Jackson, 2000; Piwoz et al., 2004).

It can be concluded that, though several outcomes have been associated with HIV/AIDS, anaemia appears to be an important marker, or risk factor for disease progression and mortality in persons infected with the HIV or AIDS. This thesis explores the nature of the relationship between anaemia and HIV/AIDS in a population in South Africa as a secondary analysis of data from a cross-sectional study.

## **1.1 OUTLINE OF THE THESIS**

The thesis has been organised into eight (8) chapters. Chapter one, gives an overview of the research and describes the general framework used to explore the factors relating HIV infection to anaemia. This chapter also states the rationale, aims/ objectives and hypothesis, and outlines the framework for developing the hypothesis in the study. In Chapter 2, HIV/AIDS, anaemia and the nature of their interaction is

reviewed. There is an overview of HIV/AIDS, which includes a brief historical perspective, an outline of its pathophysiology, epidemiology and therapeutic approaches. The effects of HIV on nutrition and aspects of the complex interrelationships amongst HIV infection, immune processes, inflammation and nutrition are presented. The nature of anaemia, its causes, pathophysiology, and other characteristics are also outlined. Chapter three sets out the methodology employed for the study, with regards to study design, subject selection, data collection and the reason for selecting specific variables for secondary analyses in this thesis. It also includes a description of structure adopted in addressing the hypothesis. Of three results chapters, the first (chapter 4) presents and discusses the preliminary results consisting of the socio-demographic characteristics of the study population in relation to HIV infection and anaemia. Chapter 5 presents and discusses the results on prevalence of anaemia and highlights effects that may serve as possible explanatory factors of anaemia in the study population. Chapter 6 explores the putative interplay between the direct and the indirect effects of HIV on anaemia whilst elucidating significant predictive factors for anaemia in the study population. Chapter 7 provides a general discussion of the main findings in the light of previously published work in the area of HIV infection and anaemia. It also presents the limitations of the study, implications for public health, and recommendations for future work. In Chapter 8 the main conclusions from the study are stated.

## **1.2 HUMAN IMMUNO-DEFICIENCY VIRUS AND ACQUIRED IMMUNE DEFICIENCY SYNDROME**

As a slow acting virus that may take years to produce illness in its victim, HIV impairs the immune system reducing resistance and increasing susceptibility to infection with other viruses, bacteria, fungi or other parasites: so-called "opportunistic infections" (OI) that arise in the setting of immune impairment. Currently there is neither a cure for HIV/AIDS nor a vaccine to protect against infection.

For an HIV infected individual the lowering of CD4+ cell counts to < 200 cells/ $\mu$ L signifies the onset of AIDS, and greatly increased risk of OI. The CD4+ lymphocyte count in the blood is used to monitor the effect of HIV on the immune system. A CD4+ cell count between 600 and 1200 cells/ $\mu$ L is considered normal and indicates that the immune system has not undergone sufficient damage to put the individual at high risk for OI. Such individuals are unlikely to require specific treatment. A CD4+ count of <350 cells/ $\mu$ L indicates impairment of immune function and should prompt consideration for antiretroviral therapy. When CD4+ counts fall to <200 cells/ $\mu$ L the individual is at imminent risk of serious OI or other complication and prompt treatment

is recommended (Dybul et al., 2002). The time required for an HIV infected person to develop full blown AIDS depends on the strain and type of the virus as well as host factors that include age, co-infections, genetic factors, health and nutritional status before and during the infection (HIV/AIDS, 2004). Antiretroviral drugs directly attack the virus and significantly reduce its replication in the body, thus reducing the viral load and slowing down the progression of the disease. There are also therapies for the prevention, treatment or cure of many OI to minimise their effects or complications.

Untreated HIV infection is chronic and progressive with recognisable stages. Acute infection occurs when the virus is introduced into a person. This is followed by the early stage during which the infected person mounts a vigorous antibody reaction to the virus and therefore is considered as sero-converted. This progresses over time to the clinical latent HIV infection or asymptomatic phase, which is followed by the late stage or late symptomatic phase, when the individual can be said to have AIDS.

### **1.3 ANAEMIA**

The term anaemia derives from a Greek word meaning “without blood”, representing a situation in which the number of red blood cells (RBC) or erythrocytes in the circulation is less than usual, and hence associated with a reduction in the haemoglobin in blood and its capacity to carry oxygen to the tissues. On this basis anaemia has been variously defined; as a reduction in the number of circulating RBC or decreased haematocrit, insufficient amount of haemoglobin, or a reduction in the oxygen carrying capacity of the blood (Phillips and Groer, 2002). However, limits of normality for haemoglobin concentration [Hb] or haematocrit (Hct) have been set by convention, below which anaemia is defined (See Appendix A). These represent the usual range of values found in a healthy population at sea level, by age and gender (WHO/UNICEF/UNU, 2001). The distribution of the haemoglobin concentration in healthy, fully iron-replete subjects is 120-160 g/L in women and 140-180g/L in men (Wintrobe, 1981).

Anaemia is one of the most common and intractable nutritional problems with consequences for health, social and economic development (WHO/UNICEF/UNU, 2001). Close to 2 billion people in the world are anaemic and prevalence of anaemia is closely associated with poverty (Tomkins and Watson, 1989; WHO/UNICEF/UNU, 2001). Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and children in resource poor settings (WHO, 2002). It has been identified as the second most common cause of disability (Murray and Lopez, 1996). There are general, non specific symptoms

associated with milder degrees of anaemia, but the functional consequence of severe anaemia may be incapacitating.

Red cells are constantly being formed and degraded within the body and, at any point in time, the RBC mass represents a balance between these two rates. A reduced red cell mass must reflect an imbalance in the rates of production and degradation or destruction of RBC and/or blood loss.

There are a large number of factors which can contribute to the development of anaemia: those which act primarily on red cell production and those that exert their influence through altered red cell loss. Factors operating on red cell production would include poor diet and nutritional factors that limit the formation of haemoglobin or the production of red cells in adequate amounts. Nutritional factors might also act to increase red cell destruction, for example poor vitamin E status increases red cell fragility and susceptibility to oxidant damage, hence increasing the likelihood of haemolysis. Red cell production may be impaired by chronic disease, medications or other factors that impair marrow function; where RBC are produced. Red blood cell destruction or losses can be increased by genetic disorders such as sickle cell disease, or blood loss that may be associated with some gastrointestinal helminthic infections (e.g. hookworms) or menstruation. It is considered that dietary deficiencies, especially, iron deficiency, account for a major proportion of anaemia in many populations (Kraemer and Zimmermann, 2007).

Irrespective of the cause, anaemia is an important indicator of both poor nutrition and health. Even mild or moderate anaemia can alter the sense of well-being or the quality of life (QOL) of victims. When severe, the effect of anaemia can be dramatic. It increases the risk of child and maternal mortality, impairs growth and cognitive development in children and leads to low school performance; reduces physical capacity and work performance in adolescents and adults (Stoltzfus, 2001); and also decreases resistance to infections in affected persons.

It has been suggested that much anaemia may derive from the synergy between the inflammatory process and its effects on the bio-availability of nutrients such as iron. Thus, although iron deficiency anaemia (IDA) may be the most obvious or prevalent form of nutritional anaemia (WHO/UNICEF/UNU, 2001), it is increasingly recognised that iron deficiency might be a final common pathway both for prevalent infection and associated nutritional problems. Infections, together with the associated inflammation, interact with nutrient availability and hence may play an important role in the initiation

and perpetration of much anaemia (Thurnham and Northrop-Clewes, 2007). Frequent exposure to endemic infections will evoke a persistent inflammatory response, thereby limiting iron availability for RBC synthesis (Thurnham and Northrop-Clewes, 2007). Infections can also lower RBC mass and haemoglobin concentrations through direct suppression of bone marrow synthesis and haemolysis. Furthermore, infections may reduce dietary intake, impair nutrient absorption, increase nutrient utilization and increase losses of nutrients (Tomkins and Watson, 1989).

Infections such as malaria, helminthiasis, tuberculosis (TB) and, now increasingly, HIV/AIDS, are among the important factors that potentially contribute to a persistent high prevalence of anaemia in developing regions such as sub-Saharan Africa (Lynch, 2005; Kraemer and Zimmermann, 2007). These infectious conditions are not uncommon in the region of study and have been shown to contribute to the cause of anaemia, especially in pregnant women (Hoque et al., 2007). In humans malaria infection by *Plasmodium* species is associated with a reduction in haemoglobin levels, frequently leading to anaemia. Though the pathogenesis of malarial anaemia is not very clearly understood, important factors ascribed to its occurrence include disordering of RBC development, intravascular haemolysis of infected RBC, together with destruction of both parasitized and non-parasitized RBC by macrophages (Roberts et al., 2005). Gastrointestinal helminths such as Ascaris and hookworms contribute significantly to IDA through the feeding activities of intestinal stages leading to chronic blood loss into the gut. With TB and HIV/AIDS, associations with anaemia has been common and many different mechanisms for anaemia, including direct and indirect effects on the bone marrow and body metabolic processes, have been suggested.

The presence of concurrent poverty, unsanitary conditions and inadequate health care increases exposure and encourages the spread of infectious diseases which may add to the risk of developing anaemia (Kraemer and Zimmermann, 2007). The wave of rapid urbanisation sweeping across the developing world can contribute to anaemia through changes in nutrition or dietary patterns, associated with social, cultural, and economic changes intrinsic to the demographic transition (Vorster et al., 1999).

Despite the emphasis placed on anaemia as a widespread problem and the efforts to reduce it, prevalence levels remain unacceptably high (Kraemer and Zimmermann, 2007; WHO/UNICEF/UNU, 2001). This is probably because the cause is more complex than a simple dietary deficiency. Recognition that the aetiology of anaemia is often complex and multifactorial shifts the emphasis for prevention to a consideration of a wider range of potential causative factors that may be independent, reinforcing or

mutually additive. These factors may vary depending on prevailing circumstances and population characteristics. Thus to identify effective interventions to correct anaemia it is necessary to have a clear understanding of the individual causative factors and the likely complexity of their interplay. This will then indicate the required mix of interventions, which may vary both absolutely and relative to each other depending on the circumstance. The need to effectively address the anaemia related problems remains urgent, and these efforts are implicit to achieving many of the targets stipulated by the global Millennium Development Goals (MDG), of the United Nations (United Nations, 2007).

#### **1.4 HIV/AIDS AND ANAEMIA**

Anaemia is one of the more important objective measures that mark risk of clinical progression in HIV/AIDS (Volberding, 2002). This is true for early as well as late disease, and seems to hold even if the anaemia improves subsequent to therapy with ARV (Volberding et al., 2004). Anaemia in HIV/AIDS is also associated with increased mortality or decreased survival (Sullivan et al., 1998; Moore et al., 1998; Lundgren and Morcroft, 2003; O'Brien et al., 2005), which may be linked to a reduction in functional capacity and QOL experienced by most persons living with HIV/AIDS (PLWHA) (Abrams et al., 2000). Low haemoglobin levels have been associated with immunosuppression (Kreuzer and Rockstroh, 1997).

In HIV infection, anaemia has been identified as a prognostic marker of disease progression or early death, independent of CD4+ T cells and viral load (Sullivan et al., 1998; Morcroft et al., 1999; Moyle, 2002). Anaemia remains a statistically significant marker of disease progression even in the presence of therapy with ARV, and seems to hold even if anaemia improves subsequent to therapy (Morcroft et al., 1999; Sullivan et al., 1998), regardless of CD4+ cell count, clinical AIDS, age, and the presence of other cell line depletions (Sullivan et al., 1998; Sullivan and Buskin, 2003).

Though infection with HIV has been primarily characterised by a depletion of CD4+ T lymphocytes, other cell line depletions including that of erythrocytes have been recognised (Volberding et al, 2004). Clinically, erythrocyte depletion characterised as anaemia is the most frequent manifestation, though thrombocytopaenia (platelet depletion) and complications of other bone marrow elements are also important (Hoxie, 1995; Davis and Zauli, 1995; Costello, 1997). These changes have generally been ascribed to impairment of the haematopoietic process, as well as to immune-mediated cytopaenias and altered coagulation mechanisms (Claster, 2002; Costello, 1997).

There are several epidemiological studies that suggest that the occurrence of anaemia tends to increase HIV disease progression and risk of death in PLWHA (Graham et al., 1993; Sullivan et al., 1998; Mocroft et al., 1999; Semba et al., 2002; Belperio and Rhew, 2004), and even changes in haemoglobin that might be described as modest (about 1 g/dL) have been associated with a significant increased risk of death (Moyle, 2002). Other epidemiological studies also suggest that anaemia in HIV/AIDS is associated with an increased progression to dementia (McArthur et al., 1993), a decreased survival or increased mortality (Moore, 1999; Semba et al., 2002). However, some intervention studies strongly suggest that reversing anaemia can prolong survival by slowing down disease progression (Graham et al., 1993; Sullivan et al., 1998; Moore et al., 1998; Moore, 1999). Anaemia in HIV/AIDS may therefore have implications for the management of the disease, which could, by and large, reflect on the health of the sufferer and by extension affect development and progress in the entire population.

The signs and symptoms of anaemia may be more evident in the later stages of HIV/AIDS (Davis and Zauli, 1995), but they may be an early feature of the disease (Fleming, 1989; Street and Milliken, 1993), or may mark the onset of infection (Snopková et al., 2005). Prevalence and severity of anaemia tends to increase with progression of the disease; being lower and often milder in the asymptomatic stage and higher and severer in clinical AIDS (Sullivan et al., 1998; Bain, 1999), with prevalence levels reaching 95% in some populations (Volberding et al., 2004). The incidence and symptoms of anaemia may have been reduced with highly active antiretroviral therapy (HAART) (Moore, 1999b; Moore and Forney, 2002), but it remains a serious problem in PLWHA. Nevertheless, its importance in the HIV infected population remains unexplained and, more often than not, anaemia goes undiagnosed in spite of its obvious clinical implications.

## **1.5 CAUSES OF ANAEMIA IN HIV/AIDS**

The causes of anaemia in HIV/AIDS remain uncertain, but studies suggest its multifactorial nature (Claster, 2002; Volberding et al., 2004) with certain population characteristics playing an important role in its determination (Volberding and Sullivan, 2002). Potential causes of anaemia in HIV disease include direct HIV infection of hematopoietic stem cells/erythroid progenitors, immune system-mediated haemolysis, neoplasms, aplastic anaemia, blood loss, opportunistic bone marrow infections as well as deficiency of erythropoietin (Kreuzer and Rockstroh, 1997). Nutrient deficiencies such as vitamin B<sub>12</sub>, folate, and iron also contribute to the development of anaemia, though it may be to a much lesser extent in infected persons living in developed

countries (Moyle, 2002), whilst medications used to treat the infection or disease or associated co-morbidities can trigger anaemia (Volberding, 2000; Volberding et al., 2004).

Perhaps one of the main reasons why anaemia is very common and frequent in the HIV population is because its plethora of causes include the direct effects of the virus, which has the ability to potentiate or interplay with other anaemia inducing factors. HIV infection alone without any concurrent medications or co-morbidities or co-infections can cause anaemia (Harbol et al., 1994; Claster, 2002). A number of studies have shown that HIV can directly impair the survival or proliferative capacity of purified haematopoietic progenitor cells (Mitsuyasu, 1994; Davis and Zauli, 1995; Redd et al., 2007).

Whilst some of the factors that interplay with HIV in the cause of anaemia may be associated with the disease, others may remain as stressors within the population. Thus, the spectrum and nature of the factors causing anaemia in HIV/AIDS may be broad but, nevertheless, often manifest as changes in the metabolic and immunologic integrity of the sufferer.

The chronic inflammatory nature of HIV/AIDS means a persistent immune inflammatory response in the body, with attendant surges in circulating cytokines and changes in inflammatory bio-markers (Janeway et al., 1999; Volberding, 2000; Claster, 2002). Opportunistic infections and other stressors associated with HIV infection can also induce inflammation. With persistent inflammation there is a tendency to maintain a continuous hypoferaemia with a concomitant suppression of erythropoiesis (Thurnham and Northrop-Clewes, 2007) and an increased release of inflammatory cytokines has a negative effect on erythropoietin levels and/or response, leading to erythropoietic failure (Hambleton, 1996).

Poor energy and nutrient intakes have always been recognised as part of the HIV infection due to changes in metabolic responses, especially as the infection progresses to AIDS (Bain, 1999). The common manifestations of weight loss, altered nutrient profiles, specific micronutrient depletions, and fat redistribution together with altered endocrine function and depleted haemopoietic cell lines, reflect the body's attempts to cope with the persistent effects of the virus and associated co-infections. These manifestations essentially describe the consequence of inadequate food intake over extended periods of time, which fits the processes associated with reductive adaptation (Ashworth, 2001). Thus, the consequences of reductive adaptation may be crucial in

restricting RBC production, as it is usually associated with the creation of an environment that tends to shorten the lifespan of RBC (Jackson, 2007; Barroso, 1999).

In HIV infection multiple nutritional deficiencies may be common, amongst which iron (Fe), riboflavin (B<sub>2</sub>), folate and cyanocobalamin (B<sub>12</sub>) deficiencies may contribute to the reduction of haemoglobin synthesis (Volberding et al., 2004). Other micronutrients like vitamins A, thiamin (B<sub>1</sub>), niacin (B<sub>3</sub>) and pyridoxine (B<sub>6</sub>) have also been implicated in limiting the production of RBC. Such deficiencies may result from dietary inadequacies, gastrointestinal pathology, malabsorption, altered metabolic handling or some combination (Friis and Michaelsen, 1998; Tang and Smit, 1998; Friis, 2005).

Other factors that may contribute to anaemia in HIV/AIDS include the presence of concurrent and endemic infections such as malaria, genetic disorders and other population characteristics, as well as the use of antiretroviral, antifungal or antibacterial agents (Hambleton, 1996; Groopman, 1998), more especially, those that have been noted to be myelosuppressive (Levine, 1999).

Whether anaemia in HIV infection can be ascribed mainly to any specific nutrient, or to a reduced energy demand stemming from an altered body composition or to the presence of infection or inflammation or to a combination of these, is an important question that remains to be answered.

## **1.6 SIGNIFICANCE OF ANAEMIA IN HIV/AIDS**

In HIV infection, underlying nutrient and energy imbalances may often manifest as a reduction in red cell mass (anaemia) and/or a reduction in lean body mass and/or a change in other physiological processes (Jackson, 2007). The appearance of hypoferaemia may signify changes in the distribution of iron in response to the inflammatory process or changes associated with other nutrients. These changes may be considered as adaptations, which help conserve energy and nutrients, or protect against pathogenic agents. It remains unclear the extent to which anaemia is reflective of other underlying processes or is in itself a direct contributor to the pathology of HIV. Although this is not the main thrust of this study, an important question that remains unanswered is "what is the real significance of anaemia in HIV infection?". Could anaemia in the HIV/AIDS population be a marker of the processes underlying the infection? Alternatively, could it be protective or contribute directly to survival? In other words, is there any advantage in being anaemic in HIV infection?

In many poor-settings anaemia will continue as an important associate of HIV infection even with ART since the many factors that are responsible for its cause are combined,

interwoven and rife. With improvements and scaling up of ART especially in developing countries the relative causes of anaemia in HIV infection may change. Lives of infected persons would be prolonged and maintaining QOL would become of utmost importance.

Clearly, the reduction or treatment of anaemia, especially, in HIV/AIDS and other pathological conditions calls for a clearer understanding of the underlying interactive processes in order to facilitate the formulation and initiation of appropriate policies and interventions leading to a resolution or alleviation of the problem. An assessment of the incidence or prevalence and determinants as well as the impact of anaemia in this population would be beneficial, serving as a basis for choosing preferred approaches for managing the disease.

### **1.7 PROBLEM STATEMENT AND RATIONALE FOR THE STUDY**

Anaemia is a substantial public health problem, especially in poor-settings, where it adds to the woes of children and women of child bearing age. In sub-Saharan Africa, where the prevalence of HIV/AIDS and malnutrition are high, anaemia is common adding to the considerable burden of morbidity and mortality. The problem of anaemia in many resource-poor settings is made worse by the fact that population characteristics such as endemic genetic disorders and the prevalence of infections like malaria and helminthiasis may add to the burden of anaemia.

Anaemia in HIV infection can be the product of a number of interactive causes or factors, and it has been difficult to determine the sequence or order of their impact in terms of causality. However, it seems clear that the virus can have a direct effect which may alter the balance between red cell production and destruction, leading thereby to anaemia. It is possible that HIV/AIDS is a major causative factor in the development of anaemia and that this anaemia is indicative of the likely severity or progression of the disorder.

However, given the susceptibility to infection and nutrient deficiency, for any HIV infected population, there is also greater likelihood for an interplay with other factors which could in themselves cause or exacerbate anaemia. It is for these reasons that this study postulates that HIV infected persons are more likely to be anaemic in comparison with uninfected persons and that those with a greater number or interacting combination of factors are more likely to be anaemic, or have anaemia of greater severity. In other words infection with HIV can increase both the frequency and degree of anaemia in infected persons.

In a population such as the one under study, where there could be distinctive anaemia causing factors, establishing HIV/AIDS as an important factor related to anaemia would be an added step towards helping in reducing the burden of both public health problems. In fact, within the region of study another important factor that could be contributing to the prevalence or incidence of anaemia is the process of rapid urbanisation occurring in the African population. The tendency for this sweeping transformation to push the incidence and prevalence of HIV infection into prominence and to provoke other important health transformations gave the impetus for the *Transition and Health during Urbanisation of South Africans (THUSA)* survey (see details in section 3.2). The high prevalence of HIV infection in this population (South African Department of Health Survey, 2007), together with the presence of factors that could be considered as predisposing to anaemia (Vorster et al, 2000), such as poor dietary practices and other infectious conditions, were indicators that influenced the use of the THUSA data as a source for the secondary analysis in this research.

Much as anaemia is recognised as an important clinical manifestation, its real significance especially in HIV infection remains enigmatic and therefore a matter for more research and discussion. The focus of treatment and care for PLWHA may have to change towards preventing or treating anaemia to maintain QOL and to enhance survival. Although the management of HIV/AIDS has improved with the use of potent ARVs and other therapies, anaemia and poor QOL persist. Owing to the impact of anaemia on the QOL of HIV infected persons, research on anaemia is beginning to receive increased attention, more so when evidence shows that recovery from anaemia can improve the QOL of HIV patients (Moore et al., 1998; Sullivan et al., 1998).

Despite the multifactorial causation of anaemia in HIV/AIDS, the direct role of the virus in the development of anaemia as well as its anaemia potentiating capabilities raises important considerations. It remains important to recognise the other processes that may be involved and the potential for multiple, complex interactions. Thus, although HIV infected persons may be intrinsically more susceptible to anaemia in comparison with non-infected persons, there is the possibility that the interacting effects causing anaemia have special potency in HIV, varying between and within population groups, and with the stage of infection. It may be that specific effects or combination of effects may be more predisposing to anaemia than others. These possibilities present a basis for a study for exploring the interaction of important factors on the risk for anaemia in the HIV population. The identification of new relationships could be useful in the development of novel strategies for both anaemia and HIV infection. It is on this basis that the objectives of the present study were developed.

## 1.8 STUDY HYPOTHESIS

The apriori hypothesis for the study postulates that, infection with HIV puts individuals at a significantly increased risk for becoming anaemic because of the direct effects of the virus and other additional and interactive indirect effects. Thus, for HIV infected persons either the factors predisposing to anaemia are greater than in uninfected persons or the many factors add up or significantly interplay to predispose infected persons to anaemia.

The statement of the main and the null hypotheses for this study are:

$H_1$ : HIV infected persons are more anaemic in comparison with uninfected persons.

$H_0$ : HIV infected persons are as likely to become anaemic as uninfected persons.

The exposures and outcomes that were considered for addressing the main hypothesis included the following:

### Exposures

- HIV status (main)
- Anthropometry (weight, height, body mass index (BMI), lean body mass (LBM), lean mass index (LMI) and skin fold thicknesses)
- Energy intake (Plus and minus alcohol as component)
- Dietary micronutrients (Vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, B<sub>12</sub>, C, E, zinc, iron and copper)
- Serum micronutrients (Vitamins A and E)
- Inflammatory markers:
  - Acute phase proteins (APP) (serum total proteins, serum albumin, serum 'globulins', serum ferritin and plasma fibrinogen)
  - Liver enzymes/by-product (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and bilirubin (Total and Direct))

### Outcome

- Anaemia (Defined by [Hb], haematocrit (Hct), serum iron, serum ferritin, total iron binding capacity (TIBC) and % transferrin saturation)

## 1.9 THE AIMS AND OBJECTIVES OF THE STUDY

The essence of this research was to elucidate the risks that HIV infection may pose to the development of anaemia in a South African population whose exposure to HIV infection and other primary factors that cause anaemia may be considered as high. In this study, HIV infection is considered a primary exposure together with other factors that may or may not be associated with the infection, such as changes in body

composition, poor dietary intake of energy or nutrients, as well as metabolic and inflammatory changes.

The findings of this work might serve as a basis in later research for developing a scheme for identifying the interactive effects associated with anaemia in HIV infected persons at higher risk for developing anaemia. Perhaps the significance of anaemia in the asymptomatic or early stage of HIV infection might be highlighted. Furthermore, identifying factors that determine anaemia in the population could be important for assessing the state or progress of HIV disease and also for predicting the survival and QOL outcomes of PLWHA later on.

The following objectives were set out to enable the exploration of the data set in order to achieve the purpose of the study.

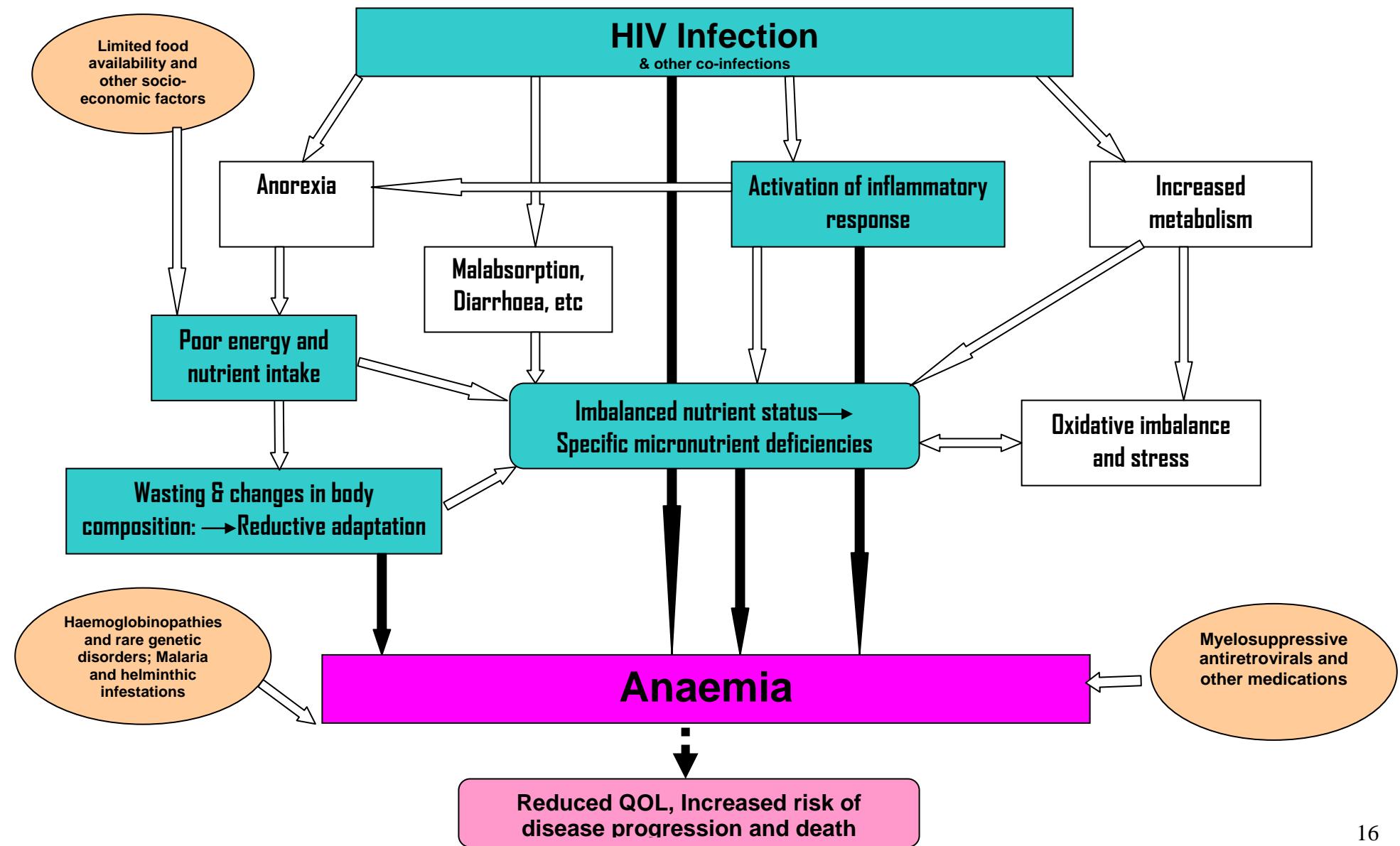
- To characterise HIV sero-status in the study population
- To identify socio-demographic indicators of the study population that may relate to HIV status and anaemia
- To characterise anaemia and relate it independently with HIV status in the study population
- To explore changes in body composition, dietary energy and nutrient imbalances and metabolic and inflammatory alterations as possible explanatory factors for anaemia in the study population
- To explore for factors that may interplay with HIV infection and may predict anaemia in the study population.

## **1.10 THE FRAMEWORK FOR THE CAUSALITY OF ANAEMIA IN THE STUDY POPULATION**

The proposed framework for this study, as depicted in figure 1.1 below, assumes a complex interaction between the factors responsible for anaemia in HIV infection. The pathways show that the main exposure of HIV infection could have both a direct and indirect effect in upsetting the balance between red cell production and destruction and to precipitate anaemia.

Within the context of this study, HIV status would be measured by the sero-status of the subjects. However, levels of infectivity or degree of severity of infection as measured by HIV titres in the blood or CD4+ cell counts would probably have been more appropriate in accounting for the effect of the exposure on anaemia.

Figure 1.1: Framework for the relationship between HIV infection and anaemia



Nevertheless, it is strongly contended that the mere presence of the virus, because of its direct involvement, can make the distinction between anaemia in infected and uninfected persons irrespective of the stage of infection.

Another consideration in the framework is the fact that a poor or inadequate energy and nutrient intake, which could be triggered either by a limited availability of food or the effects of underlying HIV and other OI on the gastrointestinal tract, can lead to anaemia in infected persons. In most infections, a reduction in energy and nutrient intake, which can be associated with anorexia and other physiological alterations often induced by the infection and, often ascribable to the inflammatory process, may occur. In most poor- settings , social considerations such as stigma and isolation can be very crucial in limiting food intake. A poor or inadequate energy and micronutrient intake, especially if prolonged, can lead to changes in body composition and wasting or weight loss, which may trigger a physiological adaptation by the body in the presence of the reduced energy – reductive adaptation. Part of the reductive adaptational process is associated with an increased destruction of RBC as well as production of fewer and poorly formed cells, which may underlie the anaemia in this case.

In this study energy and micronutrient intakes have been assessed and levels of their adequacies or inadequacies estimated. In addition, body compositional measures such as BMI, LMI and skin fold thicknesses have been used to assess reductive adaptation. However, considering the asymptomatic or 'apparently healthy, disposition of the sero-positive subjects, marked changes in their energy and nutrient intakes as well as their body compositions would not be expected to have occurred.

In HIV infection as in many others, alterations in metabolic response may result in an increased utilisation and loss of nutrients, especially micronutrients, as they are required in the intermediary metabolic process and are often eliminated with other metabolic by-products or waste as part of the excretory process. The physiological effects of HIV infection also include malabsorption and diarrhoea, which are concomitant with nutrient losses. With time, nutrient imbalances may be created as a result of these combined effects and may eventually lead to specific nutrient deficiencies and oxidative stress which, by and large, have a tremendous effect of lowering RBC production.

As the body's integrity is challenged by the infective agent, a vigorous defence is invoked usually with an increase in inflammatory cytokines, such as tissue necrotic factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), concomitant with an increase in the

body's metabolic expenditure. The liver and other members of the reticulo-endothelial system (RES) are activated and a large amount of energy and nutrients are expended. The increased protein and nutrient turnover at this stage compromises body composition and, additionally, the resulting alteration in nutrient metabolism may lead to the development of specific nutrient deficiencies which may precipitate an oxidative imbalance and stress.

The chronic inflammatory nature of HIV infection is likely to cause the release and maintenance of a persistence surge of the TNF- $\alpha$ , interleukin-1 (IL-1) and IL-6), more so when destruction of the immune system allows OI to set in. A hyper-inflammatory state, with an attendant increase in protein turnover, results in alterations in the metabolism of acute phase proteins (APPs) as well as the maintenance of a hypoferaemic state and reductions in many specific nutrients in the body that are critical for RBC production. Levels of some APP increase (positive APP) e.g. C-reactive protein (CRP) and ferritin, whilst levels of others tend to decrease (negative APP) e.g. ceruloplasmin and albumin. Because the negative APP are often carriers of micronutrients their declining levels during the APR tends to lead to a reduction in plasma or serum levels of micronutrient.

The measurement of cytokines are more likely to express the immune response but for some reasons this is cumbersome coupled with the fact that cytokines have very short half-lives. Thus, APP, are often used as proxy markers to reflect these inflammatory responses. The APPs CRP and  $\alpha$ -1-acid glycoprotein (AGP) in particular, are used very frequently to measure acute and chronic inflammation respectively, but in this study ferritin, total proteins, albumin and plasma fibrinogen, which are equally important proxy markers, have been used instead. In addition the study also relies on measurements of liver enzymes as closely depicting inflammatory response from the liver.

Within the causal inter-relationships between HIV infection and anaemia it is also suggested that factors that may not necessarily be directly ascribed to HIV but may be contributory to the development of anaemia in the population are recognisable. For instance, a limited availability of food, which is most likely to occur in any population whose prevailing socio-economic and environmental circumstances are such that they affect food security, can also induce a poor energy and nutrient intake. The fact is that certain social or politico-geographic changes, such as what may pertain in war-torn regions can have tremendous repercussions on food supply and availability. An imposition of physical restrictions or barriers on PLWHA either by upholding societal

norms or by the debilitating effect of the disease could limit their physical activity and therefore their functionality, which could subsequently lead to a reduction in their food intake.

In populations where infectious diseases such as malaria and helminthiasis are common, susceptibility to anaemia can be quite high. Furthermore, the endemicity of certain specific population characteristics like race, as well as hereditary traits, which include haemoglobinopathies (e.g. sickle cell disease) and other genetic disorders (e.g. glucose-6-phosphate dehydrogenase (G-6-PD) deficiency) as well as the use of myelosuppressive anti-microbial medications can account for the high prevalence of anaemia in some populations. Unfortunately factors that would be classified as not related to HIV infection but likely to contribute to the overall burden of anaemia in the study population, as suggested above, have not been assessed. Although some of these factors may exist in the population, not being able to account for them may be considered as a limitation of this study.

Notwithstanding the complexity in the array of causal effects, it is highly possible that a combined effect of infection with the virus and/or other co-infections, its associated nutritional, metabolic as well as inflammatory effects and other stresses related to these, would constitute a strong combination that could significantly determine the frequency and severity of anaemia in the HIV population. The outcome of this, if proven, could undoubtedly place HIV infection as an important cause of anaemia.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 INTRODUCTION – HIV/AIDS AND ANAEMIA

Advances in antiretroviral therapy (ART) have enabled people infected with HIV to live longer, making it increasingly important to understand the nature of the inter-relationship between HIV/AIDS and anaemia because of the impact that anaemia has on morbidity and mortality.

Estimates of the prevalence of anaemia in HIV/AIDS have varied from 1.6% to 95% depending on the setting (Sullivan et al., 1998; Belperio and Rhew, 2004). Anaemia occurs in all stages of HIV/AIDS but prevalence tends to rise with disease progression (Abrams et al., 2000; Belperio and Rhew, 2004). Thus, 66% to 85% of infected persons who have progressed to AIDS may have some form of anaemia (Ganser, 1988, Hambleton, 1996). Anaemia has not usually been considered to be a noteworthy complication of HIV infection, but it does appear to have significant impact both on QOL and on clinical outcome (Cella, 1996; Breitbart et al., 1998; Barroso, 1999; Abrams et al., 2000; Ludwig and Strasser, 2001; Belperio and Rhew, 2004).

Several million people worldwide are infected with HIV (UNAIDS, 2006; 2008) and anaemia is a common manifestation of HIV/AIDS (Claster, 2002; Mildvan, 2003). Thus, a large percentage of those infected with the HIV will have anaemia. Anaemia is a common problem throughout the world in its own right, especially in those developing countries where HIV is most prevalent. Hence, in these locations the numbers with HIV, or anaemia, or both HIV and anaemia will be high (Lewis, 2005). However, because there are multiple causes for anaemia it has been difficult to determine the relative contribution of HIV. This needs to be adequately defined for populations in sub-Saharan Africa in order to develop evidence informed approaches to individual clinical and population care.

Iron deficiency anaemia is one of the most common causes of anaemia worldwide (see below), but there is increasing concern about the uncritical use of iron supplements in infected individuals, in those with ongoing inflammation, or in populations where the risks of intercurrent infection might be high. Therefore, to determine our current understanding of the problem the available literature was reviewed, drawing on any available systematic reviews. The purpose was to assess the extent to which the aetiology of anaemia in HIV could be determined for populations living in sub-Saharan Africa.

## **2.2 PREVALENCE AND RISK FACTORS FOR ANAEMIA IN HIV/AIDS**

### **2.2.1 A SYSTEMATIC REVIEW OF THE PREVALENCE OF ANAEMIA IN HIV/AIDS -**

Belperio and Rhew (2004) carried out a systematic review of the literature to determine the prevalence of anaemia and its impact on the quality of life outcomes in HIV infection. The review focused primarily on anaemia of chronic disease, but did not exclude articles dealing with iron-deficiency anaemia.

The authors searched the United States (US) National Library of Medicine database (MEDLINE) and the Excerpta Medica database (EMBASE) for published articles on the prevalence and impact of anaemia in persons with HIV/AIDS. The initial MEDLINE search covered January 1966 to March 2001, while the EMBASE search included literature from January 1974 to March 2001. The search excluded non-English language and nonhuman studies, as well as editorials and abstracts. An AIDSLINE-Internet Grateful Med search (January 2000 to March 2001) was also performed.

Pertinent articles were selected by a predefined three stage process: title review, abstract review, and article review. Investigators who were trained in the method of assessment worked in pairs using defined criteria to identify and select suitable publications. Hand searches of bibliographies from accepted articles enabling identification of a limited number of additional articles were also conducted. Accepted articles were classified into 2 categories: (1) the prevalence of anaemia in patients with HIV or AIDS and (2) the effect of anaemia on the outcomes of HIV/AIDS. They found that prevalence estimates varied widely. This variation could be related to a range of factors, such as progression of HIV disease, definition of anaemia, use of antiviral medications (particularly zidovudine), location of the study (e.g., United States vs. sub-Saharan Africa), sex, pregnancy, injection-drug use, and age.

This search was repeated to include articles published up to July 2009 to determine whether more recent reports on anaemia in the HIV population support the findings of Belperio and Rhew (2004). A similar search strategy was adopted to ensure that the findings were comparable. In all a further 11 relevant papers were identified (see asterisked references in tables). For the purpose of this thesis, the results of the prevalence of anaemia in HIV/AIDS are considered.

#### **2.2.1.1 Results from the systematic review**

There were 42 articles (31 from Belperio and Rhew (2004) and 11 other authors) that reported either the incidence or prevalence of anaemia in HIV-infected patient populations (Tables 2.1 - 2.4). The incidence or prevalence was related to HIV disease

severity and specific subpopulations of infected individuals, including pre-menopausal women (plus pregnant women), injection-drug users, and children.

In the first consideration (Tabs 2.1a, 2.1b & 2.1c), prevalence of anaemia was categorised by haemoglobin thresholds for 3 separate populations: 1) hospitalised HIV-infected patients and/or patients with AIDS, 2) HIV-infected patients with and without AIDS, and 3) patients with AIDS-related complex (ARC) or HIV without AIDS. The definition of anaemia varied from study to study. Taking all these variables into account the prevalence of anaemia was consistently higher in people with AIDS than in those without AIDS. As the severity of HIV disease progressed, the prevalence of anaemia also increased.

There were 19 studies from populations in US or Europe, 4 from Africa and 1 from Asia that described the prevalence of anaemia in populations of HIV-infected patients at various stages of HIV infection (Tabs. 2.1ab&c). In most of the studies prevalence of anaemia was determined after patients were stratified on the basis of the presence or absence of AIDS. Whilst some studies were retrospective analyses of surveillance registries, others were prospective longitudinal studies. Despite the differing cut points for defining anaemia (e.g. Hb <123g/L for both sexes (Moore et al., 1991); <134 g/l men, <123 g/L – women (Patton, 1999); 80-140 g/L – men, 80-120 g/L – women (Mocroft et al., 1999)), the results clearly showed that the prevalence of anaemia was greater in patients with AIDS and those with lower CD4+ cell counts.

In three of the largest studies (Sullivan et al., 1998; Mocroft et al., 1999; Moore et al., 1991; 1992), evidence of the increasing prevalence of anaemia with HIV severity was consistently demonstrated. In the multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project conducted by the CDC, haemoglobin concentrations were abstracted from medical reviews of 31,534 patients between January 1990 and August 1996 (Sullivan et al., 1998). The prevalence of anaemia varied greatly with the stage of HIV disease. The 1-year incidence of anaemia (defined as a haemoglobin concentration <100 g/L for adults) was 3.2% in the 6,094 patients with HIV but not AIDS; 12.1% in the 2,579 patients with CD4+ cell counts of <200 x 10<sup>6</sup>/L but with no clinical symptom of AIDS; and 36.9% for the 4,642 patients with clinical AIDS. In all, 22% of the anaemia cases were associated with intravenous drug use.

In the EuroSIDA [Europe-wide cohort of patients with HIV with or without AIDS] study of a large observational, prospective cohort of European patients with HIV infection, 6,725 patients with CD4+ lymphocyte counts and haemoglobin values were evaluated

for the presence of anaemia (Morcroft et al., 1999). Patients with anaemia were more likely to have been diagnosed with AIDS in the 6 months before recruitment into the study, and patients with severe anaemia were significantly more likely to have CD4+ cell counts  $<50 \times 10^6/L$ .

The third study (Moore et al., 1991; 1992) involved a longitudinal study of anaemia in patients with HIV at 12 academic and community based sites across the United States. Between April 1987 and April 1988, zidovudine treatment was initiated in 866 patients with AIDS or ARC who had CD4+ cell counts  $<250 \times 10^6/L$  and were enrolled in the study. The prevalence of mild anaemia in patients with AIDS at enrolment was 69%, and the prevalence of mild anaemia in patients with ARC was 49%. During the first 2 years of follow-up, serious anaemia developed in 45% of patients with a baseline CD4+ count  $<100 \times 10^6/L$  and in 31% of patients with a CD4+ count  $>100 \times 10^6/L$ . The studies demonstrated an increasing prevalence of anaemia as HIV disease progresses, often in association with the use of zidovudine.

In a study in the USA by Mildvan and Creagh (2007), prevalence was higher in blacks or persons of African descent compared to whites. Findings from two of the African studies (Ssali et al., 2006; Omoregie et al., 2009) also suggested that anaemia prevalence tended to be very high in the African population.

Consistent with the results in the systematic review, earlier review studies (Zon and Groopman, 1987; Spivak et al., 1989) have reported similar trends in anaemia prevalence in other HIV/AIDS populations.

In summary, the prevalence of anaemia varied widely across studies, depending on the degree of anaemia and stage of disease, but generally, in spite of the differing cut points for defining anaemia, the prevalence of anaemia was shown to increase with progression and severity of the HIV disease.

In the second consideration, the prevalence of anaemia has been evaluated in women with HIV (Tab. 2.2). Anaemia is usually more common in women than men as it is associated with an increased demand for menstruation and pregnancy. Nine (9) relevant studies were identified (see Tab. 2.2). Of 7 studies in which anaemia was evaluated in pregnant women, 5 were conducted in Africa, 1 in Europe and 1 in Asia. In the 5 African studies, definition of anaemia was according to or close to the WHO criteria, and prevalence estimates were quite high and closely similar; 82.7%, 81.7%, 78.4%, 82.8% and 76.9% respectively (Antelman et al., 2000; Meda et al., 1998;

Ramon et al., 1999, Megan et al., 2005; Omoregie et al., 2007). In 4 studies both HIV-positive and HIV-negative pregnant women were evaluated and compared and a statistically significant higher prevalence of anaemia was found in the HIV-positives. In contrast, 2 studies conducted in Europe and USA respectively (Bucceri et al., 1997; Semba et al., 2002; Levine et al., 2001) estimated anaemia prevalences were much lower in both infected and uninfected women.

The European study was from Italy, in which 151 early-stage, HIV-positive, pregnant injection-drug users were investigated, and showed that 27% patients were anaemic compared with 15% HIV-negative, pregnant injection-drug users (Bucceri et al., 1997).

In the two US studies, anaemia was evaluated in populations of HIV-infected women (Semba et al., 2002b; Levine et al., 2001). The first study, the Human Immunodeficiency Virus Epidemiology Research (HER) Study, enrolled patients from Baltimore, Maryland; the Bronx, New York; Providence, Rhode Island; and Detroit, Michigan, and examined the prevalence and cumulative incidence of anaemia in 797 HIV-positive and 389 HIV-negative women. The median CD4+ cell count of the HIV-positive women was  $436 \pm 272 \times 10^6/\text{L}$ . The study established that anaemia was higher in the HIV-positive cohort than in the HIV-negative cohort (28.1% vs. 15.1%,  $p<0.001$ ). The prevalence of moderately severe anaemia (haemoglobin level  $<100 \text{ g/L}$ ) was 5.4% in HIV-positive women and 2.3% in HIV-negative women. Estimates of anaemia prevalence by CD4+ cell counts were 54% for  $>500 \times 10^6/\text{L}$ , 69% for 200 to  $500 \times 10^6/\text{L}$ , and 79% for  $<200 \times 10^6/\text{L}$ , demonstrating the increasing prevalence of anaemia with HIV disease progression in women infected with HIV. The second study was the Women's Interagency HIV Study (WIHS), in which the prevalence of anaemia was 37% in 2,056 HIV-positive women compared with 17% in 569 HIV-negative women. The prevalence of severe anaemia was significantly greater in the HIV-positive than in the HIV-negative women (7.2% vs. 2%,  $p<0.001$ ). Anaemia was more common in HIV-infected African American women than in HIV-infected white women (44.9% vs. 25.7%,  $p<0.001$ ).

From these studies it can be concluded that though there is a higher prevalence of anaemia in HIV-infected pregnant and non-pregnant women, it tends to increase with disease progression and also to be much higher in African populations.

**Table 2.1a: Prevalence of Anaemia in Patients with Human Immunodeficiency Virus (HIV) in Various Stages of Disease**

Study	Location	Population Measured	Use of zidovudine (%)	Definition of Anemia (hemoglobin, g/L)	Prevalence Estimates of anaemia	
					Subgroup (if applicable)	%
Moore et al. (1991)	USA	146 hospitalized patients with an AIDS diagnosis or CD4 <sup>+</sup> count of ≤200/cells x 10 <sup>6</sup> /L	16*	<123 (both sexes)		85
Patton (1999)	USA	516 HIV-infected adults, all stages of disease, in a longitudinal study of oral disease received medical care at a university hospital	42.6	Men: <134 Women: <123	All Men Women Asymptomatic HIV Symptomatic HIV AIDS	47.9 45.0 55.0 31.0 46.0 47.0
Mocroft et al. (1999)	Europe	6,725 patients from EuroSIDA, patients with HIV with or without AIDS from across Europe	Ever taken, 70–78 Current use, 13–28	Men: 80–140 Women: 80–120 <80		58.2 1.4
Keller et al. (1999)	USA	86 patients ≥50 yr hospitalized with HIV or AIDS	72 ART	Not given		77.0
Moore et al. (1998)	USA	2,343 patients in various stages of disease who received primary care in the HIV/AIDS clinic at Johns Hopkins	70–75	<95 (both sexes) 80–94 (both sexes) 70–79 (both sexes) 65–69 (both sexes) <65 (both sexes)	All	21.3 12 5 1.3 2.7
Mir et al. (1989)	England	58 patients with HIV, all stages of disease, who had bone marrow studies performed	Not reported	Men: <130 Women: <115	All AIDS ARC	68.0 92.0 41.0
Raal et al. (1988)	South Africa	Patients with AIDS admitted to Johannesburg Hospital	Not reported	<120 (both sexes)		68.2
Frontiera and Myers (1987)	USA	41 male patients with end-stage AIDS undergoing bone marrow biopsy at the University of Colorado Health Sciences Center	Not reported	<133 (mild) <100 (moderate)		95.1 56.1
Zon et al. (1987)	USA	102 patients in various stages of HIV disease, including AIDS and ARC, at New England Deaconess Hospital	Not reported	<136 (both sexes)	HIV-infected ARC AIDS	18.0 16.0 71.0
Treacy et al. (1987)	UK	20 patients in various stages of HIV disease, including AIDS and ARC (14 with bone marrow biopsy)	Not reported	Men: <140 Women: <120		75

AIDS=acquired immunodeficiency syndrome; ARC=AIDS-related complex; ART=antiretroviral therapy; EuroSIDA=Europe-wide cohort of patients with HIV with or without AIDS; Hct = haematocrit; \*Includes zidovudine and other antiretrovirals. (Source: Belperio and Rhew, 2004)

**Table 2.1b: Prevalence of Anaemia in Patients with Human Immunodeficiency Virus (HIV) in Various Stages of Disease**

Study	Location	Population Measured	Use of zidovudine (%)	Definition of Anemia (hemoglobin, g/L)	Prevalence Estimates of anaemia	
					Subgroup (if applicable)	%
Moore et al. (1991)	USA	500 patients with AIDS and CD4 <sup>+</sup> count <250 cells x 10 <sup>6</sup> /L who first received zidovudine between Apr. 1978 and Apr. 1998	100	Anemia grade 1 Men: <133 Women: <120 Anemia grade 2 <96 Anemia grade 3 <80 Anemia grade 4 <66		69.0 11.0 1.6 0.2
		366 patients with ARC and CD4 <sup>+</sup> count <250 cells x 10 <sup>6</sup> /L who first received zidovudine between Apr. 1978 and Apr. 1998	100	Anemia grade 1 Anemia grade 2 <96 Anemia grade 3 <80 Anemia grade 4 <66		49.0 7.6 1.0 0.0
Sullivan et al. (1998)	USA	31,534 HIV-infected persons in various stages of disease in the Adult and Adolescent Spectrum of HIV Disease Surveillance Project	54–74	Men: <140 Women: <120	HIV infection, no AIDS Men Women Immunologic AIDS Men Women Clinical AIDS Men Women	28.5 30.5 54.7 51.6 87.3 77.2
Turner et al. (1996)	Italy	421 patients reported to the Italian National AIDS Registry surviving ≥3 months after AIDS diagnosis	28	<80 (both sexes) 80 to <110 (both sexes)		17.8 37.2
Kozak et al. (1993)	USA	4,582 patient records with HIV-related diagnoses, with or without AIDS or ARC, discharged from short-stay Hospitals	Not reported	ICD-9 codes 280–285	All	7.3
Moore et al. (1992)	USA	863 patients with AIDS or ARC and CD4 <sup>+</sup> <250 x 10 <sup>6</sup> /L who first received zidovudine between Apr. 15, 1978, and Apr. 14, 1988 Patients with Hct <24% at baseline	100	Hb <80 (both sexes)	All CD4 <sup>+</sup> <100x10 <sup>6</sup> /L CD4 <sup>+</sup> ≥100x10 <sup>6</sup> /L	8 45 31

AIDS=acquired immunodeficiency syndrome; ARC=AIDS-related complex; ART=antiretroviral therapy; ICD-9= *International Classification of Diseases, 9th Edition*. (Source: Belperio and Rhew, 2004)

**Table 2.1c: Prevalence of Anaemia in Patients with Human Immunodeficiency Virus (HIV) in Various Stages of Disease**

Study	Location	Population Measured	Use of zidovudine (%)	Definition of Anemia (hemoglobin, g/L)	Prevalence Estimates of anaemia	
					Subgroup (if applicable)	%
*Harris et al. (2008)	Europe	12000 previously untreated patients with HIV-1 infection	Not reported	All patients (Men: <130; Women: <120) Mild anemia (110-<130/ 100-<120) Moderate anemia (80-<110/80-<100) Severe anemia <80		35.0 25.0 9.0 1.1
*Migusha et al. (2008)	Uganda	Rural cohort of 259 HIV infected persons prior to receiving HAART	Not reported	Men: <120 Women: <110	HIV positive HIV negative CD4+ <200 CD4+ ≥500	18.9 12.9 30.0 17.4
*Mildvan and Creagh (2007)	USA	Multi-centre cross-sectional study: 9690 males and females receiving care  1721 receiving no ART 7252 receiving HAART	Not reported	Anaemia Men: <140; Women: <120  Marked anaemia (Men: <110; Women: <100)	All Men Women Blacks Whites CD4+ <200 CD4+ ≥500 RNA >30000 copies RNA <500 copies	36 37.3 32.3 49.0 26.0 57.0 23.0 53.0 30.0 5.0  39.7 35.5
*Ssali et al. (2006)	Uganda and Zimbabwe	3314 patients with ARC and CD4 <sup>+</sup> count <250 cells x 10 <sup>6</sup> /L initiating zidovudine-containing regimens in the DART trial	Not reported	>9.5 8.0-<9.5 (grade 1) 7.0-<8.0 (grade 2) 6.5-<7.0 (grade 3) <6.5 (grade 4)		88 12 0.1 0.1 0.0
*Wills et al. (2004)	USA	Retrospective study in 758 HIV infected persons	Not reported	<125		30.3
*Bushkin and Sullivan (2004)	USA	HIV infected cohort	Not reported	<105 or physician diagnosis	All On HAART On AZT With AIDS CD4+ <100	13 22.6 15.1 22.1 33.2
*Jam et al. (2009)	Iran	642 HIV/AIDS patients attending clinic in Tehran	Antiretrovirals reported	<100		10.3
*Omoregie et al. (2009)	Nigeria	457 HIV infected patients	HAART reported	Men: <130 Women: <120	All On HAART HAART naive	60.6 69.2 51.2

\* Included from recent search

**Table 2.2: Studies Assessing Prevalence of Anaemia in Women Infected with Human Immunodeficiency Virus (HIV)**

Study	Location	Population Measured	Definition of Anemia (hemoglobin, g/L)	Prevalence Estimates of anaemia	
				HIV-Positive Group	HIV-Negative Group
Antelman et al. (2000)	Tanzania	1,064 HIV-infected pregnant women in their second trimester	<110.0 <85.0	82.7 27.6	NA NA
Meda et al. (1998)	Burkina Faso	218 HIV-infected pregnant women $\geq 18$ yr at $<7$ months gestation	<110	78.4	64.7
Ramon et al. (1999)	Ivory Coast	197 HIV-infected pregnant women seen at an outpatient clinic	<110	81.7	68.9 <sup>#</sup>
Bucceri et al. (1997)	Italy	151 HIV-infected pregnant injection drug-users and 164 HIV-negative pregnant injection-drug users referred to the Mangiagalli Clinic in Italy	<100	27.2	15.0 <sup>†</sup>
Semba et al. (2002)	USA	Longitudinal study of 797 HIV-positive women and 389 HIV-negative women in all stages of HIV disease (HER Study)	<120 <100	28.1 5.4	15.1 2.3
Levine et al. (2001)	USA	2,056 HIV-infected women enrolled in WIHS African American women Latinas/Hispanic women White women	<120 <100 <120 <100 <120 <100 <120 <100	37.0 7.2 44.9 9.3 24.8 4.2 25.7 4.4	17.0 <sup>#</sup> 2.0 <sup>#</sup>
*O'Brien et al. (2005)	Tanzania	1078 HIV-positive pregnant women enrolled in a clinical trial of vitamin supplementation from 1995–2003	<110 85 -109 (moderate) <85 (severe)	82.8 55.1 27.7	NA NA NA
*Uneke et al. (2007)	Nigeria	815 pregnant women attending antenatal clinic	<110 <70 (severe)	76.9 15.0	NA NA
*Sinha et al., (2007)	India	467 HIV-infected pregnant women in PMTCT trials	<100 80 -100 (mild-moderate)	38.7 36	28.4 27

HER Study = Human Immunodeficiency Virus Epidemiology Research Study; NA = not available; WIHS = Women's Interagency HIV Study.

<sup>#</sup>  $P<0.001$  <sup>†</sup> Odds ratio, 2.1 (95% confidence interval, 1.2–3.8). (Source: Belperio and Rhew, 2004); \* Included from recent search

**Table 2.3: Studies Assessing Prevalence of Anaemia in Injection-Drug Users Infected with Human Immunodeficiency Virus (HIV)**

<b>Study</b>	<b>Location</b>	<b>Population Measured</b>	<b>Definition of Anemia (hemoglobin, g/L)</b>	<b>Prevalence Estimates of anaemia</b>
van der Werf et al. (2000)	Netherlands	Cross-sectional study: 128 males and females	Men: <120 Women: <110	14.8
		Longitudinal study: 158 males and females	Men: <130 Women: <120	38
Semba et al. (2001b)	USA	205 patients in Baltimore, MD 102 patients who received HAART 103 patients who never received antiretroviral medication	Men: <130 Women: <120	40 46.1 34.0
Yoong and Cheong (1997)	Malaysia	49 patients attending the infectious disease unit of National University of Malaysia	Not defined	81.6
Semba et al. (2002a)	USA	Longitudinal cohort of 622 men and women evaluated between 1988 and 2000	All patients Men: <130 Women: <120	19.6 16.1 30.5
Semba et al. (2002b)	USA	Cross-sectional study in 136 women evaluated between 1988 and 2000	<120	44.1

HAART - highly active antiretroviral therapy. (Source: Belperio and Rhew, 2004)

**Table 2.4: Studies Assessing Prevalence of Anaemia in Infants and Children Infected with Human Immunodeficiency Virus (HIV)**

Study	Location	Population Measured	Definition of Anemia (hemoglobin, g/L)	Prevalence Estimates of anaemia	
				HIV-Positive Group	HIV-Negative Group
Adewuyi and Chitsike (1994)	Zimbabwe	46 children aged 3 mo–7 yr, admitted to the Parirenyatwa Central Hospital	<110	84.0	NA
Shaffer et al. (1990)	Zaire	28 children aged 1–13 yr	<100	50.0	27.0
			66–96	32.0	18.0
			<66	18.0	9.0
Ellaurie et al. (1990)	USA	Symptomatic children aged 2 mo–8 yr	<110	94.0	32.0
Totin et al. (2002)	Uganda	165 infants aged 9 month old HIV-positive and HIV-negative infants seen in an outpatient paediatric clinic in Kampala, Uganda	<110	90.9	76.9
			<90	35.1	
Eley et al. (2002)	South Africa	60 antiretroviral-naive children aged 13–28 mo in various stages of disease Category 1 (no immunosuppression) Category 2 (moderate immunosuppression) Category 3 (severe immunosuppression)	<110	72.0	NA
			<110	42.0	
			<110	76.0	
			<110	85.0	
			<110	91.7	NA
*Clark et al. (2004)	Uganda	225 HIV infected children 9 months old	<110		

NA = not available (Source: Belperio and Rhew, 2004)

The prevalence of anaemia has also been estimated in HIV people who inject drugs in 5 studies (Tab. 2.3). The ALIVE (AIDS Linked to Intravenous Experiences) study was a longitudinal study that enrolled 2,960 HIV-infected injection-drug users in Baltimore between 1988 and 1989 (Semba et al., 2001a). Two sub-studies addressed the topic of anaemia in HIV-infected injection-drug users. In one, anaemia was found in 44% of the 136 HIV-positive women, compared with 26% of the HIV-negative women. The second ALIVE sub-study involved both male and female injection-drug users. Of 622 injection-drug users with HIV evaluated between 1988 and 2000, 16.1% of men and 30.5% of women were found to be anaemic on enrolment ( $p<0.001$ ). In the Netherlands study, anaemia has been reported as cross-sectional prevalence rates for HIV-positive and HIV-negative injection-drug users, and also as part of a longitudinal study (van den Werf et al., 2000). Of 128 HIV-positive injection-drug users in the cross-sectional study, 14.8% had anaemia, compared with 7.8% of the 232 HIV-negative users. Of the 158 HIV-positive injection-drug users enrolled in the longitudinal study, 38% had anaemia at the first visit, and an additional 26% of participants developed anaemia during the 3-year follow-up.

Six studies were identified that evaluated the prevalence or incidence of anaemia in HIV-infected infants or children (Tab. 2.4). Of the 6 studies, 5 enrolled children from Africa, where rates of HIV infection are considerably higher than in developed countries. In one study, anaemia was evaluated in 9-month-old HIV-positive and HIV-negative infants seen in an outpatient paediatric clinic in Kampala, Uganda (Totin et al., 2002). Anaemia was found in 90.9% of 165 HIV-infected infants compared with 76.9% of uninfected infants ( $p<0.015$ ). Iron deficiency anaemia accounted for about half of the anaemia cases. In Zimbabwe, anaemia was reported in 84% of children infected with HIV (Adewuyi and Chitsike, 1994). Of 46 hospitalised infants and children, aged 3 month to 7 year, 40% had normochromic normocytic anaemia, and another 40% had hypochromic microcytic anaemia, likely due to iron deficiency. In 60 antiretroviral-naïve, HIV positive infants and children, aged 13 to 38 months, attending a clinic in South Africa, 72% had anaemia (Eley et al., 2002). The prevalence of anaemia was 42% for those who were least immunosuppressed (CD4+ cell counts  $>500 \times 10^6/L$ ), and 85% in those most immunosuppressed (CD4+ cell counts  $<200 \times 10^6/L$ ). Iron depletion was prevalent (52% had ferritin levels  $<10 \mu\text{g}/\text{L}$ ) although IDA was present in only 17% of the children. In another study in Uganda (Clark et al., 2005), anaemia prevalence in a cohort of 9 month old HIV infected infants was shown to be 91.7%.

The impact of an active opportunistic infection on the prevalence of anaemia in HIV was evaluated in a longitudinal study of 100 symptomatic paediatric patients aged 2

months to 8 years in the US (Ellaure et al., 1990). The prevalence of anaemia was 100% in 23 patients with active opportunistic infection compared with 92% in 77 children with no active infection. These studies indicate that anaemia is widely prevalent in infants and children with advanced HIV disease especially if secondarily infected, in both African and the US children.

#### **2.2.1.2 Conclusions from the systematic review**

Belperio and Rhew (2004) noted the difficulty in drawing general conclusions about the prevalence of anaemia in people with HIV from the available literature because of the wide variability amongst studies and the need for standardised criteria for studies in this area. Anaemia appears more common with disease progression but this is not clear. Progression is variously assessed on the basis of the staging of HIV disease or CD4+ cell count. In some studies all patients have been grouped together regardless of the extent to which they may have progressed to AIDS. For anaemia, there is no consistency in the criteria used to define it, with methods and cut-offs differing amongst studies, and making comparisons difficult. This may have an impact on the reliability and acceptance of the data, given the fact that laid down reference ranges for the various markers of anaemia existed (WHO/CDC, 2004). Despite these differences, the highest rates of anaemia appear with advanced HIV, or AIDS, and often in patients receiving zidovudine. The largest evaluation of the prevalence of anaemia to date (Morcroft et al., 1999) found the prevalence of anaemia for HIV-infected patients without AIDS to be 46% to 59% lower than in HIV-infected patients with AIDS.

Most of the large studies reported from America or Europe, and the few reports from Africa are predominantly in pregnant women and their infants or children as these populations were possibly more accessible. Indeed most of the studies in women and children are from Africa and here the rates of anaemia are high in non-infected populations and as high as 78% to 91% in those infected. Interpretation and generalisability of the evidence is made difficult because HIV infection continues to progress to AIDS at a high rate in these groups. Nevertheless, US data also suggest that anaemia is problematic in HIV-infected children.

#### **2.2.2 RISK FACTORS FOR ANAEMIA IN HIV/AIDS**

A major difficulty in drawing comparisons amongst studies is the substantial difference in the background factors that predispose to anaemia from one situation to another.

It is usual for there to be a higher prevalence of anaemia in women than men in the general population (Sullivan et al., 1998), making it more difficult to assess the many

studies that show anaemia to be more common in HIV-infected women than infected men (Means, 1997; Sullivan et al., 1998; Semba et al., 2002). The cut-off values for anaemia are different between the sexes, but are also different for pregnant women and children. So the observation that anaemia is more likely in infected pregnant women (van den Broek et al., 1998) or in children (Adewuji and Chitsike, 1994) has to be placed in context.

Amongst populations most anaemia is associated with poor diet and nutrient deficiencies, but dietary considerations have not been a major part of assessing the anaemia associated with HIV. The commonest diet related anaemia is related to poor iron status, or operates through limited iron availability as the final common pathway. The major environmental variable that influences iron metabolism is the presence of infection or other ongoing inflammation. It is seldom that sufficient account has been taken of the complex interplay of infections with nutrient deficiencies. These interactions vary with geography and social factors and to an extent map against the availability of better quality diets, effective treatment with antiretroviral therapy and the effective management of other co-morbidities. The use of the antiretroviral drug zidovudine (AZT), having low CD4+ cell counts (less than 200 cells/ $\mu$ l), a high viral load, a lower BMI as well as an increasing age have all been shown to constitute a high risk for developing anaemia in HIV populations (Levine et al., 2001; Creagh and Mildvan, 2002; Sharp et al., 1999; Semba et al., 2002). People of African descent, such as African-Americans or Haitians were at one time considered to be at special risk of HIV/AIDS, and possibly anaemia (Sullivan et al., 1998; Volberding, 2000). However, it is unclear whether these relations remain once social and nutritional factors have been adequately accounted for.

Most studies relating HIV/AIDS to anaemia in populations from sub-Saharan Africa, which remains the largest HIV infected population in the world, were conducted in pregnant women and children populations for two main reasons. Whilst these population groups are the most vulnerable to HIV infection and anaemia, they are also easily accessible. However, there were few studies which explored the HIV-anaemia relationship in adult men and non-pregnant women in sub-Saharan Africa. There were no studies, separately or together, that made it possible to reliably take the major factors of importance into account, to assess their relative contributions directly, or indirectly.

In many parts of the developing world patterns of ill health and morbidity relate directly to the demographic, epidemiological and nutrition transitions. As populations in sub-

Saharan Africa become increasingly urbanised the relevance of these changing patterns of exposure become increasingly important in defining risk of ill-health, ill-health itself, and opportunities for effective care. No studies have been found that systematically consider all these factors or attempt to explore how they might interact directly and indirectly, positively and negatively, in order to draw general conclusions that might be applied to the adult population in sub-Saharan Africa generally, or South Africa specifically.

In this chapter, HIV/AIDS, anaemia and the nature of their interaction is reviewed. An overview of HIV/AIDS, which includes a brief historical perspective, an outline of its pathophysiology, epidemiology and therapeutic approaches, is given. HIV effects on food and nutrition and aspects of the complex interrelationships amongst HIV infection, immune processes, inflammation and nutrition are outlined. The nature of anaemia, its causes, pathophysiology and identification are outlined. This leads to a consideration of the relationship between HIV/AIDS and anaemia, with a review of the aetiology of anaemia in HIV/AIDS and the determinants of HIV infection and/or AIDS with anaemia.

### **2.3 HIV/AIDS – BACKGROUND TO THE PANDEMIC**

Since the early 1980s the impact of HIV infection and AIDS on domestic and global health, social, political and economic outcomes has been substantial (UNAIDS, 2004; 2005; 2008). After nearly three decades the pandemic continues to challenge every effort (UNAIDS, 2008). HIV/AIDS is most prevalent in sub-Saharan Africa and the effects are exacerbated because of interactions with other common conditions such as anaemia and malnutrition, wreaking havoc on families, communities and nations. Anaemia and malnutrition on their own tend to reduce the ability of individuals to acquire, consume and utilise food. Whether the underlying cause is predominantly social or biological, the culmination is likely to be weight loss and poor nutritional status which further exacerbates any pre-existing morbid condition.

The challenge for clinicians treating HIV has been how best to manage a clinically complex illness with limited therapeutic options. By 1985, diagnostic serologic assays had been developed to test for HIV infection in asymptomatic persons, to identify new infections by seroconversion, and to screen donated blood (CDC, 1984). Although early trials of antiviral treatments and the use of immune modulators were disappointing (Byers et al., 1994; Pert et al., 1986; Mildvan et al., 1991; Schooley et al., 1990; Pedersen et al., 1990), by 1987, zidovudine (AZT, or azidothymidine), a nucleoside analogue reverse transcriptase inhibitor had been approved for use by the U.S. Food and Drug Administration (FDA) (Fischl et al., 1987).

The earlier excitement over the life-extending effects of the drug began to wane as patients treated with this single-drug therapy started to experience disease progression leading, in most cases, to death. However, because there was a level of understanding of the epidemiology, treatment, and prophylaxis of OI associated with HIV-induced immune deficiency, significant life-saving advances were made, particularly in the areas of infection with *Pneumocystis jiroveci* and *Mycobacterium avium complex* (MAC) (Shafer et al, 1989; USPHS/IDSA, 1999).

In resource-adequate settings, advances in the use of potent life-prolonging medications such as HAART and the prophylaxis and aggressive treatment of OI, has helped to reduce HIV to a chronic manageable condition (Moore and Forney, 2002; Brantley et al., 2003). Although the medications do not cure the disease, they help stabilise the immune system by suppressing viral replication and indirectly help improve nutritional status and limit progression to AIDS (Rousseau et al., 2000).

### **2.3.1 HIV/AIDS - EPIDEMIOLOGY**

AIDS has killed more than 25 million people since 1981, making it one of the most devastating epidemics in recorded history. Despite improved access to antiretroviral treatment it claimed 3.1 million lives in 2005 of which half a million were children (UNAIDS, 2005). The epidemic has hit hard in geographical areas where poverty and malnutrition are rife (UNAIDS, 2003; 2005; 2008).

It is estimated that 10% of the world's population reside in sub-Saharan Africa, but these populations carry two-thirds (63%) of the burden of HIV/AIDS, around 25 million (UNAIDS, 2005; 2008). The epidemic has resulted in a large rise in the region's death toll with HIV/AIDS becoming a leading cause of mortality, accounting for well over three quarters of all related global deaths (UNAIDS, 2005; 2006; 2008). The predominant mode of transmission in this region is heterosexual sex and over 90% of infected children acquire it through mother-to-child transmission (MTCT).

Epidemiological data suggests that the prevalence is yet to peak in some sub-Saharan African countries (UNAIDS, 2003; 2005; 2006). Since 2000, the percentage of persons living with HIV has stabilised, but the overall number of people living with the infection has increased steadily as new infections occur each year, lives are extended with ART and new infections outnumber deaths from AIDS (UNAIDS, 2008).

In Sub-Saharan Africa, there has been a continuous increase in the proportion of infected females; 17.0 million in 2003, increasing to 17.5 million in 2005 and then 17.7

million in 2006. However, in 2007, there has been a decline (Tab. 2.5) perhaps stemming from the efforts at empowering women and improving their capacities. Regional prevalence of the epidemic has shown variations within the same regions and wide variations exist at the country level where infection levels differ between areas, sexes and socioeconomic categories (UNAIDS 2006; UNAIDS 2008). In sub-Saharan Africa, Southern Africa remains the epicentre of the global epidemic where 32% to 35% of persons with HIV/AIDS in the whole world reside and 38% of all global AIDS deaths occur (UNAIDS 2006; UNAIDS 2008).

Recent developments in some South African countries show that the epidemic is non-relenting, with levels of infection among pregnant women at 20% or higher (UNAIDS, 2006; 2008). In other countries a looming epidemic is eminently shown by alarming increases in infection, a trend that actually cuts across several regions (UNAIDS, 2003; 2005; 2008). The UNAIDS update report has narrowed the cause of these rising trends, especially, in Asia to a combination of increasing intravenous drug use and commercial sex (UNAIDS, 2005; 2006).

**Table 2.5: Global summary of the HIV and AIDS epidemic for 2003, 2005 to 2007**

		2003 (millions)	2005 (millions)	2006 (millions)	2007 (millions)
<b>Number of people living with HIV</b>	Total	39.5 (34.6 – 42.3)	40.3 (36.7 – 45.3)	39.5 (34.1 – 47.1)	33.0 (30.1 – 36.0)
	Adults	35.7 (32.7 – 39.8)	38.0 (34.5 – 42.6)	37.2 (32.1 – 44.5)	30.8 (28.2 – 34.0)
	Women	17.0 (15.8 – 18.8)	17.5 (16.2 – 19.3)	17.7 (15.1 – 20.9)	15.5 (14.2 – 16.9)
	Children under 15 years	2.1 (1.9 – 2.5)	2.3 (2.1 – 2.8)	2.3 (1.7 – 3.5)	2.0 (1.9 – 2.3)
<b>People newly infected</b>	Total	4.8 (4.2 – 6.3)	4.9 (4.3 – 6.6)	4.3 (3.6 – 6.6)	2.7 (2.2 – 3.2)
	Adults	4.1 (3.6 – 5.6)	4.2 (3.6 – 5.8)	3.8 (3.2 – 5.7)	2.3 (1.9 – 2.8)
	Children under 15 years	0.63 (0.57 – 0.74)	0.70 (0.63 – 0.82)	0.53 (0.41 – 0.66)	0.37 (0.33 – 0.41)
<b>AIDS deaths</b>	Total	2.9 (2.6 – 3.3)	3.1 (2.8 – 3.6)	2.9 (2.5 – 3.5)	2.0 (1.8 – 2.3)
	Adults	2.4 (2.2 – 2.7)	2.6 (2.3 – 2.9)	2.6 (2.2 – 3.0)	1.8 (1.6 – 2.2)
	Children under 15 years	0.49 (0.44 – 0.58)	0.57 (0.51 – 0.67)	0.38 (0.29 – 0.50)	0.27 (0.25 – 0.29)

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information. Extracted from the AIDS Epidemic Update, 2005, 2006 and 2008 (UNAIDS/WHO).

### 2.3.1.1 The epidemiology of HIV/AIDS in South Africa

The present THUSA study was undertaken in South Africa, where it is estimated that 5.7 million people are living with the virus, including 280 000 children under 15 years

(UNAIDS, 2008). As in the rest of sub-Saharan Africa, women in South Africa are disproportionately affected by the epidemic and women aged 15 to 39 years are more likely to be infected as compared to men of the same age group, whilst males 40 years and above are also more likely to be infected compared to those below 40 years (UNAIDS, 2008; The South African National HIV Survey, 2008). This trend is most likely due to a number of reasons including the fact that older men prefer to have sexual relationships with younger women.

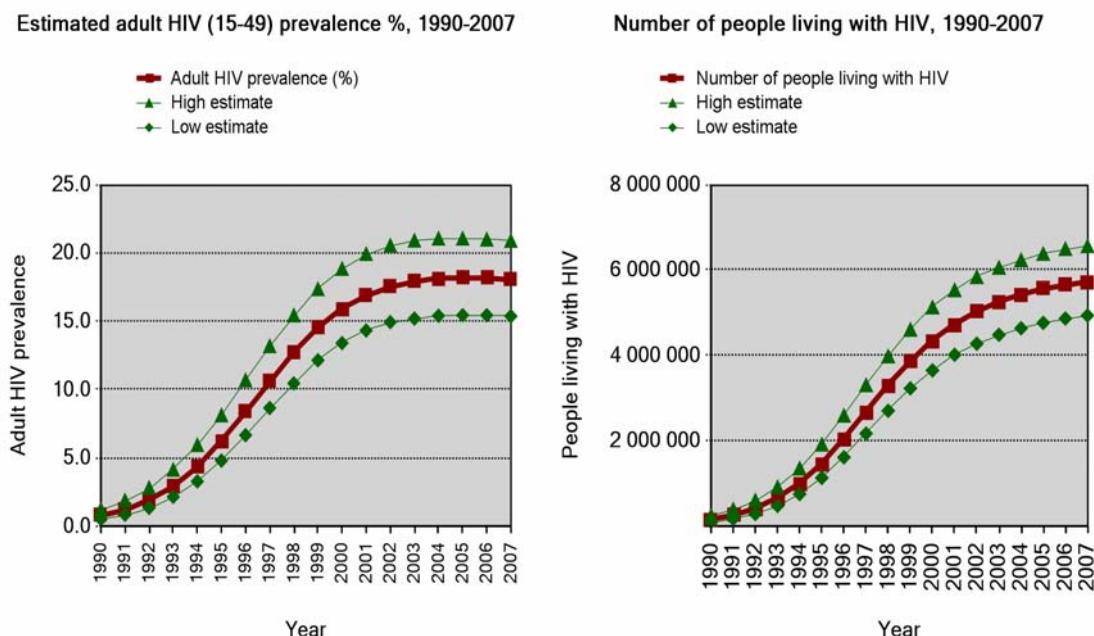
The prevalence of HIV in South Africa has risen steadily since 1990 to stabilise at 18.1% in 2007. This rise is clearly depicted in Figure 2.1 and Table 2.6 below. The table (Tab. 2.6) shows that between 2001 and 2007 the estimated number of persons living with HIV increased from 4.7 to 5.7 million, whilst the total number of children with HIV increased by 130 000 (46.4%) from 150 000 in 2001 to 280 000 in 2007. The number of AIDS orphans more than tripled from 400 000 in 2001 to 1 400 000 in 2007 (Tab. 2.6).

The estimated number of deaths from AIDS also increased steadily from 1990 to 2007 (Tab. 2.6 and Fig. 2.2). Over the period, both prevalence and deaths due to HIV have shown initial steep increases and subsequent stabilisation towards the end of 2005 and slight decline with respect to Deaths in 2006 which seems to have risen again in 2007.

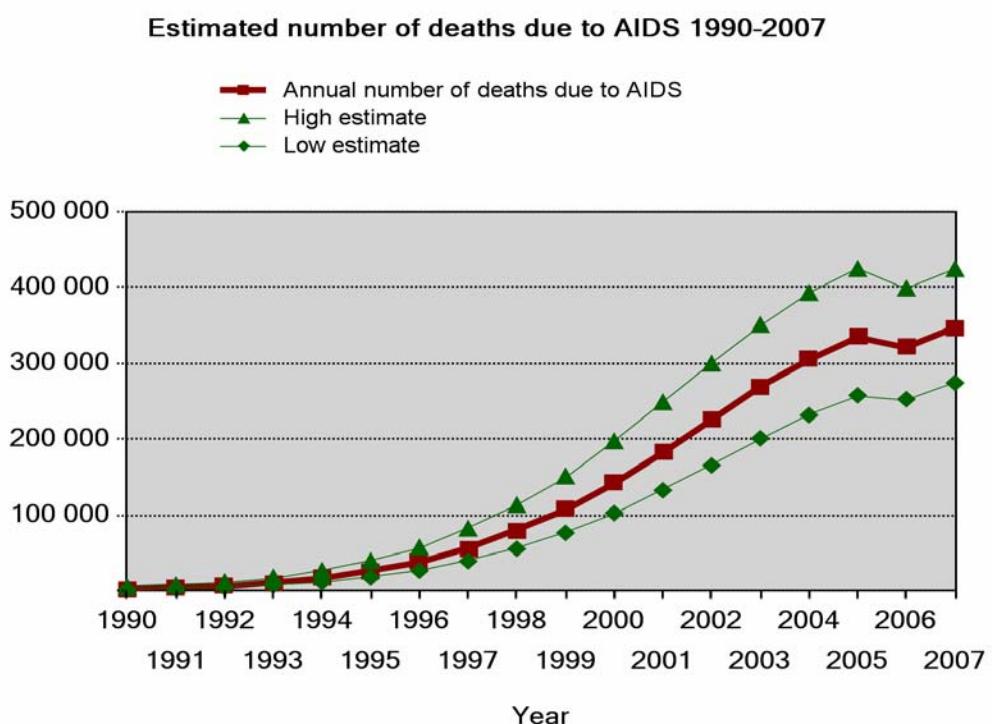
**Table 2.6: Estimates for HIV and AIDS in South Africa (Source: UNAIDS/WHO, 2008)**

Category	2001	2007
Persons living with HIV (Total)	4 700 000 (4 000 000-5 500 000)	5 700 000 (4 900 000-6 600 000)
Adults 15+ with HIV	4 600 000 (3 900 000-5 300 000)	5 400 000 (4 700 000-6 200 000)
Women 15+ with HIV	2 700 000 (2 300 000-3 200 000)	3 200 000 (2 800 000-3 700 000)
Children 0-14 with HIV	150 000 (120 000 - 190 000)	280 000 (230 000-320 000)
HIV prevalence (Adults 15 – 49)	16.9 (14.3-19.9)	18.1 (15.4-20.9)
Death due to AIDS	180 000 (130 000-250 000)	350 000 (270 000-420 000)
AIDS orphans (0-17)	400 000 (260 000-590 000)	1 400 000 (1 100 000-1 800 000)

**Figure 2.1: Estimates for Adult HIV (15-59) prevalence and number of people living with HIV from 1990 to 2007 in South Africa.**



**Figure 2.2: Estimates for deaths due to AIDS from 1990 to 2007 in South Africa.**



Provincial estimates of HIV prevalence from the South African Department of Health survey (2007) shown in Table 2.7 below puts Kwazulu-Natal at the highest (15.5%) and the Western Cape at the lowest (1.9%).

From table 2.7 below, it is evident that the national prevalence of HIV/AIDS bore a close similarity to that in the North West and Guateng provinces. In addition, females and Africans were shown to have the highest prevalences with regards to gender and race respectively.

The South African Department of Health Survey also estimated that 28% of pregnant women were living with HIV/AIDS in 2007. However, prevalence levels varied from a low of 15.2% in Western Cape Province to a high of 39.1% in Kwazulu-Natal Province (South African Department of Health Survey, 2007).

**Table 2.7: Provincial, gender and racial estimates for HIV prevalence in South Africa (Source: South African Department of Health Survey, 2007)**

Province	Number surveyed	Prevalence (%)
Kwazulu-Natal	2 729	19.5
Mpumalanga	1 224	15.2
Free State	1 066	12.1
North West	1 056	10.9
Guateng	2 430	10.8
Eastern Cape	2 420	8.9
Limpopo	1 570	8.0
Northern Cape	1 144	5.4
Western Cape	2 204	1.9
Sex		
Male	6 342	8.2
Female	9 509	13.3
Race		
African	9 950	13.3
White	1 173	0.6
Coloured	3 382	1.9
Indian	1 317	1.6
National	15 851	10.8

### 2.3.2 HIV/AIDS - TRANSMISSION AND RISK FACTORS

The primary method of spread of HIV infection worldwide is through sexual exposure. In the United States and Europe, acquisition of the virus through homosexual contact

remains important, and there is some evidence of increasing incidence of infection among young gay men and ethnic minorities (Holmberg, 1996). In most of the developing world, heterosexual intercourse with an infected person is the primary mode of transmission, accounting for approximately 70% of the overall sexual transmission (Rothenberg, 1998). Female-to-female HIV transmission has been reported, but is rare (Monzon and Capellan, 1987).

HIV is transmitted either through direct contact of a mucous membrane with a bodily fluid containing the virus (such as blood, semen, vaginal fluid, and pre-seminal fluid) or breast milk (Gayle, 2000). Transmission can occur because of unprotected penetrative sex; oral sex; blood transfusion; use of contaminated needles; exchange between mother and infant during pregnancy, childbirth, or breastfeeding; or other exposure to one of the above bodily fluids. HIV has been isolated from blood, seminal fluid, pre-ejaculate, vaginal secretions, cerebrospinal fluid, saliva, tears, and breast milk of infected individuals (Wofsy et al, 1986; Hollander and Levy, 1987; Geier et al., 1993). The DNA sequences of HIV-1 have been detected in pre-ejaculatory fluid (Ilaria et al., 1992). In genital fluids, HIV may be found in both cell-free and cell-associated compartments (Alexander, 1990). Viral concentrations in tears and saliva are comparatively low, and there may be substances in saliva that inhibit infectivity.

The use of physical barriers such as the latex condom is widely advocated to reduce the sexual transmission of HIV and meta-analysis has shown that their overall efficacy in reducing HIV transmission was 69% (Weller, 1993). Sexual activity associated with exposure to infected blood increases the risk of transmission, as does the presence of genital ulcers and/or other sexually transmitted infections or diseases (STI/Ds) (Cameron et al., 1989; Greenblatt et al., 1988; Plummer et al., 1991). In a particular study in an African population, serum HIV viral load was strongly associated with heterosexual transmission between HIV-serodiscordant sexual partners, and transmission was rare at viral loads <1,500 copies/mL (Quinn et al., 2000). The risk for women acquiring HIV through sexual contact is greater than for men.

Nonsexual HIV transmission can occur through transfusion with contaminated blood or blood products, injection drug use, occupational or accidental needle exposure (Cardo et al., 1997). HIV transmission through transfusion of contaminated blood products was recognized early in the epidemic and may still remain a significant mode of transmission in some developing countries (CDC, 1982). An infected mother can transmit the virus to the child (mother-to-child transmission, MTCT) during pregnancy, childbirth and breastfeeding. MTCT occurs in approximately 25% of live births to HIV-

infected mothers (Connor et al, 1994) and perinatal transmission can be reduced by 50% with antiretroviral regimens (Connor et al., 1994; Shaffer et al., 1999; Guay et al., 1999). Approximately one-third of cases of MTCT result from breast-feeding, and the risk increases with the duration of breast-feeding or partial breast feeding (Richardson et al., 2003).

Many people who are infected remain ignorant of their HIV status and although asymptomatic can transmit the virus during unprotected sexual intercourse or through the use of contaminated needles, razors and other devices, or during labour and delivery or through breastfeeding.

### **2.3.3 HIV/AIDS - VIROLOGY AND IMMUNOLOGY**

HIV is a member of the genus *Lentivirus*, part of the family of *Retroviridae* (ICTV, 2002). All lentiviruses have many common morphologies and biological properties. They can infect many animal species and are characteristically responsible for long duration illnesses associated with a long period of incubation (Levy, 1993). They are transmitted as single-stranded, enveloped RNA viruses.

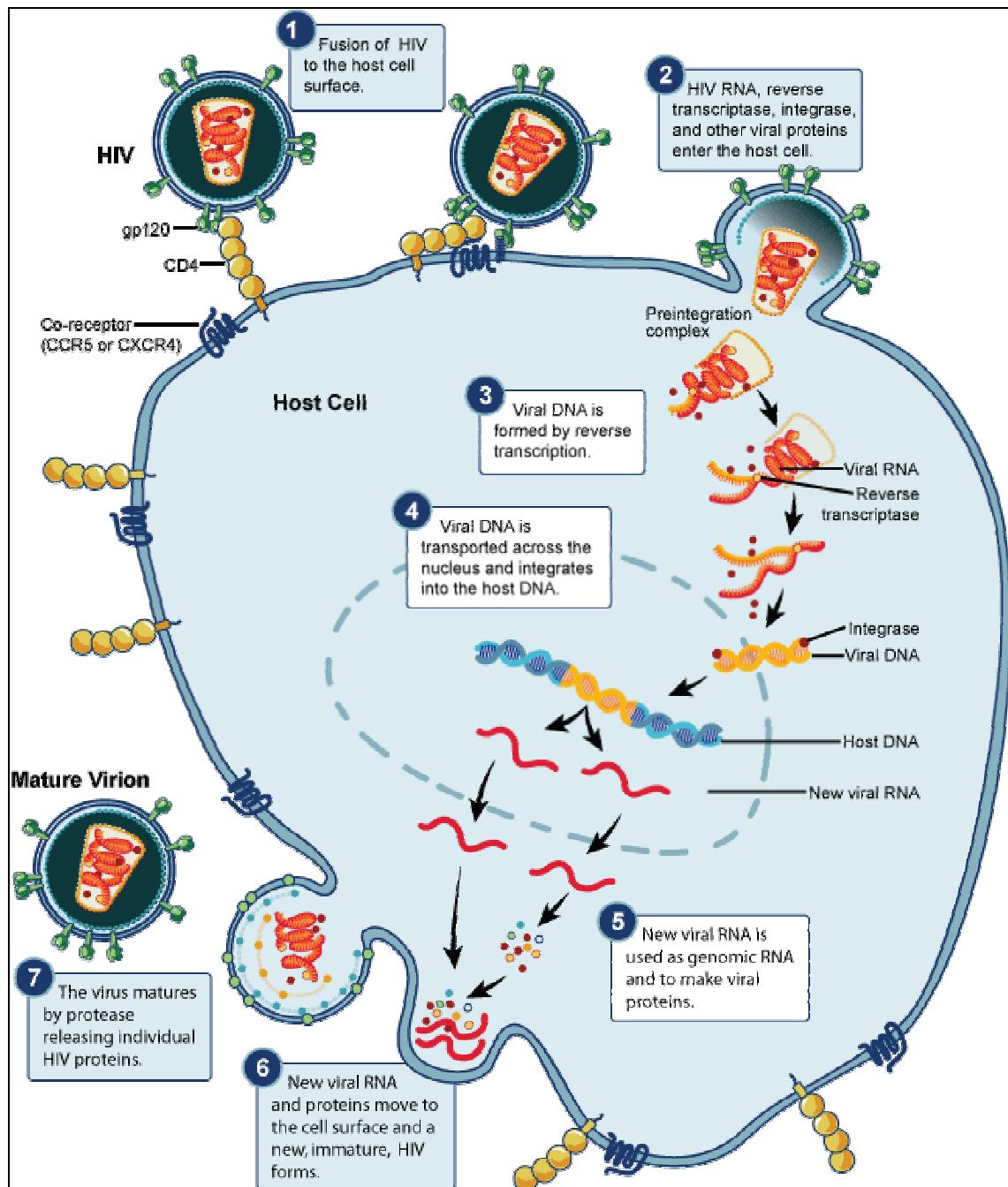
Upon infection of the target-cell, their viral RNA genome is converted into a double-stranded DNA by a virally encoded reverse transcriptase, a RNA to DNA converting enzyme, which is present in the virus particle. This viral DNA, thus produced by the reverse transcriptase is then integrated into the cellular DNA of the host for replication using cellular machinery. Once the virus enters the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function or the virus becomes active, replicates and releases a large number of virus particles which can infect other cells (Levy, 1993). Steps involved in the HIV replication cycle are illustrated in figure 2.3 below.

There are two species of HIV that infect humans; HIV-1 and HIV-2, and each of them has many strains. HIV-1, which was the first to be isolated, is more virulent and easily transmitted, and is the source of majority of HIV infections throughout the world. HIV-2 is largely confined to West Africa (Reeves and Doms, 2002).

HIV exhibits tropism and infects those cells that express the CD4 molecule on the surface (surface marker). The entry of HIV-1 into macrophages and CD4+ cells is mediated through the interaction of the virion envelope glycoproteins (gp120) with the CD4+ surface molecule on the target cells and also with its chemokine co-receptors (Coakley et al., 2005). The macrophage (M-tropic) strains of HIV-1, or non-syncitia-

inducing strains (NS1), use the beta-chemokine receptor, CCR5. The virus is able to replicate in macrophages, which express these receptors (Coakley et al., 2005). The CCR5 co-receptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype.

**Figure 2.3. The HIV replication cycle**



Source: Website of the National Institute of Allergy and Infectious Diseases (<http://www.niaid.nih.gov/diagnostics/dtpdb/attach.htm>).

Macrophages play a key role in several critical aspects of HIV disease. They appear to be the first cells infected by HIV and may continue to provide a reservoir for ongoing HIV replication when CD4+ T cells have been markedly depleted. In the central

nervous system macrophages and microglial cells are the cells infected by HIV. In tonsils and adenoids infected macrophages may fuse into multinucleated giant cells that produce copious amounts of virus (Coakley et al., 2005). The T-tropic isolates, or syncitia-inducing (SI) strains replicate in primary CD4+ T-cells as well as in macrophages using the alpha-chemokine receptor, CXCR4, for entry (Coakley et al., 2005). The alpha-chemokine, SDF-1 is a ligand for CXCR4 and suppresses replication of T-tropic HIV-1 isolates, probably by down regulating the expression of CXCR4 on the surface of these cells (Coakley et al., 2005).

Infection with HIV represents something of an immunological paradox, in that it induces a strong antiviral immune response, but simultaneously and progressively disrupting the ability of the immune system to respond to any new infections and antigens, leading ultimately to a severe immune deficiency of the cell-mediated immune system.

Persons infected with HIV can show both cellular and humoral (antibody) immune responses to the virus, but in the great majority of infected individuals these responses are unable to prevent the ultimate progression of the disease. The cellular responses are mediated by both CD4+ T cells and cytotoxic T-lymphocytes (CTLs or CD8 cells). CTLs inhibit HIV replication both directly, by recognizing and killing infected cells, and indirectly, by producing soluble chemokine antiviral factors (Walker et al., 1986; Pontesilli et al., 1998). By direct contact the CTLs kill virally infected host cells. This happens when the T-cell receptor on the surface of the CTL recognizes an epitope of an HIV protein bound to a major histocompatibility complex (MHC) class I molecule on the surface of the infected host cell. The CTL then releases enzymes that kill the infected cell or through soluble factors (e.g. macrophage inflammatory protein (MIP)-1-alpha, and MIP-1-beta), which inhibit HIV from infecting new cells by blocking their co-receptors (Malnati et al., 2000).

In viral control, CD4+ responses to HIV are important and strong HIV-specific CD4+ responses are usually associated with lower HIV viral loads (Rosenberg and Walker, 1998). The CD4+ cells respond to HIV antigens presented with MHC class II molecules on the surface of infected cells. That HIV infects CD4+ cells has the appearance of an effective evolutionary strategy with important consequences. Firstly, because productive HIV infection occurs in activated CD4+ cells, infection and killing of CD4+ cells that are responding to the HIV infection results in a selective decrease in the number of HIV-specific CD4+ cells (Rosenberg and Walker, 1998).

Secondly, as HIV continues to evolve under the selection pressure of the immune

response of the infected individual, mutations in the viral epitopes recognized by the immune system enable the virus to escape the control of even broad and robust CD4+ and CD8 HIV-specific responses (McMichael and Rowland-Jones, 2001).

The hallmark of HIV infection is, thus, the depletion of lymphocytes, which predicts an individual's risk for infection with opportunistic pathogens as well as other complications of HIV disease. The evidence shows that both increased peripheral destruction and decreased production of CD4+ cells likely play a role in the decline in CD4+ numbers (Ho et al., 1995; Hellerstein et al., 1999; Rowland-Jones, 1999; Mohri et al., 2001). This is associated with a reservoir of virus in infected macrophage populations, which are likely to be shed when other infected episodes stimulate macrophage responses.

### **2.3.4 HIV/AIDS - CLASSIFICATIONS**

The primary or acute HIV infection stage is a period of rapid viral replication that may immediately follow the individual's exposure to HIV. During primary HIV infection, most individuals (80% to 90%) develop a syndrome characterised by non specific flu-like symptoms of fever, malaise, lymphadenopathy, pharyngitis, headache, myalgia, and sometimes a rash (Khan and Walker, 1998). Because of the non-specific nature of the symptomatology the possibility of HIV infection during the first weeks may not be considered, however, during this period the individual is infectious.

Around three weeks after infection there is sero-conversion with a specific immune response to HIV-1. This begins the clinical latency phase during which a strong immune response reduces the number of circulating viral particles. Clinical latency can vary between two weeks and 20 years. During this period, the individual may be asymptomatic with no evidence of HIV-associated clinical, immunological or nutritional deficiency or abnormality. As the latency phase progresses, HIV remains active within lymphoid organs, where large amounts of the virus become trapped in the follicular dendritic cell (FDC) network. The surrounding tissues that are rich in CD4+ T-cells also become infected, and viral particles accumulate in infected cells and as free virus.

Individuals who have entered into this middle stage are still infectious. During this phase, a high level of viral replication occurs with a concomitant high CD4+ cell turnover, resulting in progressive immune dysfunction which can lead to evident clinical disease and AIDS. In this middle stage, laboratory evidence of immune deficiency can be seen even in the absence of clinical evidence. Non-specific symptoms and variable fatigue may be indicative of metabolic abnormalities and there may be some loss of

weight, which is not usually progressive.

At the late stage, clinical evidence of immune dysfunction is prominent and the patient is said to have AIDS or be actively progressing to AIDS. Malnutrition, evident as weight loss is often present and may be severe.

The CDC makes an important distinction between "HIV infection" and "AIDS", which has clinical and prognostic implications and utility in research (CDC, 1993). The CDC definition of AIDS is based on a clinical criteria, including the presence of infections, and malignancies associated with HIV infection (CDC, 1993).

The CDC also defines AIDS by a CD4+ count of <200 cells/ $\mu$ L or <14% of all lymphocytes, even in the absence of listed clinical and HIV associated conditions. In line with this definition, the World Health Organization (WHO) has developed a clinical staging system for HIV infection (WHO, 1993). For pragmatic purposes, this system weighs clinical features more heavily than laboratory evaluations, and therefore has practical use in resource-constrained areas where laboratory testing is not widely available (WHO, 1993).

### **2.3.5 HIV/AIDS – THERAPEUTIC OPTIONS**

HIV infection is a chronic infectious disease for which treatment can limit the progress of the condition, without complete elimination of the virus, or cure. Complications may be prevented, delayed or treated but it is still not clear whether the inexorable progression to AIDS can be halted. Not all persons infected with HIV have progressed to AIDS, but it is generally believed that the majority will.

The control of HIV viremia depends on host immune response as well as exogenous intervention with ARV medication. PLWHA may also take medications to treat OI or other complications. Some HIV-infected individuals use complimentary and/or alternative medicine (CAM) for management of their disease (Hsiao et al., 2003) although the evidence for their effectiveness is scanty.

The advent of potent ART in 1996 led to a revolution in the care of PLWHA. All the ARV currently approved by the FDA of the US work by inhibiting 1 of 3 steps in the life cycle of the HIV:

1. By blocking the reverse transcriptase (RT) enzyme. This is the enzyme used by the virus to convert its RNA into DNA after it enters the cell prior to entering the nucleus. All nucleoside and nucleotide analogues (NRTIs) as well as non-nucleoside or nucleotide (NNRTIs) analogues function by interfering with or

inhibiting the activity of this enzyme.

2. By blocking the protease enzyme. Protease inhibitors (PIs) inhibit the action of the HIV protease, by cleaving protein products of the viral structural genes into the functional subunits needed to create new infectious virions.
3. By inhibiting fusion of the viral and host membranes. By attaching itself to the HIV envelope glycoprotein gp41, fusion inhibitors prevent formation of the "hairpin" structure required for fusion of the HIV and host cell membranes, and thus prevent viral entry into the host cell.

The use of ARVs has dramatically reduced morbidity and mortality, but all have low efficiencies with respect to elimination of the virus from resting cells, resulting in persistence of infection despite effective suppression (Wong et al., 1997). All ARV induce drug resistance and have important side effects that challenge the need to develop new and more efficient medications. In order to optimise efficacy and to limit the chances of drug resistance, a combination of ARVs are used, referred to as combination therapy or HAART. ARV have also been associated with a range of adverse effects. Some common but mild adverse effects occur in the early stages of ART may be transient (Montessori et al., 2004).

Anaemia caused by the myelosuppressive effects of some ARV is among the more serious adverse effects together with peripheral neuropathy and hypersensitivity reactions, and metabolic disturbances such as hepatic steatosis, hyperlactataemia, lipodystrophy, hyperlipidaemia, glucose intolerance, bleeding disorders, osteoporosis, skin rash and dementia (Montessori et al., 2004).

It has been suggested that immunomodulatory approaches may offer benefit in combination with effective ART. IL-2, a cytokine released by activated CD4+ cells, that regulates T-cell proliferation and maturation, has been shown to increase CD4+ counts (Kovacs et al., 1996; Davey et al., 1999; Hecht et al., 2003), expand memory and naive CD4+ pools and decrease T-cell activation (Hecht et al., 2003) in individuals with controlled HIV viremia. However, IL-2 administration has adverse effects and no clinical benefits of IL-2 have yet been demonstrated. Techniques of immune manipulation through infusion of antigen-presenting cells that have been activated in vitro (Lu et al., 2003), infusion of expanded (Levine et al., 1996) or activated (Levine et al., 2002) CD4+ cells, and genetic manipulation of cells to induce anti-HIV CTL activity (Roberts et al., 1994) are also under investigation.

Dietary and nutritional manipulations can themselves exert powerful effects upon

immune responses. It is unlikely that sub-Saharan Africa will have the capability to adopt complex drug regimens in the near future, but there is the real possibility that improving diet might confer some benefits. Thus, although ARV are currently seen as crucial fighting the HIV pandemic and prolonging lives, there is still the need to consider and explore the use of wider strategies that combine medical therapy, psychosocial care and nutrition in managing the condition. The emergence of HIV/AIDS has added further emphasis to the constant challenge to provide sufficient food and nutrition for all people, and especially to meet basic needs of people in Africa.

Protease inhibitors (PIs), which prevented viral replication by inhibiting the activity of HIV-1 protease, were introduced in the mid-1990s, and this revolutionized the treatment of HIV (Hammer et al., 1997). As the risk to developing viral resistance grew effective combination antiretroviral therapy became the standard of care, especially, in the United States and Western Europe. Very soon thereafter, countries in which effective ART was available began to note sharply declining morbidity and mortality associated with HIV infection (Palella et al., 1998). However, in most resource-poor settings, HIV/AIDS flourishes amidst poverty and disease. In such places also malnutrition and infectious or communicable diseases are rife.

The many challenges for care also revolve around effective treatment for malnutrition, wasting/weight loss and nutrient imbalances along with anaemia, but responses have remained largely ineffective (UNAIDS, 2005, 2006; Doukas, 1992; Sullivan et al., 1998; Semba and Gray, 2001; Mildvan, 2003). Since the inception of HAART and the use of potent prophylactic medicines, anaemia and weight loss are still recorded in HIV infection but tend to be less severe and often less progressive (Volberding, 2000).

Changes in the body composition, such as lipodystrophy and associated endocrine and metabolic consequences, have also been noted (Ware et al., 2002). It is for these reasons that the need to ensure proper nutrition among persons with HIV/AIDS has to be considered an essential part of a coherent response to the HIV/AIDS pandemic, especially in Africa. The response to this complex emergency has varied amongst countries and as a general commitment greater effort is needed for the prevention and treatment of HIV (United Nations, 2007;UNAIDS, 2008).

### **2.3.6 HIV/AIDS – IMPACT OF THE INFECTION AND DISEASE**

HIV/AIDS has a great impact on the ability of households and communities to ensure food and nutrition security. Meanwhile, achieving food and nutrition security and managing nutrition-related complications carry as much importance in HIV infection as

managing the range of complications. For many in resource-poor environments achieving optimal nutritional status is a considerable challenge, which is more difficult for those living with HIV/AIDS. There is evidence that HIV infection has direct nutritional consequences; reductions in food intake, increases in resting energy expenditure, nutrient malabsorption and loss, and complex metabolic alterations that culminate in weight loss and wasting (Babamento and Kotler, 1997; Macallan, 1999).

#### **2.3.6.1 HIV/AIDS and food and nutrition security**

Food security may be defined as “the state in which all people have both physical and economic access to sufficient food to meet their dietary needs for a productive and healthy life at all times” (Bonnard, 2002), a state which can only be achieved depending on food being available, accessible and utilisable by the body. Nutrition security for a household is achieved when secure access to food is coupled with a sanitary environment, adequate health services, and adequate care to ensure a healthy life for all household members (Gillespie and Kadiyala, 2005).

Food and nutrition insecurity has been linked to the transmission of HIV and to the poor outcomes associated with the HIV disease (UNAIDS/WHO, 2002; 2003; World Food Programme (WFP), 2004). Widespread AIDS often occurs in the context of social and economic circumstances such as hunger, food and nutrition insecurity, poverty and poor social infrastructure. Conversely, where the prevalence of HIV is high, all dimensions of food and nutrition security are affected (Committee on World Food Security (CWFS), 2001).

In developing, transitional and developed countries alike, poor nutritional status arising from food and nutrition insecurity can be related to psychosocial and economic issues (American Dietetic Association (ADA), 2004). Thus, lack of education, food access, economic support and access to health care services may increase the risk of poor nutrition (ADA, 2004). HIV/AIDS and food and nutrition insecurity are entwined in a vicious cycle, with food insecurity heightening susceptibility to HIV exposure and infection, and HIV/AIDS heightening vulnerability to food insecurity (Loevinsohn and Gillespie 2003). Populations that are food and nutrition insecure tend to develop survival strategies that expose them to greater risks of infection (Loevinsohn and Gillespie, 2003; Harvey, 2003). Some may migrate to urban slums where access to health care services is limited and may be drawn into the drugs or sex trade, thus increasing their risk of exposure to infection (Loevinsohn and Gillespie, 2003; Harvey, 2003). Children may lose out on educational opportunities in order to support the family, while adults responsible for childcare become infected and die resulting in a

diminishing and crumbling social and economic infrastructure (Shah et al, 2002). Maintaining nutritional status for PLWHA becomes a daunting task, especially for the most vulnerable, because all aspects of food and nutrition security – availability, stability, access and use of food and healthcare services - are affected (CWFS, 2001). PLWHA have to deal with economic insecurity, social isolation and stigma, incarceration, limited diversity in their diets, poor cooking facilities and skills and other medical conditions and disabilities (ADA, 2004). In order to improve the health and nutritional status of PLWHA, it is required that the barriers to food and nutrition security are resolved (Ontario Public Health Association Food Security Work Group (OPHA), 2002; ADA, 2002; 2003).

#### **2.3.6.2 HIV/AIDS and nutritional status**

The case has been made for nutrition to be considered as a priority in the agenda for managing HIV/AIDS (WHO, 2003; WHO, 2005; ADA, 2002; 2003). When the nutritional needs of the body are satisfied there is enhanced resistance and decreased susceptibility to any infection, including HIV-infection. A well nourished individual is more likely to have a delayed onset or slower progression of clinical disease and greater resistance to co-infections (ACC/SCN, 1998; Williams, 1999; WHO, 2003).

Given its nature, there may be early or hidden nutritional consequence in HIV before the presence of infection becomes evident (Beach et al., 1992; Semba and Tang, 1999). In apparently healthy populations, such as the THUSA population, subclinical inflammation can alter the acute phase response which in turn results in nutrient shifts and deficiency states (Thurnham and Northrop-Clewes, 2007). As nutrients play critical roles in ensuring a competent immune system, the indirect effects of HIV infection on nutrition can further compromise immune function (WHO, 2005).

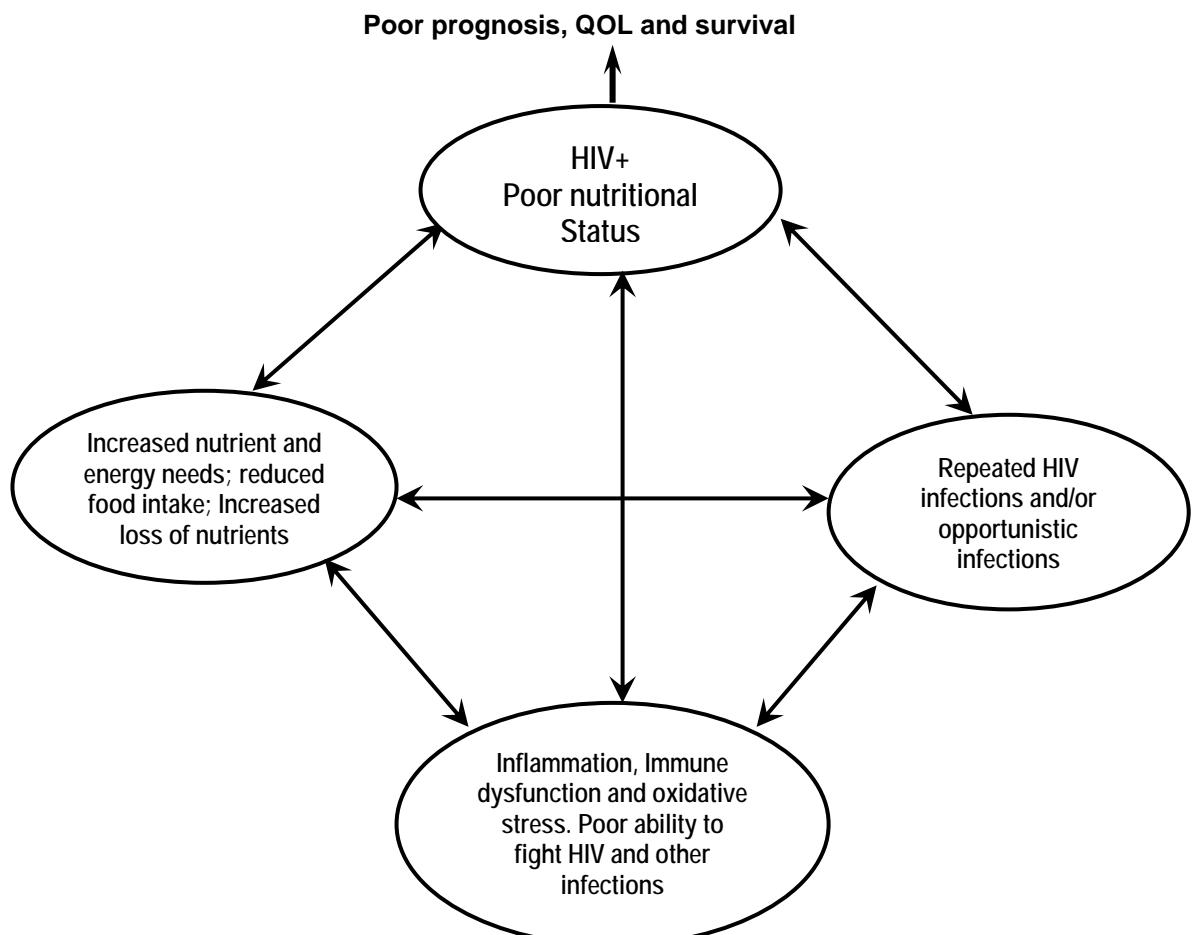
Independent of HIV infection, malnutrition can lead to immune dysfunction (Nutritionally acquired immune deficiency – NAID) (Calder and Jackson, 2000; Keusch, 2003). The maintenance of physical and chemical barriers are the first line of defence against infection, requiring intact skin surface and lining of the naso-oesophageal, gastrointestinal and urino-genital tracts (Calder and Jackson, 2000). An inflammatory response or immune activation requires a high rate of cellular turnover placing an increased demand on nutrient and metabolite utilisation (Fraker, 1994; WHO, 2005).

The availability of an appropriate mix of nutrients is necessary to optimise the response, enable the synthesis of immunoglobulins, acute phase proteins, cytokines and other cell mediators and maintain antioxidant mechanisms to protect against free-

radical damage (Allard et al., 1998). Antioxidant mechanisms include enzymes with metals (e.g. Zn, Fe, Cu, Mn and Se) as cofactors, vitamins such as vitamins E and C, and small peptides like glutathione (Allard et al., 1998).

Poor nutrition may express itself non-specifically as involuntary weight loss or wasting of body tissues (Williams, 1999) and by weakening the immune system increases the opportunity for virus replication and ease of other infection (Semba and Tang, 1999). A well-nourished, HIV sero-positive individual with a viral load that is controlled is better able to cope with the effects of HIV infection and minimise the effects of immune compromise. It is less clear the extent to which such an individual has significantly increased nutritional requirement, or if this relates at all to viral load, decline in immune function, specific treatment regimen, viral resistance or an active secondary infection (Mulligan et al., 2000). On their own, infections, including HIV, can have adverse effects on nutritional status by increasing nutrient requirements that invariably lead to nutrient deficiencies, and which if sufficiently severe, can impair the resistance of the body to the infections through a reduction in immune competence (Schrimshaw and SanGiovanni, 1997). Further, poor nutritional status affects the ability to cope with antiretroviral drugs and other medications.

**Figure 2.4: Causal inter-relationship between HIV infection and nutritional status**



The effects of HIV infection on the nutritional status of the individual may be seen as a causal web that follows a number of pathways depending on the stage and severity of the infection as illustrated in Figure 2.3 above. Thus, an HIV infected individual who starts off with a reasonable nutritional status may progress to poor nutritional status as the infection puts extra demands on the immune system, increases the body's demand for energy and nutrients, limits digestion and absorption through the gastrointestinal tract, or increases nutrient losses from the body. Separately and together these eventually lead to a poor nutritional state and weight loss in the infected individual, which further weakens the immune system, affecting prognosis, QOL, and survival.

#### **2.3.6.2.1 Mechanisms for the impact of HIV/AIDS on nutritional status**

In HIV infection, nutritional status, whether marked by loss of body weight, body cell mass, absolute and/or relative lean body mass, or changes in albumin, pre-albumin or C-reactive protein, has been shown to be a significant predictor of survival in infected adults after adjusting for CD4+ cell counts and history of secondary events (Melchior et al., 1999).

In general, there are changes in five major processes through which infection, including HIV, tends to impair the individual's nutrition or nutritional status:

- Changes in nutrient intake,
- Changes in digestion and absorption,
- Changes in demand for nutrients,
- Changes in delivery of nutrients to tissues,
- Increased losses of nutrients.

##### **2.3.6.2.1.1 Changes in intake**

Infection and illness will cause nausea, vomiting and loss of appetite or anorexia, which with other eating difficulties lead to inadequate food intake. People with HIV/AIDS most commonly eat less because of a poor appetite. OI contribute by causing nausea, malaise, and fever. Specific local infections, such as oesophageal candidiasis, may cause a sore mouth, stomach pain or pain during eating (AIDS Institute, 1995). Systemic illnesses (including HIV itself) generate a systemic acute inflammatory response, and psychological conditions (such as depression) that impair patients' ability to nourish themselves. Following an episode of illness, appetite may improve with a chance to recuperate. The need for more food of better quality is greater at this time, but people with HIV/AIDS may not be able to access extra food of quality especially against a background of poverty and food insecurity. In many instances, social considerations like stigmatisation, isolation and active drug use may play an

important role in limiting access to food for PLWHA (AIDS Institute, 1995). In some settings, stigma leads to loss of employment, or the support of family or community. People too ill to work lose their jobs and farmers who are ill are unable to grow enough food for themselves and their dependants. Infection with HIV/AIDS leads to a redirection of resources away from food to care. There is a difficult choice between paying for medicine and paying for food. Care giving can divert other family member's time and energy away from employment or food provision. Lack of help for food shopping and preparation as well as lack of or inadequate food preparation and cooking facilities are also factors that can limit food intake in PLWHA.

#### **2.3.6.2.1.2 Changes in Digestion and Absorption**

Even when food is available, it may be poorly absorbed in persons with HIV/AIDS. Intestinal malabsorption and nutrient loss is common. While severe diarrhoea and malabsorption may be due to OI or intestinal parasites such as cryptosporidium, some of the altered absorption appears to be a consequence of HIV infection itself (Kotler et al, 1990). The virus can damage the intestinal villi, and inflammation resulting from the infection can also damage gut tissue and reduce absorption (Kotler et al, 1990). Frequently, small bowel transit time is accelerated, particularly among children with severe diarrhoea and Intestinal mucosal digestive enzymes may be reduced or less active (Kotler et al, 1990). These changes in the gut affect the body's ability to utilise dietary fat and carbohydrates. A study has reported that people with HIV have high levels of faecal fat that is unrelated to fat intake or the presence of any intestinal infection other than HIV itself (Hsu et al., 2005).

#### **2.3.6.2.1.3 Changes in Demand, altered delivery or increased loss**

HIV infection and replication affect metabolism in a variety of ways some of which may be caused by the inflammatory immune response mediated by cytokines. Cytokines are chemical messengers and growth factors produced by lymphocytes and macrophages which mediate and regulate the inflammatory immune process (see also section 2.5.3.2.1). These inflammatory immune responses begin as soon as a person is infected with HIV and they increase the nutrient requirements of the host. There may be an increase in REE up to 10% or more during opportunistic or inter-current bacterial infections. Any concomitant reduction in physical activity may mean that total daily energy expenditure is not changed, or even reduced (Hsu et al., 2005).

There are endocrine or hormonal changes in patients with HIV/AIDS - such as hypogonadism, (reduced or absence of secretion of hormones from the sex glands which may occur in both males and females). In males, testosterone levels in

particular may be depressed, accompanied by a substantial loss of muscle or LBM (Hsu et al., 2005).

### **2.3.6.2.2 HIV/AIDS and the wasting syndrome**

The nutritional consequence of wasting and/or weight loss has been recognized as a frequent and major complication of HIV infection (Kotler et al., 1989), and is a common feature in PLWHA. In HIV infection, the “syndrome of wasting” is defined by a body weight loss of 10% of the usual bodyweight and associated with chronic diarrhoea and/or fever and/or asthenia (loss of body strength) with lack of other detectable causes (CDC, 1987). Wasting and/or weight loss may occur from the onset (Ott et al., 1993) or more frequently in the later stages of HIV disease (Ysseldyke, 1991).

As an AIDS-defining condition, wasting carries a high risk of mortality (Kotler et al., 1989; Kravik et al, 1997; Wheeler et al., 1998; Melchoir et al, 1999). In fact weight loss and muscle wasting which were unique identifying characteristics of the HIV disease early in the epidemic (Mhiri et al., 1992), still remain a significant clinical problem in this modern era of potent ARV (Wanke et al., 2000). The early days of the HIV/AIDS epidemic saw a rise in the syndrome of wasting even in the developed world. However, the inception of HAART brought an initial decline in its incidence, especially, in western countries, and an increase thereafter (Wanke et al, 2000).

The syndrome of wasting is usually preceded by loss of appetite, repeated infections, weight fluctuations and changes in body composition (Babameto and Kotler, 1997). Wasting and loss of body weight are nearly always associated with deficiencies of vitamins and minerals (Babameto and Kotler, 1997). Thus, wasting and/or weight loss in HIV infection develop from a number of processes that are not mutually exclusive: reduced food intake, nutrient malabsorption, metabolic changes, hypogonadism and responses to cytokines (Salomon et al., 2002).

### **2.3.6.3 HIV/AIDS and micronutrient deficiencies**

The cyclical relationship showing that infection may lead to micronutrient deficiencies and that the presence of micronutrient deficiencies affects the risk of infectious diseases (Scrimshaw et al., 1968, Friis, 2005) reflects the complex interrelationship between malnutrition and infection (Semba and Tang, 1999). In HIV infection and AIDS, micronutrient deficiencies may be very common, but are not necessarily evident, as specific signs or symptoms may often be lacking (Fawzi, 2003).

Prior to the widespread use of HAART, a high prevalence of low micronutrient levels was noted in the HIV population (Friis, 2005). Generally, micronutrient deficiencies are

common in parts of the world where access to ART is limited, such as in sub-Saharan Africa (Tang et al., 2005). Epidemiological evidence indicates that low dietary intakes or blood levels of several vitamins and minerals may be associated with increased progression of HIV disease (Friis and Michaelsen, 1998; Tang et al., 1997; Tang and Smit, 1998).

In both developed and developing populations, PLWHA tend to have decreased intake and absorption, increased urinary loss and low blood levels of specific vitamins (Vitamins A, B complex, C, E, folate and beta carotenes) and minerals (zinc, selenium and magnesium), in part a consequence of the acute phase response (Friis and Michaelsen, 1998; Tang and Smit, 1998, Friis, 2005). Many of these micronutrients play defined roles in immune function and regulatory metabolic processes (Friis and Michaelsen, 1998; Tang and Smit, 1998). Vitamin A, for instance, have been shown to play a role in the growth and function of T and B lymphocytic cells, in antibody responses and epithelial integrity and health (Tang et al., 1997). Some micronutrient deficiencies have been associated with declining CD4+ T cell counts as well as increased disease progression (Pacht et al., 1997; Tang et al., 1997; Baum et al., 1997), and also with increased risk of mortality (Semba et al., 1995a; 1995b).

Some micronutrients help maintain immune function by acting as anti-oxidants (e.g. vitamins E, C, beta carotenes and selenium and other components like glutathione). They neutralise free radicals generated during the inflammatory burst (Friis and Michaelsen, 1998; Semba and Tang, 1999). However, some other micronutrients like iron are pro-oxidants and can help to generate free radicals. Oxidative stress results from an imbalance between pro- and anti-oxidant factors, either through overproduction of reactive oxygen species or deficiency of antioxidant protection, and this stress promotes HIV replication (Friis and Michaelsen, 1998; Tang et al, 2005). In HIV infection and AIDS, a vicious cycle develops in which undernourished HIV-infected persons with micronutrient deficiencies, become further immunosuppressed and increased oxidative stress accelerates HIV replication and CD4+ T cell depletion (Semba and Tang, 1999).

There have been difficulties in establishing causal relationships between specific micronutrient deficiencies, the transmission and adverse clinical outcomes in HIV-infected persons. In part this is because the pattern of deficiencies associated with HIV infection varies across populations and according to disease stage (Fawzi, 2003; Friis, 2005). Further, structured intervention studies designed to demonstrate the effectiveness of specific therapeutic supplementation are few and inconsistent (Tang et

al, 2005). In reviewing the evidence, Tang and colleagues (2005) established that most studies in which the micronutrient status of the HIV/AIDS populations had been explored had been based upon assays for plasma or serum levels of nutrients. The problem is that for most of these measures interpretation is problematic in infected persons where low levels do not necessarily indicate poor status, but rather they may be lowered as a part of the acute phase response (Tang et al., 2005). Under such circumstances some micronutrients may be depleted, but others may shift between body compartments (Wellcome Trust and USAID, 1999).

Notwithstanding these limitations, assessment of serum or plasma levels of micronutrients has revealed that deficiencies are likely to be common among HIV infected persons, especially, in those who are under-privileged and undernourished (Kotler and Grunfield, 1995; Liang et al., 1996). Many of such studies have associated lower plasma or serum levels of vitamin A (Semba et al., 2002), vitamin E (Tang et al., 1997a) and vitamin B<sub>12</sub> (Tang et al., 1997b) with increased disease progression in HIV infection. A low vitamin A status, in particular, is associated with increased risk of MTCT of HIV (Semba et al., 1994), HIV viral load in breastmilk and vaginal secretions (Nduati et al., 1995), progression to AIDS (Tang et al., 1993) and adult survival (Semba et al., 1994). Selenium, vitamin B<sub>6</sub>, vitamin B<sub>12</sub> and Cu deficiencies have also been reported in studies on HIV infected patients (Baum et al., 1991; Beach et al., 1992). In a cross sectional study, Zn deficiency was observed among 23% of HIV infected patients (Wellinghausen et al., 1999). In contrast, a study has shown a significant association between increased zinc, vitamin A and niacin intakes and the progression to AIDS (Tang et al., 1993). Nevertheless, studies relating adverse effects of micronutrient deficiencies or lower intake seem to tip the balance of the evidence in favour of micronutrient supplementation.

It is precisely on this basis that it was proposed that a regular supplementation with micronutrients in HIV/AIDS was likely to be of benefit, especially in resource-poor communities (Fawzi et al., 1999; Semba and Tang, 1999). Indeed, supplementation trials in HIV-infected populations appeared attractive because of their affordability and relative ease of administration. However, initial results have been mixed.

Supplementations with vitamin A, for instance, have been beneficial for some HIV-related outcomes, whilst for others it was unremarkable or even adverse (Tang et al., 2005). In infected children, supplementation with vitamin A may reduce diarrhoeal morbidity (Coutsoudis et al., 1995), improve immune status (Hussey et al., 1996) and reduce mortality (Fawzi et al., 1999). In adults it did not have any impact on viral load

(Semba et al., 1998), immune status (Fawzi et al., 1998), diarrhoeal morbidity (Kelly et al., 1999), prenatal and postnatal morbidity in women (Kennedy et al., 2000) and MTCT of HIV (Coutsoudis et al., 1999; Fawzi et al., 2000). In contrast, one study has shown that a high dose supplementation may even increase mortality (Tang et al., 1996).

With vitamin E, supplementation may reduce oxidative stress and HIV viral load (Allard et al., 1998). Late-stage oral supplementation with vitamin E, however, did not affect diarrhoea morbidity or mortality (Kelly et al., 1999). With MTCT, vitamin E intake was associated with improving mastitis in breastfeeding women (Filteau et al., 1999), but similar results were found for intakes of selenium, vitamin A and beta carotene (Semba and Neville, 1999).

The case for vitamin B<sub>12</sub> and folate supplementation has not been clearly established for PLWHA but there is the need to consider these nutrients because of their potential interaction with HIV medications and their role in intermediary metabolism. There are several other micronutrients which have been associated with HIV/AIDS although the link or relationships between these micronutrients and the disease are not very clear. For instance, observational studies in industrialised countries have shown that vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub> and C deficiencies may be common in HIV-infected men, with a high or increased intake tending to slow the progression of the disease (Tang et al., 1996; Kanter et al., 1999).

The study on selenium, which is known to play an important role in reducing oxidative stress, has been a limited. However, its deficiency has been associated with HIV-related deaths (Baum et al., 1997). Oral supplementation of selenium to deficient PLWHA significantly increased antioxidant enzyme function (Delmas-Beauvieux et al., 1996). With regards to zinc, low serum levels have been associated with HIV disease progression and mortality (Baum et al., 1996), and zinc supplementation was beneficial in AIDS patients (Mocchegiani et al., 2000). However, intakes over and above the body's normal requirement from the diet and/or supplements were associated with reduced survival in HIV-infected men (Tang et al., 1996), possibly because the virus has its own requirement for zinc (Baum et al., 2000).

Because many micronutrient deficiencies occur together, the impact of multiple micronutrient supplementations on HIV-related outcomes is an important consideration. One study showed that multiple supplements slowed disease progression to AIDS and deaths, especially when given with other drug therapies (Kanter et al., 1998). In pregnant women, multiple micronutrient supplements appeared to have beneficial

effects on the offspring as well as improving the immune status of postnatal HIV-infected women (Fawzi et al., 2000).

Since HIV infection is associated with an increased oxidative stress and a subsequent decline in antioxidant nutrients, administering ARV might be expected to lead to reduction in oxidative stress and an improvement in antioxidant nutrient levels. However, this is seemingly not the case as the medications themselves increase oxidative stress and cause cellular toxicities (Tang et al, 2005). The available data suggest that any improvements in the micronutrient status or antioxidant nutrient levels with HAART is inconsistent and may be described as modest at best (Tang et al, 2005).

With the advent of HAART, HIV has become a more manageable chronic disease, and concerns around metabolic changes such as lipodystrophy syndrome and others, together with factors that affect the QOL have taken prominence over micronutrient deficiencies. Anaemia, which may manifest from many causes including the direct and indirect effects of the HIV, has also persisted, and remains a concern of importance among PLWHA (Castaldo et al., 1996; Volberding, 2000; Volberding et al., 2004).

The important role played by iron in the regulation of the body's internal milieu makes it a special micronutrient. A deficiency of iron is associated with anaemia and with impaired immune function among others. Iron deficiency anaemia is often prevalent amongst the same populations where HIV infection is common and, therefore administering iron supplements to such populations may appear reasonable. However, some caution is required since iron can act as a pro-oxidant and possibly promote viral replication. In fact some retrospective studies have suggested that a high iron status may hasten disease progression and increase mortality in infected adults (see section 2.5.3.1 on iron).

#### **2.3.6.4 HIV/AIDS and anaemia**

Since several million people worldwide are infected with HIV, and anaemia is a common manifestation of HIV/AIDS, it follows that a large percentage of those infected with the HIV will have anaemia. In developing countries, where both conditions are prevalent, It is likely that the numbers with both HIV and anaemia will be high (Lewis, 2005). Although anaemia is not historically considered a serious complication of HIV infection, it is now receiving much attention because of its significant impact on clinical outcomes and QOL (Belperio and Rhew, 2004). HIV-infected individuals with anaemia are most likely to experience debilitating symptoms that may impair their functionality,

decrease their sense of wellbeing and affect their QOL (Cella, 1996). Indeed, the impact of anaemia on the QOL and survival in HIV infection is great (Abrams et al., 2000) as anaemia induces fatigue and other symptoms which greatly impair QOL (Ludwig and Strasser, 2001). Although in HIV disease fatigue may be due to various factors, anaemia is a major contributor (Breitbart et al., 1998; Barroso, 1999).

## **2.4 THE GENERAL CHARACTERISTICS OF ANAEMIA**

Anaemia is characterised by a reduction in RBC mass (Phillips and Groer, 2002). Haemoglobin is the major carrier of oxygen in the blood and is located within the RBC. Its primary function is to deliver oxygen to the tissues of the body, thereby enabling cellular oxidative phosphorylation and generating energy for all cellular work. Any decrease in red cell mass reduces the amount of haemoglobin available and therefore reduces the amount and rate at which oxygen can be delivered to the tissues. Thus, any limitation on this fundamental biological function, with the associated reduction in oxygen supply may be expected to have deleterious effects. This state of a functionally significant reduction in cell mass characterises anaemia.

### **2.4.1 THE RED BLOOD CELL OR ERYTHROCYTE**

Erythrocytes or RBC are biconcave discs approximately 7 to 8  $\mu\text{m}$  in diameter containing approximately 300 molecules of haemoglobin. Their average life span is 120 days. With ageing the content/activity of intracellular enzymes decreases, metabolism slows, cellular integrity fails as the plasma membrane becomes osmotically fragile (Sacher and McPherson, 2000; Thurnham and Northrop-Clewes, 2007). With about 1% of erythrocytes being removed from the circulation daily, there is the need to produce the equivalent of 2,400,000 new erythrocytes per second, to maintain a steady state. Senescent red cells are removed from the circulation and broken down by the RES, such as the spleen.

Haematopoiesis refers to the production and development of erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets), from common, pluripotent progenitor stem cells, within the tissues of the hematopoietic system, consisting of the bone marrow, liver, spleen, lymph nodes, and thymus gland (Sacher and McPherson, 2000). Stimulation of the pluripotent stem cells by growth factors, like colony-stimulating factors and interleukins, give rise to myeloid stem cells that differentiate into erythrocytes, leukocytes, or platelets or lymphoid stem cells that become T- or B-lymphocytes (Sacher and McPherson, 2000). The production and development of erythrocytes (erythropoiesis) takes place under the influence of at least four cytokines; erythropoietin, interleukin-3, granulocyte-monocyte colony-stimulating

factor, and monocyte-macrophage colony-stimulating factor, that stimulate precursors to proliferate, and differentiate to form activated erythrocytes (Sacher and McPherson, 2000). The primary cytokine responsible for erythropoiesis is erythropoietin, synthesis and/or release of which is increased, mainly from cells associated with the juxtaglomerular apparatus of the kidneys, under the stimulus of tissue hypoxia (Sacher and McPherson, 2000). Erythropoietin in the circulation acts on the target tissue, the bone marrow, to stimulate the differentiation of stem cells into erythrocytes (Sacher and McPherson, 2000).

#### **2.4.2 MECHANISMS FOR ANAEMIA**

Anaemia occurs when the rate of RBC formation fails to keep pace with the rate of its degradation or loss. According to Volberding and colleagues (2004), the three main mechanisms that may contribute independently or together for anaemia to occur are:

- Lower than necessary rates of RBC production (or ineffective erythropoiesis)
- Higher than usual rates of RBC destruction (haemolysis) or
- Blood loss or bleeding (haemorrhage) which could be either acute or chronic

##### **2.4.2.1 Decreased RBC production**

If the bone marrow fails to produce enough red blood cells to keep up with the body's needs, then anaemia will result. Inadequate production may result from inadequate signal for red cell production or an unresponsive marrow or because of conflicting signals or if the demand for nutrients is too high or there is inadequate energy and/or nutrients available to meet the demand. Common nutritional anaemias are a consequence of limited availability of nutrients such as iron, vitamin B<sub>12</sub> or folates. Infection or inflammation can suppress RBC production. Some pharmacological agents can impair RBC production, for example some drugs used for cancer chemotherapy or some antiretrovirals used in HIV/AIDS treatment. Impaired renal function might limit the availability of erythropoietin.

##### **2.4.2.2 Increased RBC destruction**

If there is increased destruction of RBC or their life span is reduced from the usual 120 days then it may not be possible to maintain an adequate rate of replenishment leading eventually to anaemia (Sacher and McPherson, 2000). This may occur because of changes intrinsic to the cell itself (abnormal type of haemoglobin, e.g. sickle cell anaemia (HbSS), challenges from outside the cell such as infection, or destruction by the body's own immune system (as in autoimmune disorders and perhaps HIV/AIDS).

Sickle cell anaemia is a single gene defect of haemoglobin and under low oxygen tensions the haemoglobin polymerises, causing the RBC to become crescent-shaped and making the cells more fragile, more likely to be damaged and removed from the circulation. Glucose-6-phosphate-dehydrogenase (G-6-P-D) deficiency is an inheritable disorder in which impaired activity of the enzyme leads to cellular fragility and a liability to disruption under oxidative stress. Malaria is an example of an infection where parasitization of the RBC leads to increased damage and loss.

#### **2.4.2.3 Blood loss or bleeding**

Bleeding or blood loss is an obvious cause of anaemia and can occur for a variety of reasons. In women, there is physiological blood loss with menstruation or delivery after pregnancy. Pathological blood loss, especially through the gastrointestinal tract can be common in enteric infection such as hook worm. Other causes of blood loss, even if slight can lead to anaemia, especially when sustained over a long period of time. This is more likely to lead to anaemia where the rate of blood loss exceeds the capacity for normal replacement, or where the bone marrow is not working properly or where the fragility of red cells is increased.

### **2.4.3 CLASSIFICATION AND COMMON TYPES OF ANAEMIA**

Anaemia has been classified in many different ways, for example, based on RBC morphology or the aetiopathology or other specific characteristics (Breymann, 2002). Two systems frequently used have emphasised either aspects of RBC kinetics or morphology (Breymann, 2002).

When RBC turnover is increased there is greater release of relatively immature forms, with persistence of nuclear material into the circulation, leading to an increased reticulocyte count. On the other hand, morphological characterisation assesses the size of the RBC, reflected in the mean corpuscular volume (MCV) (Breymann, 2002). Red blood cells are small in microcytic anaemia (under 80 fl); normal size (80-100 fl) in normocytic anaemia; and larger than normal (over 100 fl) in macrocytic anaemia. Microcytic anaemia results primarily from an insufficiency in haemoglobin synthesis, most commonly from iron deficiency, but also from other defects in haem and globin (thalassaemias) synthesis or toxicities that affect synthesis (e.g. sideroblastosis) (Breymann, 2002). Normocytic anaemia occurs when the overall Hb levels are decreased, but the red blood cell size (MCV) remains normal, for example with acute blood loss, haemolytic anaemia, the anaemia of chronic disease/inflammation, or aplastic anaemia (bone marrow failure) (Breymann, 2002). .

Macrocytic anaemia is primarily due to a failure of DNA synthesis with preserved RNA synthesis, resulting in restricted cell divisions of the progenitor cells (Breymann, 2002), often due to either deficiencies of vitamin B<sub>12</sub> or folate. The use of DNA synthesis inhibition drugs (e.g. AZT), can also lead to a macrocytic anaemia.

Of the many different types, anaemia related either to iron deficiency or to chronic inflammation are more common and especially widespread in populations suffering from malnutrition and with a high prevalence of infectious diseases (Thurnham and Northrop-Clewes, 2007). Many authorities consider that iron deficiency is the most important contributor to the global burden of anaemia (Krishnaswamy, 2003; Kraemer and Zimmermann, 2007).

#### **2.4.3.1 Iron deficiency and anaemia**

##### **2.4.3.1.1 The functional role of iron in the body**

Iron is abundant in the earth's crust and a feature of living organisms with versatile biologic activity, nevertheless human deficiency is widespread (Krishnaswamy, 2003; ACC/SCN, 2000).

Iron fulfils several vital functional roles in biological systems. It provides a specific binding site for oxygen in the haem moiety of haemoglobin found in erythrocytes and myoglobin in muscle and thus serves as either a carrier or storage of oxygen required by the tissues (Hallberg, 1982). It is incorporated into haemoglobin within the bone marrow in the presence of adequate amounts of other nutrients which include vitamins A, B<sub>12</sub>, C, E and folate (Lubin et al., 1997). Iron serves as part of iron-containing enzymes, the cytochromes, which transport electrons within cells and is an integral part of important enzyme systems in various tissues (FAO/WHO, 2002). It is present in the liver as stored, but mobilisable iron (ferritin and haemosiderin) and is transported between different compartments in the body bound to the plasma protein transferrin (FAO/WHO, 2002).

The property that gives iron its functional relevance is its ability to undergo reversible one electron redox (reduction/oxidation) transfer between two common oxidative states, Fe<sup>2+</sup> (ferrous) and Fe<sup>3+</sup> (ferric) (Aisen, 1994). By catalysing the production of reactive oxygen species (unstable intermediates or oxygen species with unpaired electrons) or free radicals, iron plays a critical role in macrophage mediated cytotoxicity, providing protection against potential pathogens (Roen et al., 1995; Pacelli et al., 1995).

However, the free radicals, especially hydroxyl free radical (OH.) and superoxide (O<sub>2</sub>.) produced from the catalytic process with iron are also capable of causing destruction to cellular organic molecules such as proteins and DNA (Roen et al., 1995; Pacelli et al., 1995).

Iron is an essential nutrient for all known pathogens and many have developed complex mechanisms to acquire it, allowing them to thrive successfully in iron restricted environments (Bullen and Griffiths, 1999). Therefore, the availability of free iron in the body potentially increases their virulence and also increases the potential for excessive free radical production (Jackson, 2007). The human body has developed complex metabolic processes to absorb, transport and store iron to ensure a ready supply for bodily functions whilst limiting its easy availability to potential pathogens or for free radical formation (Lynch, 2007).

Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units each containing one haem group and one amino acid chain (globin) (FAO/WHO, 2002). The iron-containing oxygen storage protein in the muscles, myoglobin, is similar in structure to haemoglobin but has only one haem unit and one globin chain, whilst several iron-containing enzymes, i.e. the cytochromes, also have one haem group and one globin protein chain (FAO/WHO, 2002). The main iron storage protein is ferritin, which is stored predominantly in cells of the reticuloendothelial system (macrophages of the spleen, liver, bone marrow) as well as in skeletal muscle (Bothwell et al., 1979). Nevertheless, all nucleated cells are able to synthesise ferritin for managing their intracellular iron economy. Haemosiderin is an amorphous storage form of iron which is water insoluble and less rapidly available (Wixom et al., 1980).

#### **2.4.3.1.2 Intake and metabolism of iron**

Within and across populations, iron status may vary over a continuum from overt deficiency to excess, and in some pathological conditions, its distribution in various body compartments may be abnormal. A normal or healthy iron status implies that red blood cell production occurs without being limited by the availability of iron, and iron reserves are sufficient to rapidly meet the demands from limited acute blood loss (Worwood, 1997).

Under optimal nutritional conditions, iron is stored in the tissues (mainly in the RES) as ferritin and haemosiderin reserves, from where it is mobilised when insufficient iron is available to the body. In the short term this iron is provided through release of the

metal from ferritin reserves. However, the primary source of iron to the body is the diet, which is absorbed in both organic (haem) and inorganic (non-haem) forms through the gastrointestinal tract (Beard et al., 1996). Regulation of body content is determined by the rate of iron absorption, as there are no regulated mechanisms through which iron can be lost from the body.

Iron is lost passively from the body mainly through cell loss from the skin and the interior surfaces of the body - intestines, urinary tract, and airways. This generates an estimated need of about 14 µg/kg body weight/day (Green, 1968). Though dietary iron requirements for normal persons are low (Andrews, 1999), the iron needs for different groups of people may vary widely depending on their requirements for growth or to meet abnormal losses. Humans lack a regulated pathway for iron excretion and iron balance is achieved through tight regulation on its rate of absorption (Nicholas et al., 2002), which ensures that any variation in the body's needs can be met across a wide range of dietary intakes (Deiss, 1963). Critical control of absorption has been linked recently to a peptide hormone, hepcidin, produced by the liver (Fleming and Sly, 2001; Ganz, 2003; Nemeth et al., 2003).

Both haem iron and non-haem iron are normal constituents of the diet but their absorption is different (Hallberg, 1981). The dietary sources of haem iron are mainly haemoglobin and myoglobin from consumption of meat, poultry, and fish. Cereals, pulses, legumes, fruits, and vegetables constitute the main sources of non-haem iron. Absorption of haem iron from meat-containing meals varies from 10 to 40%, and is on average 25% (Hallberg, 1977; Hallberg et al., 1999). Meat promotes the absorption of non-haem iron (Hallberg, 1979). In contrast, calcium is the only dietary factor identified that influences the absorption of haem iron negatively (Hallberg, 1998).

Quantitatively, non-haem iron is often the main source of dietary iron, the absorption of which is influenced by a number of factors other than the individual's iron status (FAO/WHO, 2002). Reducing substances that keep iron in the ferrous form must be present for non-haem iron to be absorbed (Wollenberg and Rummel, 1987). Ascorbic acid (vitamin C) is the most potent enhancer of non-haem iron absorption with synthetic vitamin C increasing the absorption of iron to the same extent as the native ascorbic acid in fruits, vegetables, and juices (Hallberg et al., 1989). This effect of vitamin C has been considered as one of its important physiological roles (Hallberg et al., 1987).

Other dietary factors or ligands, such as phytates and certain iron-binding polyphenols, strongly bind ferrous ions and subsequently may inhibit absorption of non-haem iron

(Gillooly, 1983; Hallberg et al., 1989). Phytates are found in all kinds of grains, seeds, nuts, vegetables, roots (e.g., potatoes), and fruits, and inhibit non-haem iron absorption in a dose-dependent fashion (Hallberg et al., 1989). Phenolic compounds are a part of the defence system of plants against insects, animals, and humans (Brune et al., 1989). Some, such as those found in tea, coffee, and cocoa may contribute to inhibition of iron absorption (Hallberg and Rossander, 1982).

Normal individuals achieve iron balance over extended periods using a combination of three mechanisms (FAO/WHO, 2002). Firstly, iron derived from effete erythrocytes is efficiently re-utilised following degradation by the macrophages of the RES. The iron is delivered by transferrin in the plasma to red blood cell precursors in the bone marrow or other cells for reuse.

Uptake and distribution of iron in the body is regulated by the synthesis of transferrin receptors (TfR) on the cell surface. This system of internal iron transport controls the rate of flow of iron to different tissues according to their needs and effectively limits the appearance of free iron and the formation of free radicals in the circulation. Secondly, ferritin bound iron acts as a buffered reserve to meet immediate variation in iron demands. Thirdly, iron absorption from the intestines is tightly regulated to maintain reserves (FAO/WHO, 2002). Iron deficiency may develop when other factors over-ride the usual regulation associated with increased iron absorption (Hallberg et al., 1995). Of basal iron losses from the body, about half are from blood, primarily in the gastrointestinal tract (FAO/WHO, 2002).

Iron metabolism is regulated, under normal circumstances, by iron-regulatory proteins (IRP) that bind to specific portions on messenger-RNA (mRNA) and protect them from degradation (Northrop-Clewes, 2008). In a state of iron deficiency (ID), the IRP bind to mRNA and subsequently promote the expression of TfR proteins whilst repressing ferritin synthesis. When levels of iron are adequate in the body, ferritin synthesis is promoted and iron storage predominates. However, during the acute phase of inflammation, the normal control of iron metabolism is re-organised by TNF- $\alpha$  and IL-6 (Northrop-Clewes, 2008). These two cytokines are known to induce ferritin transcription and thereby increase ferritin levels despite the presence of reduced serum levels. These cytokines have been suggested to have an over-riding influence on the induction of ferritin mRNA compared to that from serum iron (Feelders et al., 1998), and serum ferritin concentrations tend to parallel the increased storage and retention by the RES .

#### 2.4.3.1.3 Characteristics of iron deficiency and iron deficiency anaemia

Iron deficiency accounts for about half of over 2 billion people suffering from nutritional anaemia. It is prevalent worldwide with infants, children, adolescents and women of childbearing age constituting the vulnerable groups, and pregnant women being the most vulnerable (Kraemer and Zimmermann, 2007). Usually ID occurs in three sequentially developing stages: depleted iron stores, iron deficient erythropoiesis and iron deficiency anaemia (IDA).

Often ID and IDA have been incorrectly used as synonyms (FAO/WHO, 2002). When a person with no mobilisable iron from reserves develops negative iron balance, an immediate impairment in the production of haemoglobin with a resulting decrease in haemoglobin and different erythrocyte indexes occurs (Wintrobe, 1981).

The absence of iron stores together with signs of an iron-deficient erythropoiesis constitutes ID, which implies that there is an insufficient supply of iron to various tissues. Initially a less than optimal level of iron available to the body leads to a decrease in iron stores; which may then progress to the state of ID. It is estimated that this occurs at a serum ferritin level  $<15 \mu\text{g/l}$  (FAO/WHO, 2002). Iron can then no longer be mobilised from iron stores and insufficient amounts of iron will be delivered to transferrin.

The binding sites for iron on transferrin contain progressively less iron, characterised as reduced transferrin saturation (Wintrobe, 1981). At a critical level of reduced transferrin saturation, the supply of iron to erythrocyte precursors fails to meet the demands for haemoglobin formation (Wintrobe, 1981). The iron-transferrin complex binds to TfR on the cell surface and the whole complex is taken up by the cell. The uptake of iron is related both to the saturation of transferrin and the number of TfRs on the cell surface (Harford, 1994). There is diurnal variation in the saturation of transferrin making it difficult to evaluate the iron status from single determinations of transferrin saturation (FAO/WHO, 2002).

The absence of iron stores (iron deficiency) can be diagnosed by showing that there is no stainable iron in the reticuloendothelial cells in bone marrow smears or more easily by a low concentration of ferritin in serum ( $<15 \mu\text{g/l}$ ) (Wintrobe, 1981). Even if absence of iron stores per se may not necessarily be associated with any immediate adverse effects, it is a reliable and good indirect indicator of iron-deficient erythropoiesis and of an increased risk of a compromised supply of iron to different tissues (FAO/WHO, 2002).

Even before iron stores are completely exhausted the supply of iron to the erythrocyte precursors in the bone marrow is compromised, leading to iron-deficient erythropoiesis (FAO/WHO, 2002). A possible explanation is that the rate of release of iron from stores is influenced by the amount of iron remaining. During the development of iron deficiency, haemoglobin concentration, transferrin concentration, transferrin saturation, soluble transferrin receptors (sTfR), erythrocyte protoporphyrin and erythrocyte indexes are altered (see appendix A). All these indicators, however, show a marked overlap between normal and ID subjects, which makes it impossible to identify the single individual with mild iron deficiency by using any of these indicators (FAO/WHO, 2002). The diagnostic specificity then increases but the sensitivity decreases, and thus the true prevalence of ID is markedly underestimated if multiple diagnostic criteria are used. In screening for ID, the more the number of tests used the higher is the diagnostic specificity but the lower the sensitivity of the procedure (FAO/WHO, 2002). Fortunately, a low serum ferritin (<15 $\mu$ g/l) is always associated with an iron-deficient erythropoiesis. However, the use of serum ferritin alone as a measure will also underestimate the true prevalence of ID but to a lesser degree than when combined criteria are used (FAO/WHO, 2002).

In ID, plasma iron levels are low but haemoglobin levels may remain normal (Butensky et al., 2004). If ID persists, a state of IDA may develop from the depletion of marrow supply of iron and haemoglobin levels may decrease (Finch, 1994). The definition of IDA has been based on cut-off values, and to an extent represents a statistical construct rather than a functional concept (Wintrobe, 1981). By definition, the prevalence of IDA is less frequent than ID, which makes the use of the latter more specific in characterising iron nutritional status (Wintrobe, 1981). Though imprecise, the IDA concept is still in use because of the ease of determining haemoglobin (Wintrobe, 1981). The use of serum ferritin has improved the diagnostic accuracy of ID (FAO/WHO, 2002). It is the only simple method available to detect early ID. However, the practical value of using ferritin is somewhat reduced by the fact that it is a very sensitive acute-phase reactant which may be increased for weeks after a simple infection with fever for a day or two (Hulthén, 1998). Several other conditions, such as alcohol use, liver as well as collagen diseases may also increase serum ferritin concentrations (Osler et al., 1998).

Other methods (Appendix A), such as the determination of sTfR levels in plasma have also been recommended in the diagnosis of ID (FAO/WHO, 2002). This method is mainly used in subjects who are already anaemic and it is not sensitive for the early diagnosis of ID. As an advantage it is not influenced by infections. Although the use of

a combination of measures, such as serum ferritin, sTfR, C-reactive protein (CRP), Alpha-1-acid glycoprotein (AGP) and alpha-anti-chymotrypsin (ACT) have been suggested to improve diagnostic accuracy, this is not always practicable in population studies (Cook et al., 1996; Northrop-Clewes, 2008). In current practice, the most practical methods are considered to be measures of haemoglobin and ferritin, with some allowance made for active inflammation.

#### **2.4.3.2 Chronic inflammation and anaemia**

##### **2.4.3.2.1 The inflammatory process and iron metabolism**

Inflammation is a general process through which the body responds to injury or pathogenic attack. It is associated with the co-ordinated and regulated release of the inflammatory cytokines IL-1, IL-6 and TNF- $\alpha$  (Janeway et al., 1999). The response is characterised by a series of local and systemic effects collectively referred to as the acute phase response (APR) (Baxendale and Gauldie, 1994). Locally there is vasodilatation, platelet aggregation, neutrophil chemotaxis and the release of histamines, lysosomal enzymes, kinins and oxygen free radicals. Systemic changes include an elevation of body temperature (fever), hormonal changes (activation of the adreno-pituitary system), leucocytosis (increase in white blood cell numbers), thrombocytosis (increase in platelet numbers), muscle proteolysis, alterations in intermediary metabolic substrates and changes in the hepatic or liver synthesis of acute phase proteins (APP) (Baxendale and Gauldie, 1994).

Teleologically, the metabolic changes that occur in the liver and peripheral tissues are thought to provide endogenous nutrients like glucose and amino acids to fuel the activated immune system (Grimble, 1992). The liver cells, in particular, respond specifically to IL-1, IL-6 and TNF- $\alpha$ , with consequential effects on the APR (Thurnham and Northrop-Clewes, 2007).

Following infection or in the presence of an inflammatory stimulus, an APR is invoked by the body which starts with the release of inflammatory cytokines such as IL-1, TNF- $\alpha$ , IL-6 and some IFNs which typically direct the inflammatory response (Tomkins, 2003). As a broad-based response to many different types of stimuli (infections and all kinds of tissue injury), the APR fulfils two important functions. Firstly, it facilitates the inflammatory and repair processes and, secondly, it protects against the potentially destructive action of the inflammatory products (Baxendale and Gauldie, 1994). Thus, in general, the APP produced during the process of inflammation may be functionally protective or anti-inflammatory, and may indicate the nonspecific presence of inflammation, tissue damage or endotoxins (Fleck and Myers, 1985).

During the APR, plasma concentrations of several nutrients and APPs are altered. Plasma levels of iron and zinc fall rapidly, but levels of copper tend to increase (Thurnham and Northrop-Clewes, 2007). The increase in serum ferritin concentration parallels that of CRP, another positive APP (Feeelders et al., 1998). In recent years a small polypeptide known as hepcidin, synthesised and secreted from liver cells in response to IL-6, has been implicated in both the hypoferaemia of infection and the increase in serum ferritin concentration (Nemeth et al., 2003; Wrighting and Andrews, 2006). Although there are studies that show a correlation between inflammation, elevated circulating cytokines and anaemia in humans and animal models (Weinstein et al., 2002), the question as to whether these cytokines act alone or regulate important pathways for red cell production remains unanswered.

#### **2.4.3.2.2 Characteristics of anaemia of chronic inflammation**

Anaemia of chronic inflammation is an acquired hypo-proliferative disorder often seen in persons with systemic illnesses or inflammatory diseases, such as infection, cancer or autoimmune conditions. The cause has been attributed to impaired erythropoiesis (Weiss and Goodnough, 2005). First described in the 1930s, it was more fully characterized by Cartwright and Wintrobe in the 1950s (Cartwright, 1966). It has been considered to be the second most prevalent form of anaemia after iron deficiency, and the most common among patients with chronic illness (Zarychanski and Houston, 2008).

In population-based studies, it has been difficult to ascertain precise estimates of the prevalence of ACI because many persons with anaemia are not investigated sufficiently to establish the cause. Further, there are no agreed criteria for the diagnosis of anaemia of chronic inflammation for research purposes, and especially, in situations where ACI is likely to be only one of many reasons why persons might have anaemia (Zarychanski and Houston, 2008). ACI varies in severity with most patients typically presenting with mild ( $> 100$  g/L) or moderate (85–100 g/L) reductions in haemoglobin concentrations (Zarychanski and Houston, 2008). In a minority of patients, there may be severe reductions in haemoglobin concentrations.

ACI has features of a biologically adaptive response, being a highly coordinated and genetically conserved response to systemic disease (Futuyma, 2008). Of the several mechanisms that may contribute independently to ACI, iron sequestration is the best studied. ACI is generally characterised by low reticulocyte count, decreased survival of matured red cells, inhibition of erythroid progenitor cells, an increase in circulating inflammatory cytokines, immune activation, suppression of erythropoietin production

and a blunted response of red cell precursors to erythropoietin (Semba and Gray, 2000; Claster, 2002; Zarychanski and Houston, 2008). Another important feature of ACI is the occurrence of a major alteration in the distribution of iron within and between tissues (Butensky et al., 2004).

As part of the host defence mechanism, under conditions of chronic inflammation, the body sequesters iron, especially in macrophages (Boelaert et al., 1996). It has been suggested that this limits the availability of iron to potential pathogens, which require it for their own growth (Weiss, 1999). There is limited mobilisation of iron from stores (Means, 1995) and a decreased absorption of iron through the gastrointestinal tract (Lee, 1983), which together limit the availability of iron for erythropoiesis (Means, 2000). Thus, the hypoferaemia associated with an inflammatory process is not necessarily associated with a reduced total body iron content, or a dietary iron deficiency, but rather a limitation on the ability to access iron (Thurnham and Northrop-Clewes, 2007). This redistribution of iron and limited availability of iron to metabolic processes can occur even when the total amount of iron in the body is normal or increased (Thurnham and Northrop-Clewes, 2007). This iron sequestration is a hallmark of ACI that could have several beneficial effects.

Early in the course of ACI, body iron stores may be normal with a mild normocytic anaemia resulting from the impaired iron cycling (Butensky et al., 2004). This may progress to retention or accumulation of iron in the reticulo-endothelial macrophages, and subsequently, serum iron and serum transferrin values become low. As time goes on the impaired intestinal absorption arising from the ACI would result in a reduced body burden of iron and hence frank iron deficiency and the anaemia becomes microcytic (Weinstein et al., 2002). However, serum or plasma ferritin levels may remain normal or high (Weiss, 1999; Volberding, 2000). In ACI bone marrow biopsies may reveal adequate or often increased iron stores with poor incorporation of iron into developing red cells (Weiss, 1999).

Among the many other causes of anaemia in HIV infection, only ACI is involved with an increase in iron stores, a condition which may pose a unique problem for HIV-infected persons (Butensky et al., 2004). Wider changes in the bone marrow are also a common feature in ACI with neutropaenia and/or thrombocytopaenia (Claster, 2002) or altered white cell function such as altered helper-T cell activity (Mitsuyasu, 1994; Volberding, 2000). It has been suggested that in HIV infection defects of this kind contribute to the increased incidence of co-infections and malignancies, which can in their own right cause anaemia (Mitsuyasu, 1994).

More recent work indicates that a small peptide hormone produced in the liver, hepcidin, may be involved in the pathogenesis of ACI (Fleming and Sly, 2001). Hepcidin has intrinsic microbial activity and its expression increases in response to inflammation (Pigeon et al., 2001; Nicholas et al., 2002). Hepcidin is a negative regulator of intestinal iron absorption and iron release by macrophages and has been implicated as having a central role in the regulation of iron homeostasis (Fleming and Sly, 2001). The expression of the mRNA for hepcidin is up-regulated in response to lipo-polysaccharides (Pigeon et al., 2001) and infections (Shike et al., 2002). Elevated expressions of hepcidin mRNA results in severe anaemia with the characteristics of ACI (Weinstein et al., 2002; Nicholas et al., 2002).

The detailed molecular basis of ACI has yet to be elucidated but cytokines such as IL-1, IL-6, TNF- $\alpha$  and interferons (INFs) regulate red blood cell production and stability (Means, 1996; Means, 2001). TNF- $\alpha$  reduces red cell synthesis and life span (Moldawer et al., 1989). IL-1 inhibits the production of erythropoietin (Faquin et al., 1992). There is some evidence that TNF- $\alpha$  and IL-1 inhibit erythroid progenitor cells (Means et al., 1993). Cytokines have also been associated with impaired mobilisation of iron from reticulo-endothelial cells (Means, 1995); IL-1 can increase ferritin synthesis and, with TNF- $\alpha$  increase ferritin secretion into plasma (Rogers et al., 1990; Tran et al., 1997). This tends to limit the availability of iron for erythropoiesis. Hepcidin, as an iron-regulatory hormone, constitutes an important link between host defense, inflammation and iron metabolism. In humans, hepcidin synthesis is markedly induced by infection and inflammation (Nicholas et al., 2001; Shike et al., 2002), with IL-6 as the key inducer of its synthesis during inflammation (Nemeth et al., 2004).

## **2.5 INTER-RELATIONSHIP BETWEEN HIV INFECTION AND ANAEMIA**

As persons with HIV infection continue to live longer, primarily as a result of advances in ART, it becomes important to recognise the inter-relationship between the infection and anaemia particularly because of the impact that anaemia has on morbidity and mortality in the HIV/AIDS population. In this regard, assessing the incidence and prevalence of anaemia becomes an imperative and a pre-requisite for identifying factors that might be contributory to the anaemia, especially, in the HIV/AIDS population.

### **2.5.1 POSSIBLE AETIOLOGY OF ANAEMIA IN HIV/AIDS**

As in the normal population, the causes of anaemia in HIV infection and AIDS can be ascribed to many different factors (Bain, 1999; Henry, 1998; Claster, 2002). These factors may act separately and/or together to tip the balance between red cell

production and red cell destruction or loss. However, the anaemia of HIV has been noted to ensue principally as a result of a common process called erythropoietic failure (Moore et al., 1998; Bain, 1999), though the basis for this failure is not well understood. Anaemia in HIV/AIDS may also be induced or exacerbated by treatment or drug therapy or ART (Bain, 1999; Groopman and Itri, 1999). The involvement of the virus itself has been considered as direct with other related effects described as indirect.

#### **2.5.1.1 Direct effects of HIV**

Specific HIV strains can act directly to inhibit the differentiation of stromal cells (progenitor cells) in the bone marrow leading to a reduction in the production of red cells and other bone marrow elements (Claster, 2002; Levine, 1999; Redd et al., 2007). The haematopoietic stem cell is the common progenitor of both the myeloid and lymphoid lineages in the bone marrow. Hence, the haemato-suppressive effect on early progenitor cells could contribute to the anaemia and other cell line depletions in HIV, and to the inability of the bone marrow to reconstitute a functional pool of mature CD4+ T-cells (Davis and Zauli, 1995).

This direct effect will contribute to an increased incidence of OI, malignancies and comorbidities, which constitute complications that in their own right could directly suppress haematopoiesis and cause or exacerbate anaemia (Volberding, 2000; Volberding et al, 2004). Several opportunistic organisms that infiltrate the bone marrow and disrupt erythropoiesis have been associated with anaemia in HIV/AIDS. The most common infectious agents associated with HIV-related anaemia include *Mycobacterium avium complex (MAC)*, *Mycobacterium tuberculosis*, *Histoplasma*, *Cryptococcus*, *Coccidioides*, *Pneumocystis carinii*, *Parvovirus B19* and *Leishmania* (Hambleton, 1996). Anaemia may be directly induced in HIV by neoplasms, especially lymphomas, as they infiltrate the bone marrow (Sipsas et al., 1999).

#### **2.5.1.2 Indirect effects of HIV**

##### **2.5.1.2.1 Inflammation as a possible cause of anaemia**

HIV infection and AIDS are associated with chronic inflammation, which may lead to a continuous release of inflammatory cytokines such as IL-1, TNF- $\alpha$ , IL-6 and some IFNs that typically direct the inflammatory response (Tomkins, 2003). The direct effects of HIV on the immune system also allows OI to thrive, which also contribute to the sustenance of the APR. TNF- $\alpha$  and other inflammatory cytokines, which may inhibit erythropoiesis have been suggested as the cause of anaemia associated with chronic disease or anaemia of chronic inflammation (Maciejewski et al., 1994; Means, 1996). The basis for this anaemia is that, apart from lowering blood nutrients, inflammation

blocks iron absorption and impairs iron utilisation, thereby lowering RBC production (Jackson, 2007, Thurnham and Northrop-Clewes, 2007) (see also section 2.5.3.2).

#### **2.5.1.2.2 Reductive adaptation as a possible cause of anemia**

In an infection, there is likely to be a competitive metabolic demand by the body for energy and nutrients (Macallan, 1998; Jackson, 2007). To combat the infectious agent and maintain the body's integrity, energy and nutrient demands of many organs are increased (Calder and Jackson, 2000), which eventually leads to changes in nutritional status (Jackson, 2007). Whether the limited availability of food is associated with food insecurity from poor socio-economic and environmental factors, or the effects of HIV and poor appetite, a reduction in food intake, weight loss and changes in the body composition are likely to occur. If prolonged or severe, loss of weight may be associated with other complex adaptive changes in tissues and organs characterised as reductive adaptation (Ashworth, 2001; Jackson, 2007). Anaemia may appear as one part of the reductive adaptation response and be ascribed to the production of poor quality cells with a shorter lifespan, in part attributed to their existence in a challenging environment conferred by the presence of infection and nutritional or metabolic compromise (Jackson, 2007).

#### **2.5.1.2.3 Nutritional deficiencies as a possible cause of anaemia**

Like other cells, mature RBC result from the multiplication and differentiation of precursor, requiring the continuous availability of a full complement of nutrients and metabolic intermediates (Jackson, 2007). Any limitation in one or more of these nutrients would impair the ability to maintain RBC production, especially when the demands for those nutrients directly involved in the production of haemoglobin far outstrip that of other cells (Jackson, 2007). The particular need for iron for haem production and the unusual amino acid composition of haem and globin are important when considering the nutrient needs for RBC production (Jackson, 2007).

Red blood cell production depends on the iron status of the individual as well as factors associated with ID or iron excess. In HIV and other infections, the impact of reductive adaptation and the inflammatory process can result in potentially available iron being reduced, normal or increased (Jackson, 2007). Within the context of reductive adaptation iron may accumulate in the tissues because of ineffective utilisation for red cell production (Golden and Ramdath, 1987; Powers and Thurnham, 1981). Furthermore, limited availability of other nutrients, such as vitamin A, riboflavin or copper, for RBC production, may also lead to an ineffective utilisation and an increase in stored iron (Nemeth and Ganz, 2006). Whilst a deficiency of vitamin C is likely to

affect the absorption of non-haem iron in the diet (Halberg et al, 1987), a deficiency of vitamin B<sub>12</sub>, which is necessary for erythropoiesis and DNA synthesis, could lead to a reduction in RBC production (Powers and Bates, 1987). Riboflavin deficiency can impose limitations on absorption and utilisation of iron (Powers and Bates, 1987), and a deficiency of pyridoxine (B<sub>6</sub>) may affect haem synthesis.

Haem is formed from porphyrin (derived from glycine in the molar ratio of 1:4), and a limitation in the availability of glycine caused by micronutrient deficiencies would constrain its formation (Jackson, 2007). A limitation in glycine availability also affects glutathione synthesis (Persaud et al., 1996), a reduction of which in RBC may enhance its vulnerability to oxidative stress (Jackson et al., 1987; Jackson et al., 2004). Additionally, limitations in the availability of other antioxidant nutrients, which have commonly been observed in HIV infection (Tang, 2005), may add to this enhanced vulnerability of RBC. Any one of these factors, together or separately, can cause loss of cellular integrity and functionality in RBC, which would facilitate their premature removal from the circulation (Jackson, 2007).

#### **2.5.1.2.4 Other possible causes of anaemia**

A loss of cellular integrity can lead to an increased vulnerability of the RBC to haemolysis. In HIV/AIDS, haemolysis of RBC could be induced by the presence of red cell autoantibodies or the haemophagocytic syndrome or a disseminated intravascular coagulation or thrombotic thrombocytopenic purpura (Levine, 1999; Coyle, 1997; Rule et al., 1996; Rarick et al., 1996). Additionally, the presence of inheritable disorders such as sickle cell disease, glucose-6-phosphate dehydrogenase deficiency (G6PD), thalassaemia and other minor or rare haemoglobinopathies which predispose to haemolytic anaemia may also cause or exacerbate anaemia in HIV/AIDS (Nestel, 2002; Phillips and Groer, 2002). Anaemia could also be induced by oxidant drugs or medications (including ARV, anti-fungal and anti-bacterial agents) (Volberding, 2000; Volberding et al., 2004). Apart from their haemolytic effects some ARVs can also suppress the bone marrow and reduce erythropoiesis whilst others are particularly toxic to the hepatocytes (Levine, 1999).

In places where malaria and helminth infections, especially hookworm, are endemic, they may contribute immensely to anaemia (Ssali et al., 2006). Additionally, chronic anaemia in HIV infection is also associated with a decreased production of or blunted response to endogenous erythropoietin (Spivak et al., 1989) as well as hypogonadism (Dobs, 1998) which may lead to a decrease in red cell production.

However, and especially, as the disease progresses, a more obvious cause of anaemia in HIV persons, arises from the loss of blood (Volberding et al., 2004). This may occur in neoplastic conditions (e.g. Kaposi sarcoma) in the gastrointestinal tract as well as from other gastrointestinal lesions due to opportunistic infections.

### **2.5.2 COMMON TYPES OF ANAEMIA IN HIV/AIDS**

In PLWHA, IDA and ACI are the most common occurrences (Butensky et al., 2004). Though IDA may be present in HIV/AIDS, the development of anaemia in most HIV-infected persons has often been ascribed to ACI because of the attendant reduction in red cell production and a suppression of reticulocyte response commonly associated with chronic infections (Sears, 1992; Coyle, 1997).

Since HIV/AIDS is essentially an inflammatory disease, it is not surprising that ACI commonly occurs in both HIV-infected adult and children populations (Balbaryski et al., 1998; Monroe et al., 2000). Although most of these studies do not differentiate between IDA and ACI, findings from bone marrow evaluations confirm that ACI is of major occurrence in adults with HIV infection (Frontiera and Myers, 1987). Nevertheless, it is not uncommon to find both IDA and ACI existing together (Eley et al., 2002; Butensky et al., 2004).

### **2.5.3 HIV/AIDS AND IRON METABOLISM**

Infection with HIV has been associated with profound immune deterioration as well as a progressive deposition of iron in tissues and organs (Boelaert, 1996). The direct effects of the virus as well as the presence of chronic or acute inflammation or dietary inadequacies stemming from reduced intake or malabsorption could account for the altered iron status in infected persons (Butensky et al., 2004).

Anaemia, in advanced HIV infection, has been characterised by an accumulation of iron in tissues and cells, which may predispose to microbial infections. This would, as a matter of importance, require caution in the use of iron as a therapeutic agent or supplement during the management and intervention of anaemia in PLWHA.

Oxidative stress, resulting from the response of the body to HIV and other infections and from the inflammation associated with such infections, may lead to a depletion of antioxidant molecules, some of which are important for the production of haemoglobin. Together with the inflammation, oxidative stress can lead to an alteration in iron status by causing an increase in red cell destruction with a concomitant sequestration of iron into storage as well as an attendant decrease in red cell production (Thurnham and

Northrop-Clewes, 2007). Although HIV is associated with an altered iron status it is not very clear whether the altered iron status is due to dietary iron deficiency or a blockade of the iron absorption or the inflammation or process of inflammation or the relative contributions of these processes. The influence of dietary iron intake on the absorptive regulatory process in the infected person may also be an important issue to consider (Thurnham and Northrop-Clewes, 2007).

Although earlier studies have depicted lower prevalences for IDA in HIV-infected adult populations (Mir et al., 1989; Castella et al., 1990), a more recent study has confirmed a rising prevalence that occurs predominantly in infected women (Semba et al., 2002). Women together with children, who are infected with the virus, account for about half the anaemia seen in these population groups (Totin et al., 2002; Semba and Gray, 2001). The major contributors to IDA in HIV/AIDS may include inadequate dietary intake of iron (Hiltgartner, 1991) and malabsorption of iron, which are often associated with HIV disease (Castaldo et al., 1996; Tarallo et al, 1993). In both instances, the inflammatory process may play a central role, in that, whilst it is known to inhibit iron absorption it also induces anorexia which ultimately reduces dietary iron intake (Beresford et al., 1971). Clearly, in a population whose intake of iron is inadequate, IDA is very likely to exist irrespective of whether the inadequacy is brought about by the infection or socio-economic or environmental circumstances.

## **2.6 CONCLUSIONS FROM LITERATURE REVIEW**

The main purpose of this section has been to explore the inter-relationship between HIV/AIDS and anaemia and the many factors that might contribute to the development of anaemia during HIV infection.

It has been proposed that people with HIV have a greater propensity to anaemia, and that anaemia itself indicates a more serious clinical state. The main hypothesis considered for the study was that taking all other factors into account, "HIV infected persons are more anaemic in comparison with uninfected persons".

In the light of the above, the literature review has provided definitions respectively for the main outcome of anaemia and exposure of HIV/AIDS as well as other aspects that relate the two. The various effects of HIV/AIDS on the body that are most likely to be a part of this linkage have been considered. In reviewing the causes of anaemia, emphasis has been placed on its multifactorial causation and the important, complex interplay amongst these factors. HIV infection by its direct and indirect linkages with RBC production has been identified as a potential contributor to the public health

problem of anaemia. Despite important advances in ART, anaemia in HIV remains a problem (Mildvan, 2003). With improved suppression of HIV as newer therapies evolve, and as the lifespan of HIV-infected persons are prolonged, the risk for PLWHA to develop anaemia of chronic inflammation (ACI) is likely to be greater (Volberding, 2000; Belperio and Rhew, 2004).

Furthermore, the persistence, even in the HAART era, of malnutrition and other nutritional deficiencies (e.g. iron, vitamins A and B<sub>12</sub> and folate deficiencies) which impair production of red blood cells means that anaemia has to be considered within a wider aetiological context in many populations. This study seeks to focus on the role of HIV infection in the aetiology of anaemia and to identify the factors that interplay to increase the predisposition of infected persons to anaemia. These are important considerations for sub-Saharan African populations where the factors that predispose to anaemia are multiple.

In effectively tackling the HIV pandemic, it will be important to identify factors that exacerbate infection or affect the well-being and survival of PLWHA. Any research directed at identifying those at increased risk of developing anaemia will add to the efforts at improving the quality of life and reducing the effects of HIV. This study seeks to provide some insight to the development of anaemia in HIV//AIDS by elucidating the complex interactions between HIV infection and anaemia in a sub-Saharan African population.

## CHAPTER THREE

### 3.0 DATA AND METHODS

In the previous chapter a review of the literature on HIV and anaemia highlighted the need to explore the relationship between these two important public health problems, especially, in a population where many factors intricately interplay in the relationship. The importance of elucidating HIV eliciting effects as likely to increase the risk of anaemia in the infected population is the main thrust of this research work.

This chapter outlines the methodologies for capturing and analysing the data to address the study hypothesis. It describes the design, sampling and data collection strategies. In addition, modalities for selecting variables and the statistical tools for their analyses are outlined. Lastly, a post hoc power analysis is considered for outcomes that presented without statistically significance between the two groups in the study population.

#### 3.1 INTRODUCTION

HIV infection can be a multiple risk for anaemia because of the many effects of the virus that can be related to the cause of anaemia, and which may interplay in bringing about the condition. In a sub-Saharan African population where factors predisposing to anaemia are rife, infection with HIV would significantly increase the burden already imposed by these factors on anaemia. Identifying those at risk and understanding the nature of the interactive factors would, therefore, be an important step in gauging appropriate interventions or therapies.

Following from the main hypothesis which stated thus, “HIV infected persons are more anaemic in comparison with uninfected persons”, the study objectives were set as follows:

- To characterise HIV sero-status in the study population
- To identify socio-demographic indicators of the study population that may relate to HIV status and anaemia
- To characterise anaemia and relate it independently with HIV status in the study population
- To explore changes in body composition, dietary energy and nutrient imbalances and metabolic and inflammatory alterations as possible explanatory factors for anaemia in the study population
- To explore for factors that may interplay with HIV infection and may predict anaemia in the study population.

Based on the objectives above, a series of explorative analyses, of the data set derived from the THUSA survey, would be employed in elucidating the various propositions in the study.

In the next sections a brief description of the original data-set together with the methodology used in its collection are presented. Variables that best fit the definitions of the exposures and outcomes and which may be identified in the link between HIV and the outcome of anaemia as well as those that may constitute effect modifiers or confounders are highlighted. Finally, the various research instruments or statistical tools used in analysing the study data are also presented.

### **3.2 STUDY DATA**

The secondary data, which is subject of the analysis in this thesis, was extracted from the larger THUSA survey. However, for a better understanding of the work in this thesis and to put in perspective the relevance of the research, a summary or overview of the survey and methods employed in the original data source are presented.

#### **3.2.1 THE THUSA SURVEY**

The THUSA survey was a large study that was carried out in the North West Province of South Africa spanning from 1996 to 1998. The study had a major aim of monitoring the impact of urbanisation on the determinants of health in South Africans in order to provide vital information for appropriate health intervention (Vorster et al., 2000; Vorster et al., 2004).

Apart from being an acronym for *Transition and Health during Urbanisation of South Africans*, THUSA takes its meaning from a word in the Setswana language that is translated as 'help' (Vorster et al., 2000; Vorster et al. 2005). The actual motivation for the THUSA survey was the rapid process of urbanisation of, especially, Africans in South Africa, with its attendant acculturation and modernization (Vorster et al., 2000; Vorster et al. 2005). A process that was thought to be associated with the triple burden of morbidity and mortality in South Africa: i.e. Infectious diseases (including HIV/AIDS); non-communicable diseases (NCDs); and violence and injuries.

The North-West Province, where the survey was carried out, is one of the nine provinces of South Africa. It is located rather centrally to the north of the country and is bordered by Northern Cape to the southwest, Orange Free State to the southeast, The Limpopo to the northeast, Gauteng to the east and to the north by 4 districts in Botswana (see provincial map in figure 3.1. Source: Wikimedia commons, 2006). It

ranks 6th in terms of its size as well as population, which is in the region of 3.4 million people. Among the languages that are predominantly spoken in the Province are Setswana (65.4%), which is the most widely spoken, followed by Afrikaans (7.5%) and then isiXhosa (5.9%). With regards to race, the majority of inhabitants are blacks, constituting about 91.5% (Statistics South Africa, 2007).

**Figure 3.1: Map of South Africa showing the North West Province (Source: Wikimedia commons, 2006)**



### **3.2.2 DESIGN OVERVIEW OF THE THUSA STUDY**

Beyond achieving the purpose of examining the changes in health determinants of Africans in the North West Province undergoing rapid urbanisation, the THUSA study was designed with much broader aims of informing preventive strategies, as well as policy and programmes, together with the purpose of identifying areas that needed further research (Vorster et al., 2000; Vorster et al. 2005).

During the conceptual stages, a basis for developing the framework for the study was developed, which involved the identification from the literature of measurable potential exposures and outcomes for describing the determinants of health during urbanisation (Vorster et al., 1999).

A cross-sectional comparative design was adopted based on the dictating circumstances of limiting resources and urgency for information (Vorster et al., 2000), more so, the authors of the study found the use of a total randomized sampling technique as unnecessary as the study was not aimed at reporting the prevalence and risk factors of diseases (including HIV/AIDS) (Vorster et al., 2000; Vorster et al. 2005). Additionally, although a proportional sample stratification of subjects was planned, the research team was forced to adopt a disproportional stratification approach in some regions due to the non availability of reliable subjects. This was due to the rigorous exclusion criteria which critically reduced the number of potential subjects in certain households. Thus, some participants were voluntarily recruited into the study. This quasi-random mode of selection may probably be a source of potential bias to the study as females were more willing to volunteer.

### **3.2.3 SUBJECT SELECTION AND EXCLUSION CRITERIA**

A community-based sample of 1854 'apparently healthy' men and women, aged 15 years and above was recruited from 37 randomly selected sites representing all health districts in the Province (Vorster et al., 2000).

A model for sampling and subject recruitment was developed by the Statistical consultative Services of North West Province based on available data on the population density of the Province with all subjects having an equal chance of being selected.

The subjects were stratified into five levels or stages of urbanisation. The criteria for categorising subjects into levels or stages were mainly on the basis of the type of employment or jobs they had and where they lived (housing or settlement type).

The first level (level 1) comprised of typical rural people living in a traditional rural African village with a tribal head. Level 2 were farm dwellers living and working in commercial farms; Level 3 were made up of people living in informal housing areas or 'squatter camps' adjacent to all major towns and cities. These subjects represented subjects in the most rapid transition phase as they moved recently into the informal housing areas from mainly rural areas. These could also be described as peri-urban settlers. The level 4 subjects were from the old established African townships with brick houses, running water and electricity available who worked as labourers in various institutions and industries and, finally, the level 5 subjects represented the upper class urban subjects who were mainly professionals (teachers and nurses), government employees, politicians or came from businesses/corporate industrial

environment (Vorster et al. 2005). Generally, levels 1 and 2 subjects could be described as rural whilst level 3 was more or less transitional and the levels 4 and 5 were considered urban (Vorster et al. 2005). Subjects were also grouped into year of recruitment which spanned 1996 to 1998.

With regards to exclusion, the criteria covered pregnant and/or lactating women, subjects below 15 years of age, those suffering from or diagnosed with known debilitating diseases or who were having an acute or chronic illness or infection (including HIV/AIDS, diabetes, hypertension or cardiovascular diseases (CVD), epilepsy, TB, etc), as well as those on any form of chronic medication, inebriated/high or whose body temperature was above 37.5°C (i.e. febrile). In addition visitors to the area as well as subjects who fasted for less than 8 hours were excluded.

### **3.2.4 DATA COLLECTION**

A variety of quantitative and qualitative research techniques were employed by a multi-disciplinary team who were trained to collect the data. The research team comprised of both scientists, with Natural and Social Science backgrounds, and multilingual speaking field workers who collected quantitative and qualitative information through interviews or questionnaires at located sites (clinics or health centres).

The questionnaires were specially designed or adapted and validated for the population under study, and the multi-lingual fieldworkers who administered them were specially trained to obtain information on socio-demography, anthropometry, physical activity, psychosocial variables and dietary intake.

As much as possible each subject was interviewed in a language of his/her choice. Also, qualified and experienced phlebotomists and other medical staff were used in the collection of blood and other bodily samples from each participant on the field, and the samples were then transported to the laboratory for subsequent biochemical analysis. For the clinical assessments and screening, this was done by qualified medical staff trained for that purpose. All the data was collected at specific clinics or health centres where subjects were asked to attend after the selection.

#### **3.2.4.1 Socio-demography physical activity and psycho-social variables**

A questionnaire on demographic, socioeconomic and other lifestyle habits including type of housing, access to electricity, water source, sanitation, health history, family structures, education, income etc was completed by the researchers and/or field workers. Habitual physical activity was assessed with a physical activity questionnaire designed and validated for this population (Kruger et al. 2000). Other variables that

served as indicators of psychological well-being were collected with a psychological questionnaire. This questionnaire was based on existing scales which were adapted and validated for the population (Costa et al, 1992; Cohen et al. 1983). For the current study socio-demographic considerations included in the analyses were sex (female or male), age (40 years and below or above 40 years), housing or settlement type (rural or urban), monthly income earnings (R1000 and lower [lower] or R1001 to 3000 [middle] or R3001 and above [higher]), educational attainment (none to standard 6 [lower] or std. 7 to std. 10 plus trade [middle] or std. 7 to std. 10 plus higher academic achievement [higher]), marital status (never married or married or divorced or widowed), first language spoken (Tswana or Afrikaans or Xhosa or Zulu or other), smoking status (smoker or non-smoker) and alcohol consumption status (consumer or non-consumer).

#### **3.2.4.2 Clinical examination**

At all field locations, two trained nursing sisters examined each subject for signs and symptoms of malnutrition. Areas of examination included the thyroid, hair, skin, tongue, nails, glands, eyes, teeth, gums and lips. Oral temperatures were taken with a clinical thermometer and blood pressure was taken at 10 minute intervals with a sphygmomanometer (Tycoo ®, USA) having an adjustable cuff with subjects remaining seated and calm.

#### **3.2.4.3 Dietary intake**

An important goal of the THUSA study was to identify differences in dietary intakes and health status of a population who were at various stages of urbanisation. In line with this, information on habitual dietary intake was obtained from a validated quantitative food frequency questionnaire (QFFQ). Prior to the study there was a need to develop a culturally sensitive dietary assessment tool which was suitable for the study population as other groups had made similar observations in the same population using tools that were considered culturally insensitive (Buzzard and Sievert, 1994; Coates and Monteilh, 1997). The QFFQ used in this study was first developed and tested for reproducibility and validity by MacIntyre and other workers (MacIntyre et al. 2000a; MacIntyre et al. 2000b). The processes in the development and testing for reproducibility and validity of the QFFQ has been fully described by MacIntyre et al., (2001).

From the food data obtained using the QFFQ, energy and nutrient intakes were calculated using a computer programme approved by the Medical Research Council of South Africa (Tygerberg, South Africa) and based on the South African food composition table (Langenhoven et al., 1991). Adjustments were made for

underreporting of energy intake according to the method described by Willet et al. (1995). Resting energy expenditure (REE) or basal metabolic rate (BMR) was estimated from the weight, age and gender of each subject using the Schofield equation (Schofield, 1985).

#### **3.2.4.4 Anthropometric measurements and indices**

Anthropometric measurements including weight, height, skin fold thicknesses and body circumferences (waist and hip) were taken in triplicates, by staff trained by the Institute of Bio-kinetics, Potchefstroom University for Christian Higher Education (PU for CHE), using methods that were standardised by a specialist staff (level II Anthropologist). In all cases the measuring equipment was calibrated and the necessary precautions taken to avoid biases. Other anthropometric indices like BMI, waist-to-hip ratio (WHR) and so on were derived from earlier measurements. For instance, BMI was derived from the weight and height measurements and was computed as the weight in kilograms divided by the height in metres squared ( $\text{kg}/\text{m}^2$ ).

##### **Body weight and height**

Body weight was measured using a calibrated electronic scale (precision Health Scale, A+D Company, Japan) with subjects wearing light clothing. The measurement scale was placed on a flat surface and zeroed. Subjects were asked to stand on the scale and the readings were called out by the interviewers to the recorders. For each subject, the measurements were taken three times.

Height measurements were taken using an anthropometer (Invicta, 1465, UK). Subjects were measured standing in an erect position and with the head in a Frankfurt horizontal plane. The subjects were measured with shoes or caps removed and readings made in a position to avoid parallax error. This was repeated three times without the measurer knowing what the previous recordings were. Subject's heights were measured in centimetres.

##### **Waist and Hip circumferences**

Waist circumference was measured at the narrowest area below the rib cage and above the umbilicus as viewed from the front while the subjects was standing in the anatomical position. An inelastic but flexible standard tape was used to measure the area at the end of a normal expiration. The hip circumference was measured at the point of greatest circumference around the buttocks with the subject standing in the anatomical position. The same inelastic, but flexible standard tape was used.

### **Skinfold measurement**

The following skinfolds were measured in triplicate and the mean values were calculated and reported:

- Triceps
- Subscapular
- Abdominal
- supraspinal
- Thigh
- Calf
- Iliac-crest

Standardised skinfold callipers were used (John Bull, British indicators, Ltd).

### **Girth measurements**

An elastic, but flexible standard tape was used to measure relaxed upper arm, tensed upper arm, forearm, thigh – 1 cm below gluteal fold, calf and wrist girths.

### **Body mass index (BMI)**

Subjects BMI (or Quetelet) were computed from the weight and height using the Quetelet formula,  $BMI = \text{weight (kg)}/\text{height (m)}^2$ .

### **Waist-to-hip ratio**

Waist-to-hip ratio was calculated from the measured waist circumference (in cm) divided by the measured hip circumference (in cm). This index provided knowledge on regional body fat distribution which is a valuable guide in assessing health risk for cardiovascular disease and diabetes (Kaplan, 1989).

### **Body density**

Body density (BD) was calculated using the formula by Jackson et al. (1980):

$$\begin{aligned} \text{BD of women} = & 1.0992921 - (0.0009929 \times \text{sum of skinfolds: triceps, thigh, supra-iliac}) \\ & + (0.0000022 \times [\text{sum of Skinfolds: triceps, thigh, supra-iliac}]^2) - (0.0001392 \times \text{age}) \end{aligned}$$

$$\text{BD of men} = 1.1043 - (0.001327 \times \text{thigh skinfold}) - (0.00131 \times \text{subscapular skinfold})$$

### **Fat percentage**

The formulae for percent fat (%Fat) were as follows (Sloan, 1967):

$$\% \text{ fat for women} = (5.03 / \text{BD}) - 4.59$$

$$\% \text{ fat for men} = (4.95 / \text{BD}) - 4.5$$

### **Fat mass**

Fat mass (FM) was derived as follows (Mc Ardle et al., 1994):

$$\text{Fat mass} = (\% \text{ fat} / 100) \times \text{body mass}$$

### **Lean body mass**

Lean body mass was derived as follows (Mc Ardle et al., 1994):

$$\text{LBM} = \text{body mass} - \text{fat mass}$$

#### **3.2.4.5 Biochemical measurements**

For biochemical or laboratory determinations, fasting blood, urine and cell samples were collected for serum, plasma, and DNA preparation as well as for HIV testing. For the purposes of this thesis only blood sample collection and analysis would be described.

#### **Method of blood sample collection and analyses**

A baseline blood sampling by trained personnel was preferentially carried out between the hours of 08:00 and 11:00 to control for the effects of environmental temperature and circadian rhythm on the level on variables and also to keep the period of fasting relative constant. Each respondent was fasted overnight for not less than 12 hours, i.e. they were not allowed to eat anything between those hours. A total of 75 ml of blood was drawn from the vena cephalica of each subject with sterile equipment (including butterfly infusion set, Johnson & Johnson, 21G 19mm needles and syringes) with no or minimum stasis. These blood samples were then prepared immediately for use or stored appropriately.

Samples collected were analysed using a large range of methods in different laboratories resulting in about 50 different variables indicative of nutritional and health status and risk for NCD. However, for the purposes of this study descriptions of analysis would be restricted as much as possible to those relevant in addressing the study hypothesis.

Sera as well as citrated or ethylenediaminetetraacetic acid (EDTA) plasma samples were, however, prepared in the field (4°C) using a refrigerated centrifuge (Universal 16R™ Hettich centrifuge, Tuttlingen, Germany). To obtain serum, a portion of the whole blood collected from each subject was left for 30-60 minutes to clot, after which centrifugation under refrigerated conditions separated the serum from the cells. In the same way anti-coagulated blood (containing citrate or EDTA) was centrifuged to obtain a separation between plasma and the blood cells. Aliquots of sera and plasma were

then harvested into Eppendorf tubes, labelled and stored at -20°C for 2-4 days on the field and subsequently transferred to a temperature of -84°C in the laboratory pending further biochemical analysis.

Analysis of processed blood samples involved the determination of serum proteins; total proteins, albumin, 'globulins' (obtained from the difference between total proteins and albumins); bilirubin (Total and Direct); electrolytes; glucose; lipids and enzymes using the DAX system (discrete analyzer, Technicon DAX48; Miles Inc. Diagnostic Division, Tarrytown, NY, USA). Furthermore, serum micronutrients (Vitamins A, B<sub>6</sub>, B<sub>12</sub> and E and iron), ferritin, transferrin, as well as iron binding capacity (TIBC) and erythrocyte folate were determined using a number of methods which included high performance liquid chromatography (HPLC), immunological and colorimetric methods.

With the use of standardised and portable equipment, Hct and [Hb] were determined in the field from EDTA-anti-coagulated blood. Haematocrit was determined from a haematocrit centrifuge with its tubes (Hettich Zentrifugen; Haematocrit 24D-78532; Tuttlingen, Germany). Haemoglobin was determined in a hemocue using the colorimetric method from Boehringer Mannheim (Germany). Measurement of plasma fibrinogen was by the Clauss method using the ACL 200 (Instrumentation Laboratory, Milan, Italy) system. HIV status, a parameter which was included after the study was over, was determined using an enzyme-immunologic method (Enzymum-Test., anti HIV 1+ 2+ subtype, Boehringer Mannheim, Germany). In all the measurements, the protocols were followed using appropriate standards and necessary precautions were taken to ensure validity and accuracy (Vorster et al. 2005).

### **3.2.5 ETHICAL CONSIDERATIONS**

Ethical approval for the study was given by the ethics committee of the North-West University, Potchefstroom. However, it was conducted with the full cooperation of the North-West Department of Health and Social Services as well as the communities from which the subjects were recruited. In compliance with the ethical norms, full information on the study procedure as well as its objectives were provided and explained to the subjects in their home language and each subject was then made to sign a consent form (illiterate subjects were made to sign with a cross). Subjects received lunch after the glucose tolerance test and their travel expenses were paid.

Further ethical approval was given by the same ethical committee for anonymous HIV testing of the samples after the study was completed. This meant that participants were not aware of their HIV status at the time of the study

### **3.3 MODALITIES FOR IDENTIFYING CONFOUNDERS AND EFFECT MODIFIERS**

In the epidemiological and public health context, there are several biological and social mechanisms that can be associated with HIV infection as an exposure and which can be linked to anaemia as an outcome. There are usually two ways by which a second factor associated with the main exposure factor affects the outcome relationship. It might either act as a confounder or as an effect modifier, or sometimes as both (Margetts and Nelson, 2008). A confounder is known to distort the relationship in any direction, i.e. increases or decreases the association between exposure and outcome (Margetts and Nelson, 2008). It does not reveal the real level of association between the main exposure and the outcome and it is not part of the causal pathway. This means that they must be adjusted for during analyses. On the other hand, effect modifiers are part of the causal pathway and must therefore be dealt with differently. They change the levels of risk between the main exposure and the outcome at the different strata of the effect modifier. One way of dealing with them is by the use of stratified analysis.

Stratified analysis considers each level of the effect modifier and measures the outcome to identify the strata with the highest level of risk. For instance age and sex may have strata with a higher risk for HIV infection and anaemia, e.g. younger females have a higher chance of being infected because of the high risk of HIV infection associated with females and younger persons, and anaemic because of the association of females with anaemia. It is therefore important to consider such effect modifiers in the analysis in order to identify such strata or groups for possible public health interventions.

### **3.4 STATISTICAL RESEARCH TOOLS USED IN ANALYSES**

Selection of appropriate variables from the secondary data set would be undertaken and statistical analysis done using various tools described below. Results would be compared between the HIV infected and uninfected groups and also between males and females within the two groups. The original data has been laid out in an Statistical Package for Social Science (SPSS) compatible format and therefore allowed statistical analysis using the SPSS statistical software (version 15.0, SPSS Inc. Chicago, IL, USA).

#### **Normality test**

For each selected variable a normality test was first done using either a one sample Kolmogorov-Smirnov normality test or by plotting histograms against a normal

distribution curve. A large selection of the variables were in significant agreement with the normal distribution curve, however, for a few others, which were skewed, values considered to be potential outliers (those above  $\pm 3SD$ ) were removed before a semblance of normality was shown. Variables that had few cases and were likely to make analysis spurious were discarded whilst skewed data were log transformed before analysis.

Data for the HIV infected were separated from the uninfected group and each was considered independently of the other as they were subjected to the normality tests. Apart from a few HIV infected variables with low sample sizes which showed skewedness, the rest conformed to the previous normal determinations for data on the whole population.

Skewed data or those that showed a semblance of normality after removing the outliers were log transformed. For all outcome and exposure variables, various parametric and non-parametric tests as outlined below, were used appropriately to assess changes and differences within and between groups that were compared. For each normalized variable standard descriptive statistics were presented in tables for the two HIV groupings as well as sub-groupings of sex where appropriate, as means with their 95% confidence interval or as rates (percentages), and statistical comparisons were made between the groupings as described below.

### **Tests of significance**

To address the hypothesis of the study and to establish a relationship between exposures and outcome, an independent sample t-test, chi-square, and a univariate analysis of variance (uniANOVA) were used where appropriate to test any differences between the groups for the exposure and outcome variables. Whilst the independent sample t-test tested for differences between the means of the two sample populations, the chi-square did same for the rates or percentages derived for each population. The use of a univariate ANOVA allowed the adjustment of potentially confounding socio-demographic and anthropometric variables which served as explanatory factors for the effect of the exposures on the main outcome of anaemia.

### **Pearson's Correlation**

To establish the linear relationships between specific exposure variables on the main outcome of anaemia a Pearson's correlation was applied. A Pearson's Correlation is a technique used to test the direction and strength of the relationship between two variables. In other words, it's a device to show whether any one set of numbers has an

effect on another set of numbers. Correlation between two variables reflects the degree to which the variables are related. When measured in a population the Pearson Product Moment correlation is designated by the Greek letter rho ( $\rho$ ). When computed in a sample, it is designated by the letter "r" and is sometimes called "Pearson's r." Pearson's correlation reflects the degree of linear relationship between two variables. It ranges from +1 to -1 and a correlation of +1 means that there is a perfect positive linear relationship between variables.

### **Logistic regression analysis**

In order to explore the factors that determined or influenced the overall outcome of anaemia multivariable logistic regression models were employed. These provided crude and adjusted odd ratios which assessed risk levels. Using a stratified logistic regression model variables that were considered of importance in predicting anaemia in each of the significant socio-demographic strata were obtained.

Logistic regression allows one to predict a discrete outcome, such as group membership, from a set of variables that may be continuous, discrete, dichotomous, or a mix of any of these. The dependent variable in logistic regression is usually dichotomous, that is, the dependent variable can take the value 1 with a probability of success  $\theta$ , or the value 0 with probability of failure  $1-\theta$ . This type of variable is called a Bernoulli (or binary) variable. The independent or predictor variables in logistic regression can take any form. That is, logistic regression makes no assumption about the distribution of the independent variables. They do not have to be normally distributed, linearly related or of equal variance within each group, and the relationship between the predictor and response variables is not a linear function.

The goal of logistic regression is to correctly predict the category of outcome for individual cases using the most economical model. To accomplish this goal, a model is created that includes all predictor variables that are useful in predicting the response variable. Several different options are available during model creation. Variables can be entered into the model in the order specified by the researcher or logistic regression can test the fit of the model after each coefficient is added or deleted, called stepwise regression. Stepwise regression is used in the exploratory phase of research and backward stepwise regression appears to be the preferred method of exploratory analyses, where the analysis begins with a full or saturated model and variables are eliminated from the model in an iterative process. The fit of the model is tested after the elimination of each variable to ensure that the model still adequately fits the data. When no more variables can be eliminated from the model, the analysis has been completed.

### **Deming regression analysis**

The Deming regression analysis is a method of linear regression that finds a line of best fit for a set of related data. It differs from simple linear regression in that it accounts for error in observations on both the x- and the y-axes (Linnet, 1993). In other words whereas the ordinary linear regression method assumes that only the Y measurements are associated with random measurement errors, the Deming method takes measurement errors for both axes into account. In statistical terms the Deming regression analysis assumes that the co-efficient of variation of each comparative method is the same, i.e.  $\lambda = \text{Var}(x)/\text{Var}(y)$  is constant across the range of values investigated.

The Deming regression analysis is also called an "errors-in-variables regression analysis" or "estimating a structural relationship" (Linnet, 1993). It is based on the assumption that the ratio of the variances of the errors of observations for the two variables is known and in which the errors are independent and normally distributed.

### **3.5 POST HOC POWER ANALYSIS FOR INDEPENDENT DATA**

A post hoc analysis, which is arguably and generally an unacceptable method, is usually done on comparative data that may reach statistical significance but for which the comparative importance prior to the investigation were not perceptible (Margetts and Nelson, 2008).

The weakness of many experimental studies is that the presence of an apparent difference between two groups may fail to reach significance as a result of misclassification errors in deciding which hypothesis is correct. Such studies can be said to be statistically underpowered (Margetts and Nelson, 2008). This may constitute a major limitation of a study, particularly one in which analyses are done on a secondary data source (in this case the THUSA survey). Often with a predetermined sample size, there is a greater likelihood that the results would be statistically underpowered, particularly when the data is stratified for analysis.

Statistical power relates to the sampling error and is defined as the probability that the null hypothesis would be rejected by a test when the alternative hypothesis is true or the probability of accepting the alternative hypothesis when it is true (Margetts and Nelson, 2008). Inherent in any well designed experiment is the small risk of rejecting the null hypothesis when it is actually true, which is referred to as the Type I error or significance level ( $\alpha$ ), or accepting the null hypothesis when actually the alternative hypothesis is true (Type II error). These two types of errors have to be contended with in power calculations. Statistical power is very much dependent on the size of the

sample which also allows for the determination of the degree of certainty in a relationship in order to show significant differences between two groups (Margetts and Nelson, 2008), in this case between infected and uninfected persons. The comparative limitation in size would also mean that potential confounders (e.g. age, gender and ethnicity) may not be equally distributed between the groups being compared and this could lead to bias and subsequent misinterpretation. However, since the impact of any potential confounder could be removed by appropriate sampling or by adjusting or controlling for the confounders at the analysis stage, this has to be taken into consideration during analyses.

According to the Yale University Education (2003), the factors that are likely to affect the statistical power of an experimental design include:

- The type of statistical test used in the analysis: Some tests are designed with more power than others and therefore may give higher significant results.
- The sample size: A larger sample size would provide greater power, but a very large sample size may not be cost effective as it would require a lot of money and/or time.
- The size of the experimental effect required: The magnitude of the difference in the null hypothesis would affect the power (i.e. a smaller difference would result in a smaller power or vice versa).
- The levels of errors in the experimental measurements: Errors act as 'noise' and can hide the real effects. It is, therefore, important to ensure that a high accuracy and precision are achieved in an experimental design in order to reduce the noise and to increase statistical power.

In well-designed studies the power to detect the desired effects are usually set at 80% ( $\beta=0.2$ ), 90% ( $\beta=0.1$ ) or even 95% ( $\beta=0.05$ ) to minimize the chance of a non-significant test result when the effect is actually present (Margetts and Nelson, 2008). However, a value of 80% is more frequently accepted.

Using the given sample size and taking the two HIV groups in this study into consideration the power has been calculated in a post hoc analysis as outlined in appendix C.

## RESULTS SECTION

This section of the thesis presents the outcomes from the analyses of secondary data extracted from the original THUSA survey. The aim of this section is to present and briefly discuss the findings whilst highlighting those that are important and relevant for addressing the main hypothesis.

### GENERAL INTRODUCTION

Anaemia remains a significant marker of disease progression and survival, especially in HIV infection and AIDS. In sub-Saharan Africa and many resource-poor settings, the rising trend of HIV infection may continue to pose a threat to the burden of anaemia, whilst anaemia in the HIV/AIDS population may spell disease progression, a reduction in survival or risk of death. The significance of anaemia in HIV/AIDS is perhaps seen in the impact it has on the QOL and survival of infected persons. To be able to characterise the causes of anaemia or identify persons at greatest risk for developing anaemia in a population is a prudent idea. Doing so would, among other things, facilitate the charting of a pathway that could help prevent or target the treatment of anaemia as a means to effectively manage HIV disease.

The overall aim of this study is to elucidate the increased risk for anaemia posed by HIV infection. It is postulated that the direct effects of the virus may interact with other indirect anaemia-inducing effects to enhance the predisposition of infected persons to anaemia. Perhaps, it is this unique direct as well as interactive effect of the virus that accounts for the common and frequent occurrence of anaemia in the HIV/AIDS population.

Thus, the hypothesis for this study was stated as; “Persons infected with HIV are more anaemic than persons uninfected”, which served as the basis for setting up the modalities, outlined in the preceding sections, to address the proposition.

Data for this study was taken from the THUSA survey (see Chapter 3), which was a cross-sectional population based study. It broadly served as a project to help monitor the impact of transition or urbanisation on the determinants of health in South Africans, with the purpose of providing pertinent information for appropriate health interventions. The primary aim of this present study, undoubtedly, closely aligns with the broad aims of the THUSA study, as the findings from this study could highlight the increased risk HIV could impose on victims by making them highly susceptible to anaemia and generally reducing their work capacity and QOL, which could have implications for national development.

From the proposed framework for the relationship between HIV infection and anaemia (Fig.1), exposures or factors identified as most likely to cause or contribute to the cause of anaemia in sero-positive persons were considered. These included the direct effects of the virus (in this study denoted by HIV sero-status) as well as indirect effects that can be captured under the processes of reductive adaptation, nutrient deficiencies and inflammatory and/or metabolic alterations, and also including a number of other related or unrelated socio-demographic factors that may be part of the causal link.

Several variables were selected for analyses which included the following: socio-demographic and anthropometric measurements; energy and micronutrient intakes derived from a record of food intakes from carbohydrate, protein and fat as main sources (energy from alcohol consumption was also incorporated). Other variables were biochemical measurements, including levels of 2 important serum micronutrients (Vitamins A and E), Hb, hct, total serum proteins, serum albumin, serum 'globulins', plasma fibrinogen, total and direct bilirubin, serum Fe, TIBC, serum ferritin and % transferrin saturation. In addition, levels of serum liver enzymes (AST, ALT, ALP and GGT), high levels of which may normally indicate insidious injury or damage to the liver cells or other related tissues, served as proxies to measuring inflammatory stress.

In this study anaemia is defined mainly on the basis of [Hb], however, Hct and other more specific iron status indicators (serum Fe, serum ferritin, TIBC and % transferrin saturation) are also used to define various characteristics of anaemia in the study population.

Tabulated results are, in general, compared between groups; HIV sero-negative or uninfected (HIV-) versus HIV sero-positive or infected (HIV+), or between subgroups; males versus females and anaemics versus non-anaemics. Where results are presented as rates any differences between the groups would be accounted for by the chi-square ( $\chi^2$ ) statistics. For results presented as means, a 95% confidence interval (95% CI) would be indicated and any differences between groups tested for using a univariate ANOVA before and after adjusting for significant confounders or effect modifiers. Binary logistic regression models are used to predict the effect of exposures on the main outcome. Each model takes into account categorical variables and adjusts for confounders and/or covariates. Factors that emerge with significant predictive values are considered as explanatory factors. For all statistical tests, probability values less than 0.05 ( $p<0.05$ ) would denote statistical significance. Results for this study have been presented in 3 separate chapters as outlined below in order to ensure a logical arrangement of findings for addressing the study hypothesis.

4.0 A PRELIMINARY ANALYSIS OF THE SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

5.0 DETERMINATION OF HIV ASSOCIATED FACTORS THAT MAY SIGNIFICANTLY CONTRIBUTE TO ANAEMIA IN THE STUDY POPULATION

6.0 EXPLORATIVE EVALUATION OF FACTORS THAT MAY INTERPLAY WITH HIV OR PREDICT ANAEMIA IN THE STUDY POPULATION

In the first results chapter, baseline socio-demographic characteristics are examined and any associations or risk levels that may relate socio-demography with HIV infection and/or anaemia in the study population explored. This preliminary analysis would highlight significant socio-demographic variables within the causal relationship between HIV and anaemia that could mask the effects of the main exposure of HIV infection on the outcome of anaemia. Significant influences from such confounders or effect modifiers would be appropriately adjusted for or taken into consideration in the categorisation of persons at increased risk of anaemia.

The second results chapter identifies the size of the problem of anaemia in the study population and explores various factors that may help explain the association of HIV infection with anaemia.

And the third results chapter further explores for various factors that may independently or interactively (with HIV infection) predict anaemia in the study population.

## CHAPTER FOUR

### 4.0 A PRELIMINARY ANALYSIS OF THE SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

#### 4.1 INTRODUCTION

This chapter is intended to highlight any differences in socio-demographic characteristics between the two HIV status groups and their sub-grouping, since these factors could serve as confounders or effect modifiers in the relationship between HIV and anaemia. It is important to consider significant socio-demographic differences as explanatory factors for the relationship between HIV and anaemia and to account for them in subsequent analyses.

In this chapter, socio-demographic data for the study population have been analysed and compared using basic descriptive statistics and appropriately presented in tables as rates (percentages). The results generated from this cross-sectional data analysis does not only define the baseline socio-demographic characteristics of the study population but more importantly could be the basis for further longitudinal research that would help establish the causal relationships between HIV infection and anaemia as well as how these may relate to other health issues in the study population.

Many socio-demographic variables may be associated with HIV infection and anaemia independently. It is also very likely that associations between socio-demographic variables and HIV infection or anaemia may vary according to other prevailing environmental factors as well as specific population characteristics. The causal relationship between HIV and anaemia therefore becomes a web in which all these factors may be considered.

The socio-demographic characteristics that have been examined and presented in this section, in relation to HIV infection and anaemia status, include gender, age grouping, settlement type, educational attainment, monthly total income earned, marital status, first language spoken, smoking and alcohol consumption status.

Whilst HIV status is based on the serological identification of viral antigens in the blood (HIV sero-positive, HIV+) or otherwise (HIV sero-negative, HIV-), anaemia status was based on the use of cut-offs for haemoglobin according to the WHO criteria (see Appendix A). Persons with Hb levels below conventionally accepted cut-offs were classified as anaemic whilst those with Hb levels above were classified as non-anaemic.

In the next section levels of association between socio-demographic variables in the study population and HIV and anaemia statuses, using proportions are explored.

#### **4.2 ASSESSING THE RISK FOR SOCIO-DEMOGRAPHIC FACTORS WITH HIV INFECTION AND ANAEMIA USING PROPORTIONS**

Tables 4.1, 4.2 and 4.3 below present summaries of the socio-demographic characteristics compared between HIV status grouping, gender and anaemia subgroupings of the study population respectively. Chi-squared statistics have been used to test for any differences in proportions and any values of  $p<0.05$  denoted statistical significance.

From table 4.1 it is evident that out of the total of 1821 'apparently healthy' adult subjects, whose data was included in various analyses, 216 (11.8%) were HIV sero-positive or infected whilst the rest (1605, 88.2%) were HIV sero-negative or uninfected. Obviously, the prior consideration of the study population as an 'apparently healthy' one becomes questionable upon this finding. The disproportion in subject numbers, between HIV sero-positive and uninfected persons can be accounted for by the inherent design of the original study, which was not biased towards selecting sero-positive persons. In fact, the original survey was not intended to measure prevalence of diseases (including HIV) and therefore subject selection was designed to be random.

Generally, the table (Tab. 4.1) shows that though more females were recruited for the study than males (57.9% cf. 42.1%), there were no significant differences between gender proportions for the two groups ( $p=0.623$ ). Even though there was an obvious inherent bias that excluded pregnant women in the selection of subjects, the high female to male ratio could be ascribed to the willingness of females to make up the numbers during the selection process. However, in terms of gender HIV prevalence, there were slightly more males (12.3%) who were sero-positive than females (11.5%) in the study population, but this difference was not significant.

The table (Tab. 4.1) also shows that the HIV sero-positive population were on average significantly younger ( $p=0.001$ ) than their uninfected counterparts with proportions of persons below 40 years higher in the sero-positive population than in the uninfected population. This also reflected in the prevalence of HIV infection being higher in those aged 40 years and below (13.8%) than in those above 40 years (9.2%).

It is further evident from Table 4.1 below that there were proportionately more HIV sero-positive persons residing in urbanised settlement types, i.e. comprising of settlers

in upper class areas, townships and squatter camps, than in those considered to be rural, i.e. settlers in typical rural areas and farms (70.4% cf. 29.7%,  $p<0.001$ ). Thus, HIV prevalence rates were shown to be higher in the urbanised settlement (14.5%) than in the rural settlement (8.3%).

**Table 4.1: Socio-demographic characteristics by HIV status (All values are frequencies with percentages in parentheses except age in years)**

Variable		HIV status				
		HIV-	HIV+	*p value	Total sample	% HIV+
Sample size (n)		1605 (100%)	216 (100%)	-	1821 (100%)	11.8%
Sex	Male	685 (42.7%)	96 (44.4%)	0.623	781 (42.1%)	12.3%
	Female	920 (57.3%)	120 (55.6%)		1040 (57.9%)	11.5%
Age (years)	Mean (95% CI)	37.98 (37.25-38.70)	34.94 (33.14-36.76)	0.001	37.62 (36.94-38.29)	—
Age Grouping	15-39 yr.	919 (57.4%)	147 (68.1%)	0.003	1066 (58.5%)	13.8%
	40 and over	682 (42.6%)	69 (31.9%)		751 (41.5%)	9.2%
Settlement type	Rural	710 (44.2%)	64 (29.7%)	<0.001	773 (42.5%)	8.3%
	Urban	896 (55.8%)	152 (70.3%)		1048 (57.5%)	14.5%
Highest Qualification	lower	860 (54.0%)	100 (46.3%)	0.027	960 (53.1%)	10.4%
	middle	573 (35.9%)	98 (45.4%)		671 (37.1%)	14.6%
	higher	160 (10.0%)	18 (8.3%)		178 (9.8%)	10.1%
Total income per month	lower	1185 (73.8%)	162 (75.0%)	0.945	1347 (74.0%)	12.0%
	middle	316 (19.7%)	41 (19.0%)		357 (19.6%)	11.5%
	higher	102 (6.4%)	13 (6.0%)		115 (6.3%)	11.3%
Marital status	Never married	316 (19.7%)	62 (28.7%)	0.309	368 (20.2%)	16.9%
	Married	244 (15.2%)	34 (15.7%)		278 (15.3%)	13.2%
	Divorced	13 (0.8%)	2 (0.9%)		15 (0.8%)	13.3%
	Widowed	65 (4.1%)	7 (3.2%)		72 (3.9%)	9.7%
First Language Spoken	Tswana	1198 (74.6%)	153 (70.8%)	0.104	1351 (74.2%)	11.3%
	Afrikaans	32 (2.0%)	4 (1.9%)		36 (2.0%)	11.1%
	Xhosa	163 (10.2%)	27 (12.5%)		190 (10.4%)	14.2%
	Zulu	30 (1.9%)	4 (1.9%)		34 (1.9%)	11.8%
	Other	179 (11.2%)	27 (12.5%)		206 (11.3%)	13.1%
Smoking status	Yes	544 (33.9%)	75 (34.7%)	0.819	619 (33.9%)	12.1%
	No	1060 (66.1%)	141 (65.3%)		1201 (66.0%)	11.7%
Alcohol intake status	Yes	618 (39.9)	97 (45.8)	0.102	715 (40.6)	13.6%
	No	932 (60.1)	115 (54.2)		1047 (59.4)	10.9%

\*Derived from chi-squared test,  $p<0.05$  denotes statistical significance

With regards to educational attainment, a little over one half of the study population (53.1%) had lower status (i.e. none or up to standard (std.) 6 educational attainments), in other words, their educational backgrounds were considerably poor. When compared to their uninfected counterparts, HIV sero-positive individuals tended to be highly represented in the middle level (Std. 7 to 10 (+ trade)) educational attainment, whilst uninfected individuals were more likely to have both lower and higher (i.e. std. 7

– 10 and beyond) educational attainments. These differences were quite significant ( $p=0.027$ ), and reflected the higher prevalence rate for HIV infection in the middle education attainment group (14.6%) compared to those for higher or lower educational attainments (10.4% and 10.1% respectively).

When monthly income from all sources was considered, about three quarters (74%) of the study population had lower earnings (up to a thousand rands (R1000) per month), with about 20% and 6% earning middle (R1001 to R3000) and higher ( $>R3000$ ) respectively. The differences in earnings did not differ significantly between the two sero-status groups ( $p=0.945$ ). However, a large proportion of lower income earners overall may be taken as a reflection of low economic status and the fact that it defines a sizable proportion of the population makes it widespread. Despite the marginal differences in proportions of monthly income earnings between the two sero-status groups, they were not significant. There were also no differences in HIV prevalence rates between the income groupings. This may probably indicate that HIV infection was not restricted to any particular income group and that lower income earners were as likely to be sero-positive as higher income earners.

Table 4.1 has further shown that in spite of the low number of subjects recorded for marital status, HIV sero-positive subjects are proportionately higher in the never married (28.7% cf 19.7%) and lesser in widowed (3.2% cf 4.1%) categories compared with their uninfected peers, whilst proportions for married and divorced categories were closely similar between the two groups. However, the observed differences were not significant ( $p=0.309$ ). In terms of HIV prevalence, the rates were 16.9% for never married, 9.7% for widowed, 13.2% and 13.3% for married and divorced categories respectively in the study population.

For first languages spoken, a large majority (74.2%) of the entire study population spoke Tswana and the proportions between HIV sero-positive persons and their uninfected counterparts was not significantly different ( $p=0.104$ ). The proportions for those who spoke Xhosa, Zulu and Other as first languages were also closely similar between the two groups, but like with the previous comparison, the differences were not significant ( $p=0.104$ ). In general a high proportion spoke indigenous first languages, which actually reflected the indigenous ethnicities that characterised the study population (Statistics South Africa, 2007), and confirmed the predominance of Africans or blacks in the study population. With regards to HIV infection rates persons who spoke Xhosa (14.2%) and Other (13.1%) as first languages were proportionately higher.

When smoking status was considered, about a third (33.9%) of the study population were smokers and, even though the proportion of HIV sero-positive subjects who smoked was marginally higher compared to uninfected smokers (34.7% cf. 33.9%) the difference was not significant ( $p=0.819$ ). Between smokers and non-smokers there was also a marginal difference in the HIV prevalence rates (12.1% cf 11.7%) with that of smokers tending to be slightly higher but not significantly different.

The proportion who consumed alcohol was shown to be higher in the HIV sero-positive group compared with their uninfected peers, which also reflected in a marginally higher HIV prevalence rate in alcohol consumers than non-consumers. Nevertheless, the differences were not significant.

In table 4.2 below, socio-demographic characteristics between males and females of the two groups are compared. From the table it is evident that both HIV sero-negative and sero-positive males and females are proportionately higher in the  $\leq 40$  years age group than in the  $>40$  years group. Within the groups, the differences in age grouping proportions between males and females are not significant. However, HIV sero-positive persons, irrespective of gender, have a higher proportion of younger persons than their uninfected counterparts, with infected females being significantly ( $p=0.006$ ) more likely to be younger.

With regards to educational attainment, proportions were slightly different for the various levels for between and within groups. Whilst HIV sero-positive males tended to be proportionately higher in the higher educational attainment category and lower in the lower educational attainment category than their uninfected peers, sero-positive females, were highly represented in the middle level as compared to their uninfected peers. However, these respective differences for between and within gender groups were not significant.

In terms of monthly total earnings, females in both groups were significantly more likely to be represented in the lower earning category than the middle and higher categories compared to their male counterparts. When gender comparisons between infected and uninfected groups were considered there were no significant differences in earnings.

The table (Tab. 4.2) further shows that there were no significant differences between sexes for each of the groups with regards to settlement type. However, HIV infected females like their male counterparts were significantly more likely to reside in urbanised settlement types compared to their uninfected counterparts ( $p=0.015$  and  $p=0.001$ ).

Regarding first languages spoken, table 4.2 also showed that over two-thirds (70% and above) of both males and females irrespective of HIV status were represented in the Tswana speaking category. Though fewer proportions were represented in the other first spoken languages, differences between gender within and between the infected and uninfected persons were not significant.

Differences between males and females for smoking status was very significant ( $p<0.001$ ) irrespective of HIV status, with a higher proportion of males more likely to smoke. However, when gender comparisons for smoking status between infected and uninfected groups were considered, proportions were similar and the differences marginal and not significant.

The trend for alcohol consumption was similar to that of smoking. Males, irrespective of HIV status, were significantly more likely to consume alcohol ( $p<0.001$ ) compared to females. However, when sero-status groups were compared, there were no significant differences between genders for alcohol consumption even though proportions were slightly higher for the HIV infected males and females.

**Table 4.2: Socio-demographic characteristics by gender (Values are shown as frequencies with percentages in parentheses)**

Characteristic		HIV-		HIV+		Chi square (p-value)			
		Male (a)	female (b)	Male (c)	female (d)	a*b	c*d	a*c	b*d
Size (n)		685 (42.7%)	920 (57.3%)	96 (44.4%)	120 (55.6%)				
Age grouping	<40 yrs	396 (58.1%)	522 (56.8%)	63 (65.6%)	84 (70.0%)	0.602	0.493	0.163	0.006
	≥40 yrs	285 (41.9%)	397 (43.2%)	33 (34.4%)	36 (30.0%)				
Educational attainment	lower	360 (52.6%)	500 (54.3%)	43 (44.8%)	57 (47.5%)	0.734	0.853	0.193	0.134
	middle	131 (19.1%)	190 (20.7%)	22 (22.9%)	34 (28.3%)				
	higher	187 (27.3%)	225 (24.5%)	31 (32.3%)	29 (24.1%)				
Total income per month	lower	476 (69.5%)	709 (77.1%)	67 (69.8%)	95 (79.2%)	0.003	0.044	0.554	0.386
	middle	156 (22.8%)	160 (17.4%)	19 (19.8%)	22 (18.3%)				
	higher	52 (7.6%)	50 (5.4%)	10 (10.4%)	3 (2.5%)				
Settlement type	rural	296 (43.2%)	414 (44.6%)	24 (25.0%)	40 (33.3%)	0.476	0.183	0.001	0.015
	urban	389 (56.7%)	506 (55.0%)	72 (75.0%)	80 (66.6%)				
First language spoken	Tswana	496 (72.4%)	702 (76.3%)	68 (70.8%)	85 (70.8%)	0.331	0.465	0.107	0.355
	Afrikaans	17 (2.5%)	15 (1.6%)	2 (2.1%)	2 (1.7%)				
	Xhosa	79 (11.5%)	84 (9.1%)	9 (9.4%)	18 (15.0%)				
	Xulu	12 (1.8%)	18 (2.0%)	1 (1.0%)	3 (2.5%)				
	Other	78 (11.4%)	101 (11.0%)	15 (15.6%)	12 (10.0%)				
Marital Status	Never married	146 (21.3%)	170 (18.4%)	30 (31.3%)	32 (26.7%)	0.007	0.391	0.587	0.616
	Married	107 (15.6%)	137 (14.9%)	16 (16.7%)	18 (15.0%)				
	Divorced	6 (0.9%)	7 (0.8%)	1 (1.0%)	1 (0.8%)				
	Widowed	15 (2.2%)	50 (5.4%)	1 (1.0%)	6 (5.0%)				
Smoking status	Yes	386 (56.3%)	158 (17.2%)	55 (57.3%)	20 (16.7%)	<0.001	<0.001	0.874	0.890
	No	299 (43.7%)	762 (82.8%)	41 (42.7%)	100 (83.3%)				
Alcohol intake status	Yes	397 (60.7%)	221 (24.7%)	62 (66.7%)	35 (29.4%)	<0.001	<0.001	0.269	0.263
	No	257 (39.3%)	675 (75.3%)	31 (33.3%)	84 (70.6%)				

\*Derived from chi-squared statistics, p<0.05 denotes statistical significance

**Table 4.3: Socio-demographic characteristics by anaemic status (Values are shown as frequencies with percentages in parentheses)**

Characteristic		HIV-		HIV+		Chi square (p-value)			
		Non-anaemic (a)	Anaemic (b)	Non-anaemic (c)	Anaemic (d)	a*b	c*d	a*c	b*d
Age grouping	<40 yrs	488 (57.5%)	411 (57.4%)	71 (68.3%)	74 (67.3%)	0.943	0.884	0.041	0.05
	≥40 yrs	360 (42.5%)	305 (42.6%)	33 (31.7%)	36 (32.7%)				
Educational attainment	lower	461 (54.4%)	376 (53.0%)	52 (50.0%)	47 (42.7%)	0.124	0.155	0.169	0.09
	middle	313 (36.9%)	249 (35.1%)	47 (45.2%)	50 (45.5%)				
	higher	74 (8.7%)	84 (11.8%)	5 (4.8%)	13 (11.8%)				
Total income per month	lower	630 (74.1%)	527 (73.5%)	80 (76.9%)	80 (72.7%)	0.951	0.771	0.822	0.972
	middle	167 (19.6%)	143 (19.9%)	18 (17.3%)	23 (20.9%)				
	higher	53 (6.4%)	47 (5.8%)	6 (6.6%)	7 (6.2%)				
Settlement type	rural	448 (52.7%)	250 (34.8%)	41 (39.4%)	22 (20.0%)	<0.001	0.006	0.014	<0.01
	urban	402 (47.3%)	468 (65.2%)	63 (60.6%)	88 (80.0%)				
Sex	male	389 (45.8%)	280 (39.0%)	53 (51.0%)	42 (38.2%)	0.007	0.063	0.323	0.87
	Female	461 (54.2%)	438 (61.0%)	51 (49.0%)	68 (61.8%)				
First language spoken	Tswana	671 (79.0%)	502 (70.1%)	76 (73.1%)	76 (69.1%)	0.081	0.622	0.394	0.24
	Afrikaans	15 (1.8%)	17 (2.4%)	1 (1.0%)	3 (2.7%)				
	Xhosa	74 (8.7%)	86 (12.0%)	15 (14.4%)	12 (10.9%)				
	Xulu	14 (1.6%)	15 (2.1%)	2 (1.9%)	2 (1.8%)				
	Other	75 (8.8%)	96 (13.4%)	10 (9.6%)	16 (14.5%)				
Marital Status	Never married	178 (47.5%)	133 (52.6%)	35 (53.0%)	27 (69.2%)	0.523	0.384	0.761	0.25
	Married	147 (39.2%)	94 (37.2%)	25 (37.9%)	9 (23.1%)				
	Divorced	8 (2.1%)	5 (2.0%)	1 (1.5%)	1 (2.6%)				
	Widowed	42 (11.2%)	21 (8.3%)	5 (7.6%)	2 (5.1%)				
Smoking status	Yes	313 (36.8%)	213 (29.7%)	46 (44.2%)	28 (25.5%)	0.003	0.008	0.143	0.36
	No	537 (63.2%)	504 (70.3%)	58 (55.8%)	82 (74.5%)				
Alcohol intake status	Yes	335 (39.4%)	266 (37.0%)	59 (56.7%)	38 (34.5%)	0.340	0.003	0.007	0.61
	No	515 (60.6%)	452 (63.0%)	45 (43.3%)	72 (65.5%)				

\*Derived from chi-squared statistics, p<0.05 denotes statistical significance

Table 4.3 above is a comparative summary of socio-demographic characteristics between anaemic and non-anaemics within and between the HIV sero-status groups. Both non-anaemics and anaemics, irrespective of HIV status, are represented largely in the ≤40 year group than the >40 year group. Age grouping differences between anaemic and non-anaemics were not significant within the groups but both HIV sero-negative anaemics and non-anaemics were significantly more likely to be ≤40 years old, i.e. younger.

For educational attainment, there were no significant differences between and within the sero-status groups for anaemic and non-anaemic peers and the trend was not different from the comparisons between HIV sero-negatives and sero-positives.

Much like the HIV sero-status group comparisons total monthly earnings were similarly distributed between anaemics and non-anaemics. Both anaemics and non-anaemics, irrespective of HIV status, were more represented in lower income category, and though there were marginal differences these were not statistically significant.

With regards to settlement type, anaemics, irrespective of their HIV status, were significantly more likely to be urbanised than rural. However, between the HIV sero-negatives and sero-positives, both anaemics and non-anaemics in the latter group were significantly more likely to be urbanised.

The proportion of females to males was significantly higher in the anaemics compared to their non-anaemic counterparts disregarding their HIV status. However, when HIV status was the basis of comparison the gender differences between anaemics and non-anaemics were not significant.

Trends between anaemics and non-anaemics for first languages spoken and marital status were similar to those obtained when compared between HIV sero-status groups. Both anaemics and non-anaemics were more represented in Tswana as first language speaking and never married categories. Differences between anaemics and non-anaemics for these socio-demographic characteristics were marginal and were also statistically not significant.

When smoking status was considered, the results showed that anaemics, irrespective of their HIV status were significantly less likely to smoke as compared to non-anaemics, but the differences between anaemics and non-anaemics across the two HIV sero-status groups were statistically not significant.

In terms of alcohol consumption, the proportion of consumers was lower in both sero-status groups. However, whilst there was no difference between sero-negative anaemics and their non anaemic peers, sero-positive anaemics were significantly less likely to consume alcohol. Between the anaemics, HIV sero-negative persons were significantly more likely to consume alcohol, whilst the difference between the non-anaemics was not significant.

### **Summary of section 4.2**

Many socio-demographic variables were considered for analysis, which included sex, age grouping, settlement type, educational qualification, first language spoken, marital status, monthly total income, smoking status and alcohol consumption status.

So far, the results have shown that the prevalence of HIV infection in the study population is 11.8%. In addition, though females were higher in proportion compared to males; they were as likely to be infected as their male counterparts. HIV sero-positives were significantly more likely to be younger and also to be urbanised compared to their sero-negative peers. Generally, there were more persons represented in the lower monthly earnings, lower educational attainment, Tswana as first spoken language and never married categories, with smaller proportions in the other categories. Although differences between the two groups were marginal they were not significant. There were also no statistically significant differences between the two groups for proportions who smoked or consumed alcohol, albeit, marginal differences were shown.

With regards to gender comparisons, both males and females, irrespective of their HIV status, are more likely to be in the  $\leq 40$  year group, but infected persons, especially the females have a significantly higher tendency to be in this age group. In terms of settlement type infected males and females were significantly more urbanised than their uninfected counterparts. Differences between infected males and females were, however, not significant even though infected males were proportionately more represented than their female counterparts in the urbanised settlement type. The comparisons between males and females for first languages spoken, educational attainment and marital status followed the same trend as for comparisons between the two sero-status groups reported earlier. Within and between the sero-status groups, gender differences for these socio-demographic characteristics were marginal and not significant. Females, irrespective of HIV status, were significantly more likely to be lower earners and less likely to smoke or consume alcohol, but between the two sero-status groups, there were no significant gender differences in these parameters.

Considering socio-demographic characteristics between anaemics and non-anaemics, both HIV sero-positive categories were significantly more likely to be in the age group ≤40 years. However, within the sero-status groups there were no significant differences in age groupings between anaemics and their non-anaemic peers. With regards to settlement type, anaemics, irrespective of the HIV status were significantly more likely to be urbanised. Furthermore, infected anaemics and non-anaemics were significantly more likely to be urbanised compared to their uninfected counterparts. Within the groups females were significantly more likely to be anaemic than non-anaemic, but gender comparisons between the groups did not show any significant differences for males and females respectively. Both anaemic HIV sero-positive and sero-negative persons were significantly more likely to be urbanised but the non-anaemic and anaemic infected persons showed a significant likelihood of being urbanised when compared to their uninfected peers.

Trends for educational attainment, total monthly earnings, first language spoken and marital status were very similar to the earlier ones reported for sero-status and gender comparisons. The differences in these socio-demographic characteristics comparing anaemics and non-anaemics within and between HIV statuses were marginal and not significant. Anaemics, irrespective of their HIV status were also significantly less likely to smoke compared to non-anaemics. However, whilst infected anaemics showed a significant likelihood for not consuming alcohol as compared to their non-anaemic counterparts, the latter were also significantly more likely to be alcohol consumers compared to their non-anaemic sero-negative counterparts.

It is evident from the above results that some socio-demographic characteristics were significantly different between the two HIV sero-status groups, which are also reflected within the gender and anaemic status comparisons. Clearly these significant differences may be indicative of influence, which means that the significant socio-demographic characteristics could be associated with HIV infection and, therefore, may serve as explanatory factors in the relationship between HIV infection and anaemia in the study population.

#### **4.4 KEY FINDINGS - CHAPTER FOUR**

The key findings in this chapter include the following:

- The prevalence of HIV infection in the study population was 11.8%.
- HIV sero-positive persons were significantly more likely to be younger: Significantly, sero-positive females were more likely to be younger than males and, anaemics, irrespective of HIV sero-status, were also more likely to be younger.

- HIV sero-positive persons were significantly more likely to be urbanised. Sero-positive males and females were significantly more likely to be urbanised compared to their sero-negative counterparts. Anaemics were generally more likely to be urbanised but both sero-positive anaemics and non-anaemics were significantly more likely to be urbanised compared to their sero-negative peers.
- A sizable proportion of the study population, irrespective of HIV sero-status, gender and anaemic status, were more likely to have lower educational attainment, earn lower monthly income, speak Tswana as first language and never married. However, compared to males, females were significantly more likely to be lower income earners.
- Females, irrespective of sero-status, were significantly more likely to be anaemic.
- Although there were no differences between the sero-status groups for proportions who smoked or consumed alcohol, males were significantly more likely to smoke or indulge in alcohol consumption. Anaemics generally were significantly less likely to smoke, whilst seropositive anaemics in particular were less likely to consume alcohol compared to their non-anaemic counterparts.

The next section briefly discusses the findings in this chapter in order to highlight the significance of socio-demographic factors as part of the link in the causal web of HIV and anaemia as proposed by the framework in chapter one (Fig. 1.1).

#### **4.5 DISCUSSION OF KEY CHAPTER FOUR FINDINGS**

This chapter compared socio-demographic characteristics between infected and uninfected groups and also between male and female as well as anaemic and non-anaemic sub-groupings in the study population. The main purpose was to account for any socio-demographic explanatory factors that may influence or modify the effects of HIV and other exposures on the outcome of anaemia. In regarding these significant explanatory factors as confounders in the relationship between HIV and anaemia, they would have to be adjusted for in subsequent analyses. Similarly, the gender related differences observed within and between the groups may suggest an effect modifying influence that may also have to be accounted for in subsequent analyses.

As an initial step, it is important to point out that because the original THUSA study was not designed to measure prevalence of disease (Vorster et al., 2000: Vorster et al. 2005) reference to the THUSA population as 'apparently healthy' is justified by the rigorous selection criteria that ensured that unhealthy persons took part in the study. However, the subsequent serological classification of a group as HIV sero-positive or

infected may defeat this consideration on hind sight. Much as sero-negatives or the uninfected population may still be 'apparently healthy', sero-positives would then essentially fall into an asymptomatic classification as they were found to be free of symptoms of disease. This is important because it depicts the early stage of infection within the sero-positive population. Nevertheless, this categorisation into two HIV sero-status groups provides reasonable justification for the present study.

The HIV prevalence of 11.8% in the study population may be relatively higher than the recently documented prevalence rates in six out of the nine provinces of South Africa (South African Department of Health Survey, 2007; UNAIDS/WHO, 2008), which also includes that for the North West Province (10.9%) (Tab. 2.7). However, it is important to state that the observed HIV prevalence rate in the study is not a true representation, then and now, of the larger population from which the subjects have been chosen for several reasons. Compared to the HIV prevalence at the time of the study, the HIV prevalence in the THUSA population is an underestimation of the true prevalence, whilst it overestimates the current provincial prevalence.

Among the many reasons to suggest a possibly higher HIV prevalence in the province are, firstly, the targeting of an 'apparently healthy' population as inherent in the design of the THUSA study. Because the initial selection criteria was biased towards excluding persons with debilitating illnesses or known medical conditions (like diabetes and hypertension), as well as conditions that may be HIV or AIDS defining, a number of potential HIV infected individuals would be missed. In addition, subject selection also excluded pregnant women, children below 15 years, persons 'under the influence' and those with febrile conditions, another criteria that adds to the missing of potential HIV infected persons, which is likely to underestimate the actual prevalence within the population. In fact, pregnant women and children constitute the most vulnerable in sub-Saharan Africa (including South Africa) and other poor-settings, and in these places HIV prevalence rates in these population groups tend to be especially high (UNAIDS/WHO. 2006; 2008). However, the study prevalence overestimates the current provincial prevalence, which is indicative of a gradual decline in infection rates in South Africa since the study was undertaken.

Despite this possible mis-estimation, the level of HIV prevalence recorded in this study probably underscores the fact that HIV infection has been a public health problem and, perhaps, still remains so in the North West Province of South Africa where the study was undertaken, considering that the current rate is at par with the national estimated HIV prevalence of around 10.8% (South African Department of Health Survey, 2007).

Another important consideration in this study is the identification of the stage of infection for HIV sero-positives. Studies have shown that HIV disease progression is associated with an increased prevalence and severity of anaemia (Spivak et al., 1989, Belperio et al., 2004). This means that the stage of a person's HIV infection can be a very important determinant of the level or severity of anaemia. Thus, identifying the stage of infected persons can be considered a very important step towards assessing the level of anaemia in the population.

With regards to staging, guidelines have been provided by the CDC (CDC, 1993). With the CDC criteria in mind, HIV infected persons in the study population could be more appropriately classified as stage I or asymptomatic for a number of reasons. Firstly, the rigorous selection criteria ensured that those selected were 'apparently healthy' until they were serologically identified with HIV. In addition, persons who were in other stages of infection would have been excluded as they would most probably have shown clinical signs for exclusion. It is most likely that a large number of the infected subjects, if not all, were at an early stage of HIV infection as they were in a relatively good health, unaware of their HIV status and were enjoying a normal life as everyone else.

Demographic surveys carried out in many countries, and especially, where the population is in transition and HIV is endemic, suggest that females, younger persons as well as urbanised settlers are more likely to be infected (UNAIDS. 2006; 2008). The significant likelihood for HIV sero-positive persons in this study population to be younger and urbanised may lend support to this suggestion.

Whilst younger persons are more likely to be involved in risky behaviours that may expose them to the infection, the conditions for acquiring HIV tend to be rife in urban areas (UNAIDS. 2006; 2008). The general trend in certain socio-demographic characteristics of the study population tends to highlight the vulnerability of the study population not only to HIV infection but also to anaemia.

In South Africa and indeed in the North West Province a rapid wave of urbanisation is sweeping across (Vorster et al., 1999) with attendant changes in the socio-demographics of the population. Urbanisation, and especially where it is rapid, often comes with socio-economic, nutritional and other environmental changes which may greatly affect income and subsequently reduce people's ability to acquire food and other basic amenities (Vorster et al. 1999). Under such circumstances younger persons tend to migrate from the rural areas to live in transitional or urban settlements

(or slums) as they seek greener pastures (UNAIDS, 2006). Nutritional and lifestyle changes occur, as individuals are compelled to cope and survive. Changes include the consumption of cheap or affordable and often poor quality foods, the commercialisation of sex and abuse of drugs including alcohol, which may influence the predisposition of such persons to HIV and other infections (Vorster et al., 2000). In South Africa, these changes have been noted as part of a series of effects that has culminated in what is referred to as the 'triple burden' of morbidity and mortality (Vorster et al., 2000; Vorster et al. 2005).

In this study, the general effect of urbanisation on socio-economic status is reflected in the very large numbers of the study population (about two-thirds) that were earning lower monthly incomes as well as a large majority whose educational attainments could be described as poor. Indeed, poor socio-economic circumstances, such as can be associated with this predominantly African (black) study population, may constitute part of the vicious cycle that allows poverty, HIV infection and anaemia to perpetuate (Semba, 2003). Apart from the higher predisposition to HIV and other infections conferred by this racial characteristic (Shisana et al., 2005), it is also very likely that the interactions between poverty, nutrition and other less perceptible racial factors could heighten the vulnerability of the group.

With a large proportion of females, who are significantly likely to be younger, poorer, never married and also poorly educated, the high HIV prevalence rate in the study population could be reasonably explained. Apart from their higher biological vulnerability to HIV infection, many socio-demographic factors associated with females, including the significant characteristics of being younger, lower monthly earnings and poorer educational attainments add to their vulnerability. Although it has not been established in this study, in many poor settings and regions experiencing rapid transition, younger females often drop out of school and usually remaining unmarried have to fend for themselves. A large number may reside in or migrate to urban slums in search of greener pastures and most of them may end up in sexual compromises often fanned by peer pressure, drugs and alcohol, which could enhance their risk to HIV infection (UNAIDS, 2008).

Since many potential effects of HIV infection may be part of its causal relationship with anaemia, any factor that may affect the prevalence or severity of HIV infection or modify its effects within the population could be connected as an integral part of the linkage between HIV and anaemia. In this study population, even though females are significantly more likely to be anaemic, this gender characteristic may not be

considered as part of the link between HIV and anaemia, because sero-positive females were as likely to be anaemic as their sero-negative peers. Nevertheless, the reasons why females are more likely to be anaemic can be ascribed to biological attributes such as menstrual blood losses and pregnancy/delivery associated iron losses (Volberding et al., 2004) as well as poor dietary practices which tends to reduce iron intake.

Another socio-demographic attribute of anaemics in general, as far as this study is concerned, is that they were more likely to reside in an urbanised settlement. Considering the changing nutritional and socio-demographic circumstances in a rapidly urbanising settlement, many young persons would have diets that are impoverished. They may resort to coping mechanisms which includes having fewer meals and eating cheaper foods often lacking in nutrients (UNAIDS, 2006). These could eventually make them nutrient deficient. In addition, socio-demographic changes could lead to inadequate provision of amenities as well as a deterioration of sanitation and health (Calder and Jackson, 2000), which would increase vulnerability to infectious diseases. This implies that in a rapidly urbanising environment persons who are young and poor, often belonging to the female population, are more likely to be nutrient deficient and therefore more susceptible to infectious diseases. Altogether, these would make such individuals more likely to be anaemic. Thus, the significant likelihood of infected persons or anaemics to reside in urbanised settlements may point to the fact that living in an urbanised settlement could be an important socio-demographic consideration for the relationship between HIV and anaemia.

Perhaps other important socio-demographic attributes in the study population, which could be invoked to explain the association of anaemia with females is that males were significantly more likely to smoke and consume alcohol than females. Smoking generally creates a hypoxic environment within the respiratory tract as the smoker continually floods the airways with carbon dioxide and other substances. An increase of carboxy-haemoglobin that often ensues creates a hypoxic condition, which eventually serves as a stimulus for haemoglobin synthesis. Thus, smoking may increase the Hb levels by about 0.3 g/dL (WHO, 2004), which tends to make smokers less likely to be anaemic compared to non smokers. The finding that non-smokers in this study are significantly more likely to be anaemic may lend support to this assertion.

With regards to intake of alcohol, some moderate levels of consumption has been shown to boost food intake (Yeomans, 2004), and with this may come an increase in nutrient intake, which may also lead to an improvement in Hb and RBC syntheses.

This could explain why males, who are more likely to drink, are also less likely to be anaemic. The fact that sero-positive anaemics were significantly less likely to drink alcohol could perhaps be an added reason to explain their anaemic status.

At this point, it is important to state that the socio-demographic factors considered in this study only form part of a constellation of factors that may relate HIV infection to anaemia in this population. According to the framework for this study some factors other than socio-demographic are likely to cause anaemia independently or interact with many others to increase the susceptibility of sero-positive persons to anaemia. These other factors and their interplay or interaction with, especially, HIV infection, would be the matter for exploration in the next two chapters.

## CHAPTER FIVE

### 5.0 DETERMINATION OF HIV ASSOCIATED FACTORS THAT MAY SIGNIFICANTLY CONTRIBUTE TO ANAEMIA IN THE STUDY POPULATION

In the previous results chapter, socio-demographic variables have been compared between HIV infected and uninfected groups as well as gender and anaemia status sub-groupings in the study population. The levels of association between socio-demographic factors with HIV infection and anaemia have also been explored.

In the following sections factors that have been proposed to constitute a part of the link within the causal web of HIV infection and anaemia in the study population would be examined to see if there are any independent or interactive associations.

#### 5.1 INTRODUCTION

Some studies have shown that HIV may directly influence the proliferation of erythrocyte progenitor cells to cause a reduction in RBC numbers. Many indirect or side effects of HIV also tend to affect the host's nutritional status as well as other metabolic processes, which may subsequently reduce RBC proliferation. As a result of these effects, infection with HIV may become a multiple risk for the development of anaemia in a sufferer.

Many biological factors can be considered as being part of the multiple causation of anaemia in HIV infection. However, in this study a direct effect of the virus, depicted by differences in anaemia prevalences by sero-status groupings, as well as indirect effects that may be constituted as reductive adaption, increased nutrient deficiency and metabolism, and inflammation or inflammatory response, would be considered for exploration as part of the explanatory factors between HIV infection and anaemia.

The initial explorative step in this chapter to establish an overall effect begins with the determination of anaemia prevalences including characteristics and relationship with iron status in the study population. This would then be followed by further explorative analyses to establish independent risk relationships between each of the considered factors, as exposures with HIV infection, on the measured outcome of anaemia.

The objectives set for the explorative analyses were as follows:

- To relate anaemia with effects of HIV (status) in the study population (Determination of anaemia prevalences)
- To relate body compositional and energy changes with HIV and anaemia statuses in the study population.

- To relate micronutrient imbalances with HIV and anaemia statuses in the study population
- To relate inflammation with HIV and anaemia statuses in the study population

## **5.2 RELATING ANAEMIA WITH AN OVERALL HIV EFFECT (STATUS) IN THE STUDY POPULATION**

In this section the independent association between HIV and anaemia is highlighted by the determination of sero-status prevalences and other characteristics of anaemia in the study population.

The [Hb] and Hct cut-offs used in defining anaemia were, <13 g/dL or <12 g/dL and <39 or <36 for adult men or premenopausal women respectively (see table A1, Appendix A). Generally accepted cut-offs for the iron status markers were also considered for exploring the different characteristics of anaemia in the study population.

### **5.2.1 COMPARING MARKERS OF ANAEMIA BETWEEN HIV SERO-STATUS GROUPS AND ANAEMIA STATUS SUB-GROUPS**

The table below (Tab. 5.1) represents the summary of the results that compares the means of the different markers used in defining anaemia between sero-status groups and their anaemia sub-grouping.

From table 5.1, mean levels of [Hb] and Hct were lower in the HIV infected group compared to their uninfected counterparts. However, only Hct differences were significant ( $p<0.001$ ) both before and after the significant socio-demographic confounders of gender, age grouping, settlement type, smoking and alcohol consumption, which meant that the effect of HIV infection on Hct was significant. Both [Hb] and Hct remained significantly lower ( $p<0.001$ ) in the anaemic compared with their non-anaemic peers. Therefore, the effect of anaemia on these parameters was also significant. With regards to interaction between HIV and anaemia, there was no significant effect on Hct levels. However, the interactive effect on [Hb] which was marginally significant before the adjustment became insignificant after the adjustment.

Iron (Fe), TIBC and ferritin were marginally lower, whilst % transferrin saturation was marginally higher in the sero-positives, but there were no significant differences, even after adjusting for the socio-demographic confounders, between sero-positives and sero-negatives.

**Table 5.1: Markers of anaemia and/or iron status compared between anaemic and non-anaemic sub-groups using ANOVA**

	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (n=798)	Anaemic (n=683)	Non-anaemic (n=104)	Anaemic (n=106)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
Haemoglobin (g/dL)	14.14 (14.03-14.26)	11.16 (11.07-11.24)	14.02 (13.84-14.60)	10.79 (10.47-11.11)	0.061	0.189	<0.001	<0.001	0.050	0.094
Haematocrit (%)	44.79 (44.49-45.10)	41.07 (40.73-41.42)	43.65 (42.76-44.55)	39.13 (37.99-40.27)	<0.001	<0.001	<0.001	<0.001	0.243	0.409
Serum Fe (µmol/L)	18.08 (17.51-18.66)	15.35 (14.76-15.93)	18.00 (16.22-19.78)	15.29 (13.81-16.78)	0.911	0.392	<0.001	<0.001	0.984	0.718
TIBC (µmol/L)	66.82 (65.94-67.69)	68.66 (67.55-69.76)	64.59 (62.61-66.58)	68.83 (65.34-72.31)	0.315	0.087	0.003	0.034	0.241	0.322
% transferrin saturation	27.71 (26.77-28.64)	23.32 (22.34-24.29)	28.66 (25.51-31.81)	23.84 (21.30-26.38)	0.459	0.690	<0.001	<0.001	0.828	0.870
Serum Ferritin (µg/L)	114.07 (103.54-124.61)	100.71 (91.51-109.91)	110.15 (87.95-132.35)	87.61 (68.45-106.78)	0.396	0.620	0.073	0.384	0.647	0.928

Statistical significance is denoted by p<0.05. \*Adjusted for gender, age grouping, settlement type, smoking and alcohol consumption. Data values are means (95% confidence interval – CI)

However, the differences between anaemics and non anaemics were highly significant except for ferritin (Tab. 5.1). This meant that whilst there was no significant effect of HIV infection, anaemia had a highly significant effect on these parameters. The interactive effects between HIV infection and anaemia on these iron status markers were also not significant.

In the next section the prevalence or levels of anaemia in the study population, using the main markers of [Hb] and Hct, and considering various sub-groupings would be presented.

### **5.2.2 PREVALENCE OF ANAEMIA IN THE STUDY POPULATION**

The prevalence of anaemia, defined by both [Hb] and Hct, and some specific iron status markers are compared between HIV infected and uninfected groups and other significant socio-demographic sub-groupings. Grades of anaemia defined by [Hb] are also compared between the sero-status groups. Based on the assumption that Hct relates to 3 times [Hb] (Sacher and McPherson, 2000; Bain and Bates, 2001), a Deming regression<sup>10</sup> rather than a normal linear regression fit is used to estimate a more realistic Hct cut-off to be used to determine anaemia prevalence.

#### **5.2.2.1 Prevalence of anaemia - defined by WHO cut-offs for Haemoglobin concentration and Haematocrit**

Table 5.2 below shows prevalence of anaemia in the study population (defined by [Hb] and Hct) and compared between HIV sero-positive and uninfected groups as well as within significant socio-demographic sub-groupings. Also shown are grades of anaemia between sero-status groups.

From table 5.2, it is evident that prevalence of anaemia was higher in HIV sero-positives than in sero-negatives, however, the differences became significant when anaemia was defined by Hct cut-offs ( $p<0.001$ ). The higher prevalence in HIV sero-positives was in agreement with prevalence values obtained for both sero-positive males and females, which were higher compared with those of their sero-negative counterparts. This was irrespective of the marker used to define anaemia. However, only HIV sero-positive females had significant Hct-defined prevalence compared to their sero-negative counterparts. Irrespective of sero-status, anaemia prevalence in females was higher than males, which rather confirmed the earlier finding that females in the study population were significantly more likely to be anaemic than males. In contrast, anaemia prevalences defined by Hct were generally lower compared to those defined by [Hb] (Tabs. 5.2). However, the differences in prevalence between the sero-

status groups using the Hct definition were significant whilst the [Hb] definitions were not. Because of the lower but significant prevalences obtained with Hct definitions and considering that Hct cut-offs could relate to  $3 \times [\text{Hb}]$ , There is a high likelihood that this relationship would deviate from what is conventionally accepted. A DR analysis would therefore be used in the next section to establish cut-offs, which would be used to determine prevalences that may better reflect the relationship between the two markers.

**Table 5.2: Prevalence of anaemia in socio-demographic sub-groupings and grades of anaemia in the study population**

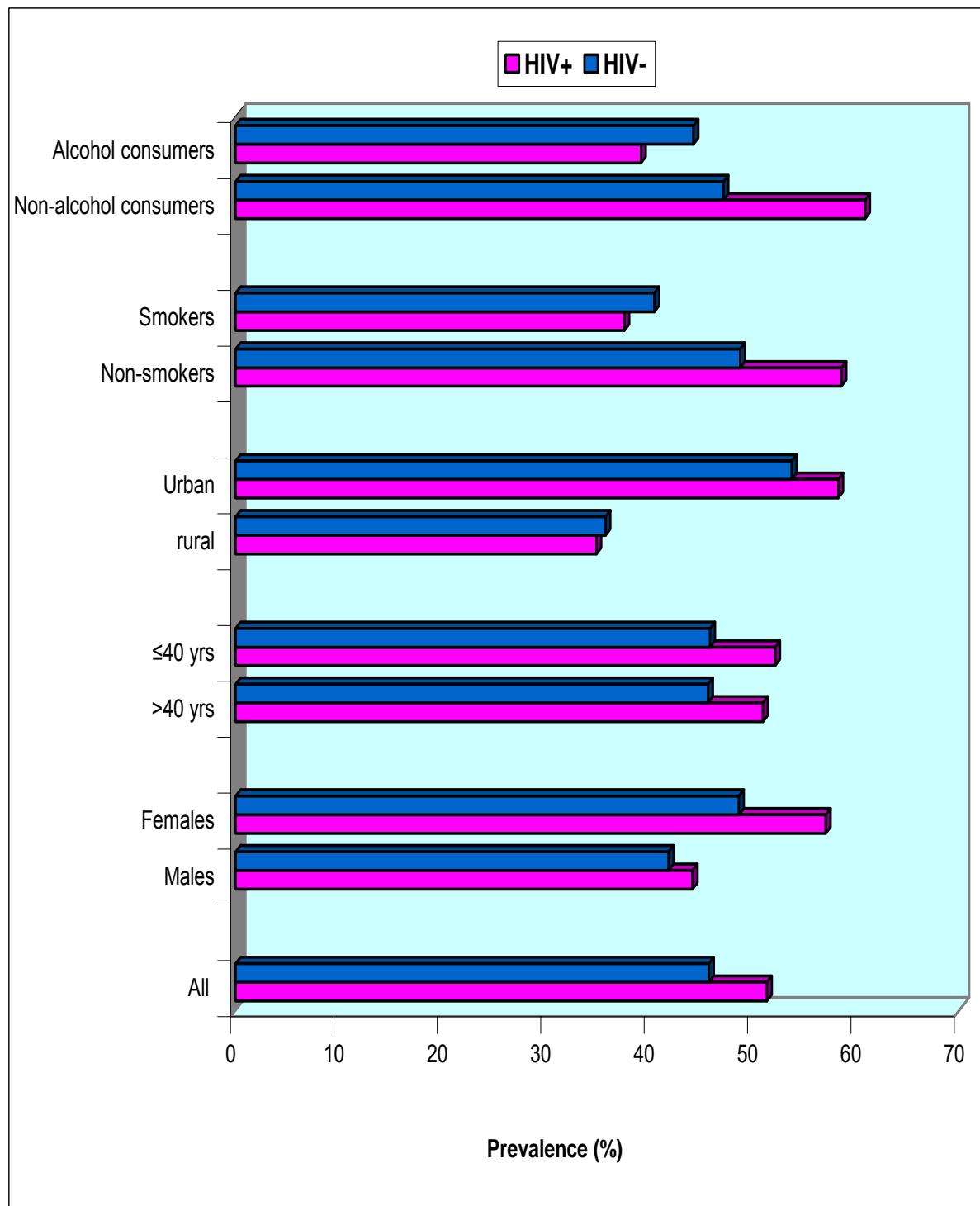
Category	[Hb] as indicator				Hct as indicator			
	HIV-	HIV+	Overall	p value	HIV-	HIV+	Overall	p value
Socio-demography								
All	45.8	51.4	46.4	0.123	11.2	20.0	12.9	<0.001
Males	41.9	44.2	42.1	0.663	6.9	9.5	7.3	0.374
Females	48.7	57.1	49.7	0.066	14.4	28.7	16.1	<0.001
>40 yrs	45.7	51.0	46.5	0.234	10.9	20.6	12.2	0.001
≤40 yrs	45.9	52.2	46.5	0.317	11.7	18.8	12.4	0.084
rural	35.8	34.9	35.7	0.887	9.4	15.4	10.0	0.103
Urban	53.8	58.3	54.5	0.307	12.6	21.8	14.0	0.003
Non-smokers	48.8	58.6	49.6	0.024	13.7	25.0	15.0	0.001
Smokers	40.5	37.6	40.2	0.663	6.3	10.8	6.9	0.152
No-alcohol intake	47.2	60.9	48.7	0.006	13.4	26.8	14.9	<0.001
Alcohol intake	44.3	39.2	43.6	0.349	8.6	11.5	9.0	0.366
Grades of anaemia								
Mild	31.1	32.4	31.3	0.278	-	-	-	-
Moderate	12.9	15.3	13.2	0.163	-	-	-	-
Severe	0.7	3.2	1.0	0.009	-	-	-	-

Statistical significance is denoted by  $p < 0.05$ .

Table 5.2 above also shows that, with regards to age grouping, settlement type, smoking and alcohol consumption statuses, anaemia prevalences (defined by [Hb]) were higher in HIV sero-positives except for rural settlers, non-smokers and persons who consumed alcohol, where prevalences were lower than in their sero-negative peers. Anaemia prevalences in sero-positive non-smokers and persons who did not consume alcohol were significantly higher ( $p=0.024$  and  $p=0.006$  respectively) than for their sero-negative counterparts. However, all other differences in prevalence between the sero-status groups were not significant.

With Hct defining anaemia, prevalences for sero-positives for all the significant socio-demographic variables were higher than for their sero-negative peers. Almost all of the comparative differences were highly significant except for those between the categories of males,  $\leq 40$  years age grouping, rural settlers, smokers and alcohol consumers. The figure below (Fig. 5.1) illustrates the comparative prevalences between sero-status groups as well as between socio-demographic sub-groupings.

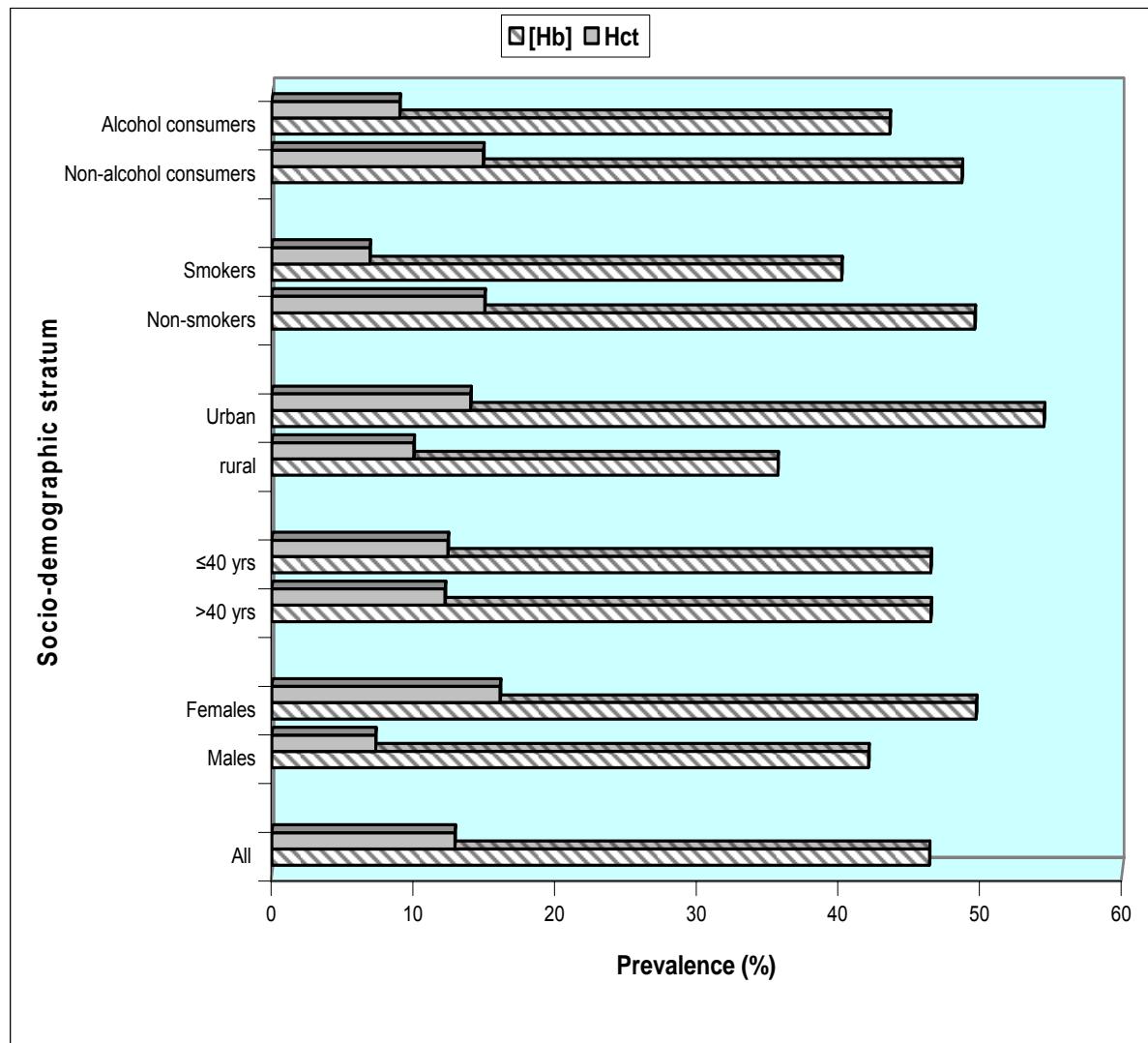
**Figure 5.1: Prevalence of anaemia (defined by [Hb]) in the study population**



Using [Hb] to define anaemia clearly identified a larger proportion of individuals with anaemia than with Hct. These differences have been illustrated in figure 5.2 below for the various demographic categories within the sero-status groups. By the WHO standards of defining anaemia, [Hb] is a more sensitive marker as the illustration below seems to confirm. Nevertheless, there was significant agreement between the two markers with a partial correlation of  $r=0.547$  ( $p<0.001$ ), albeit, the Hct definition was more likely to show significant differences in anaemia prevalence between the

groups. Despite the significant agreement shown between the two markers, the correlation coefficient was less than 0.8, which statistically suggests a less accurate expression of the relationship. It is very likely that the normally assumed relationship, which estimates Hct as three times Hb (Bain and Bates, 2001; Sacher and McPherson, 2000), does not hold for this population. The of this assertion is explored by the use of a DR analysis in the next section. Deming regression fits would then be used to derive cut-offs for Hct and then the prevalence of anaemia would be determined using these new cut-offs.

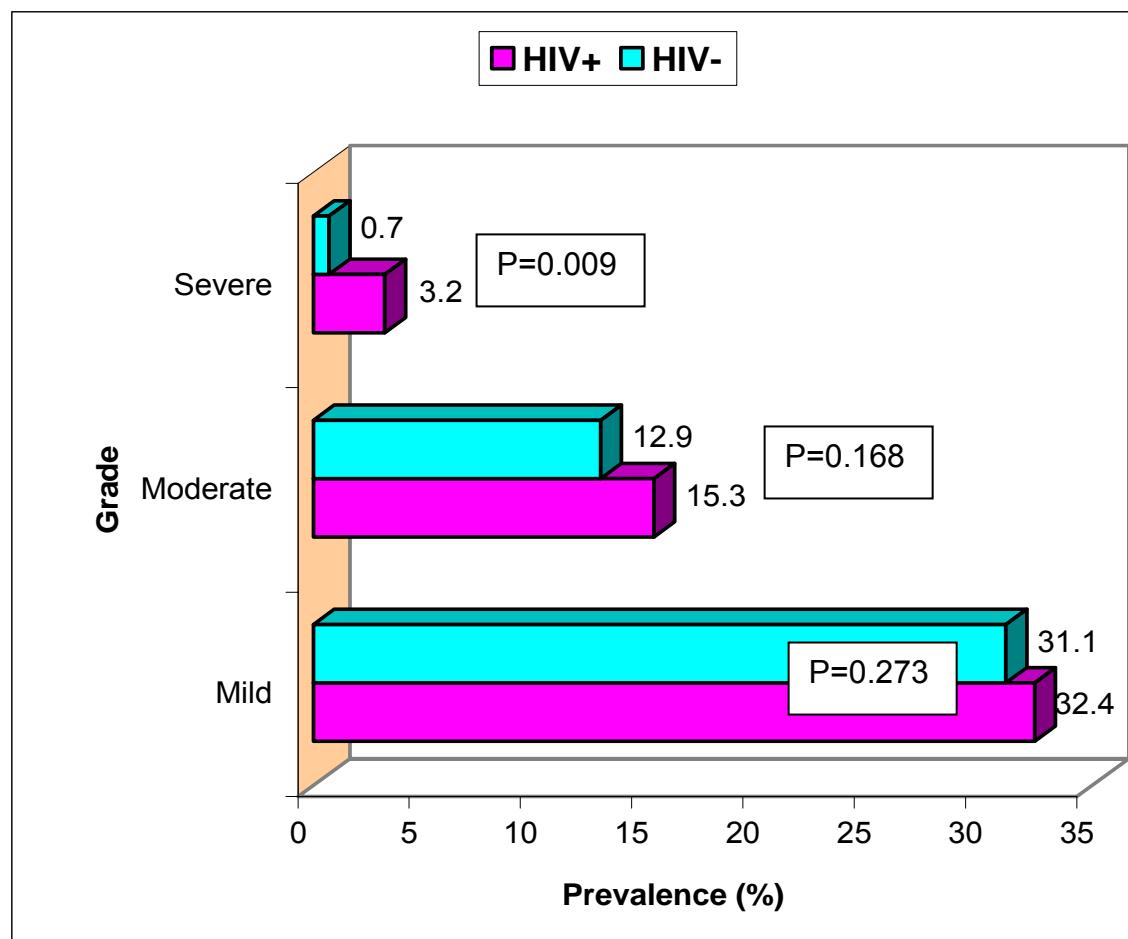
**Figure 5.2: Comparing prevalences of anaemia defined by [Hb] with those defined by Hct in the study population**



Again, as illustrated in figure 5.3 below, [Hb] defined cut-offs for anaemia have been used to categorise 3 stages of anaemia in HIV infected and uninfected persons. These values are also presented in table 5.2 above for mild, moderate and severe grades. The figure clearly depicts higher prevalences for mild, moderate and severe anaemia in the sero-positive group than in their uninfected counterparts.

Generally, about 31% of the overall study population were mildly anaemic (about 70% of anaemia), whilst 13.2% and 1% were moderately and severely anaemic respectively. This means that mild anaemia was the most prevalent grade of anaemia in both sero-status groups. Whilst the differences between the two sero-status groups for mild and moderate anaemia prevalences were not significant, HIV sero-positives were significantly ( $p=0.009$ ) more likely to be severely anaemic as compared to their uninfected counterparts, but the numbers considered in this category were very small (HIV-, 11 [0.7%]; HIV+, 7 [3.2%]). However, this tendency for severe anaemia may imply that HIV infection could contribute to the high levels of anaemia in the study population and may also be associated with its exacerbation in the sero-positive population.

**Figure 5.3: Grades of anaemia (defined by Hb) compared between sero-status groups**



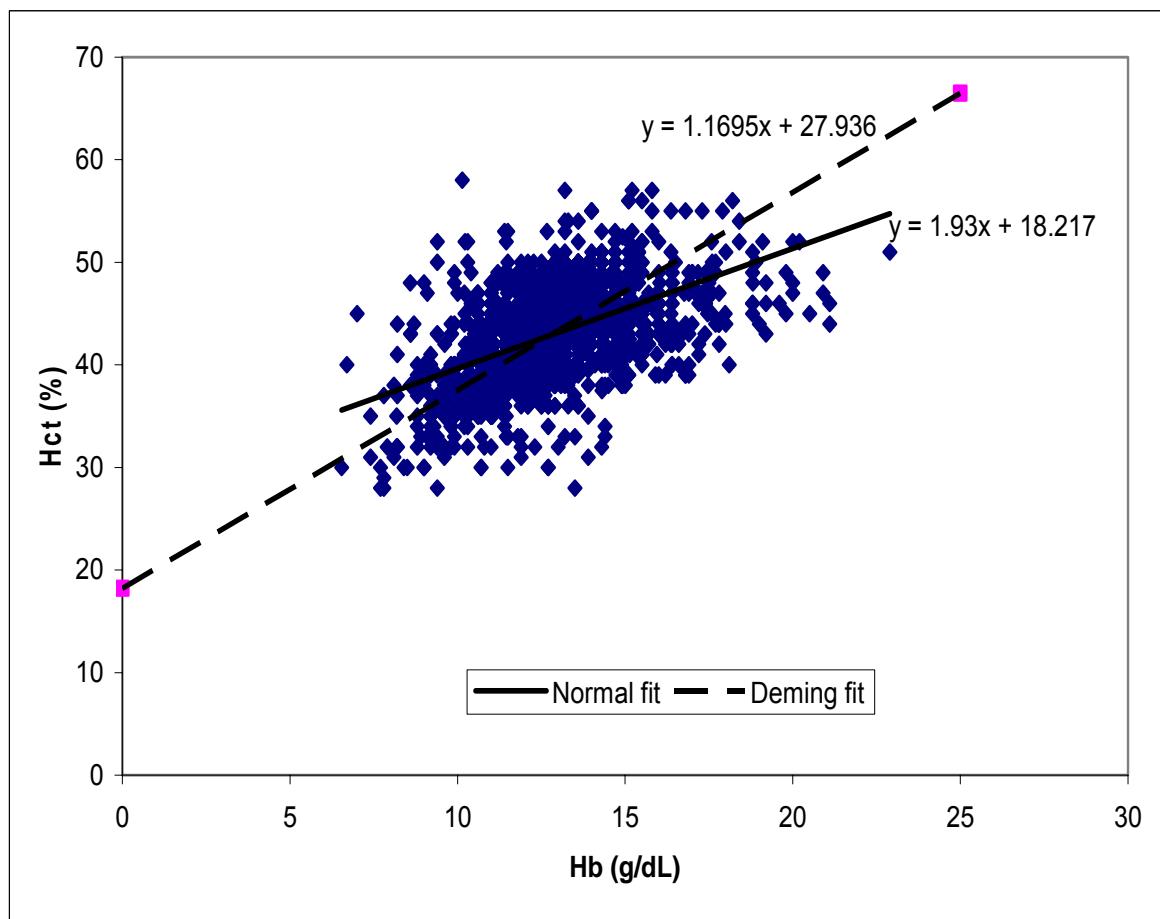
#### 5.2.2.2 Prevalence of anaemia - estimated from Deming regression derived haematocrit cut-offs

The scatter plots below (Figs. 5.4 [a, b & c]) represent relationships between [Hb] and Hct for the whole study population, males and females respectively. Regular linear

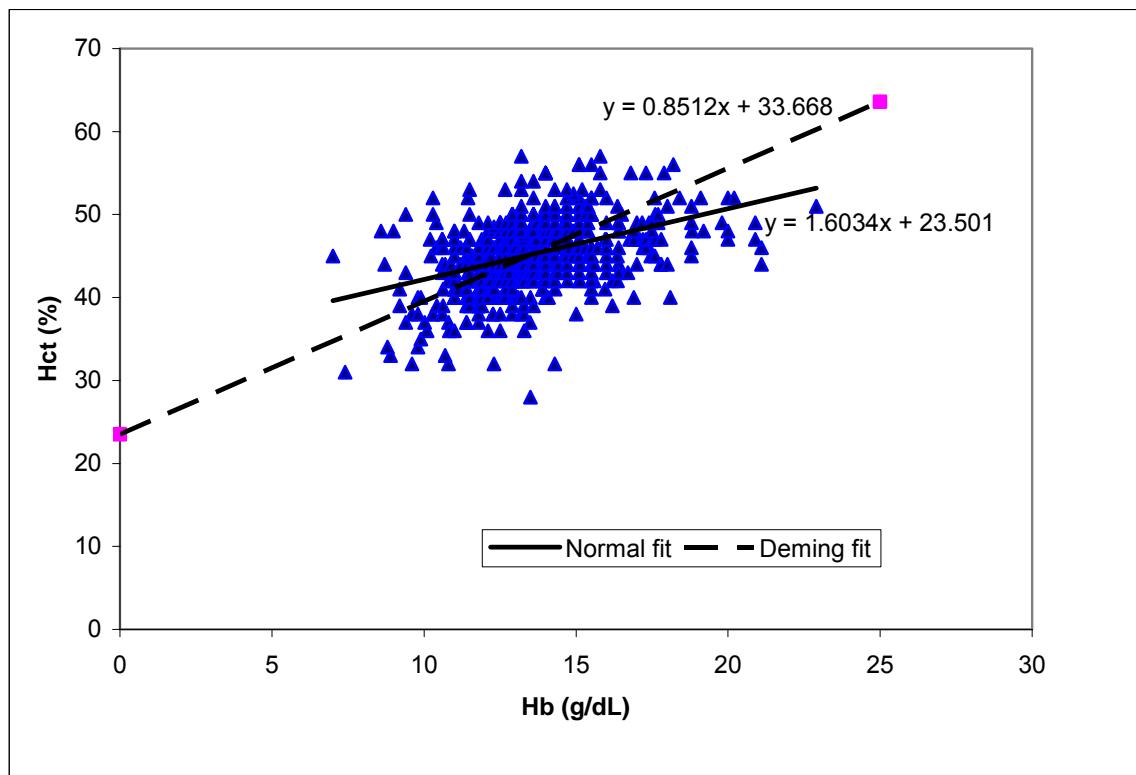
regression fits are also shown alongside DR fits derived from DR analysis, which unlike the normal linear regression accounts for errors on both x and y axes. The essence of this manoeuvre is to derive a relationship between [Hb] and Hct that better predicts one from the other.

Apart from the differences in the predictive powers of the two regression fits in figures 5.4b and 5.4c below, they both do not seem to be in agreement with multiplying the [Hb] by three to obtain an approximate Hct level as may pertain in normal clinical practice (Bain and Bates, 2001). At and below the cut-off for [Hb] the Deming regression line estimates the ratios as close to 1:3.4 in both males and females (considering [Hb] cut-offs of <12 and <13 g/dL for males and females respectively as the x variates). This perhaps suggests that the conventionally accepted values based on the 1:3 ratio is more likely to under-estimate prevalence of anaemia when Hct values are used.

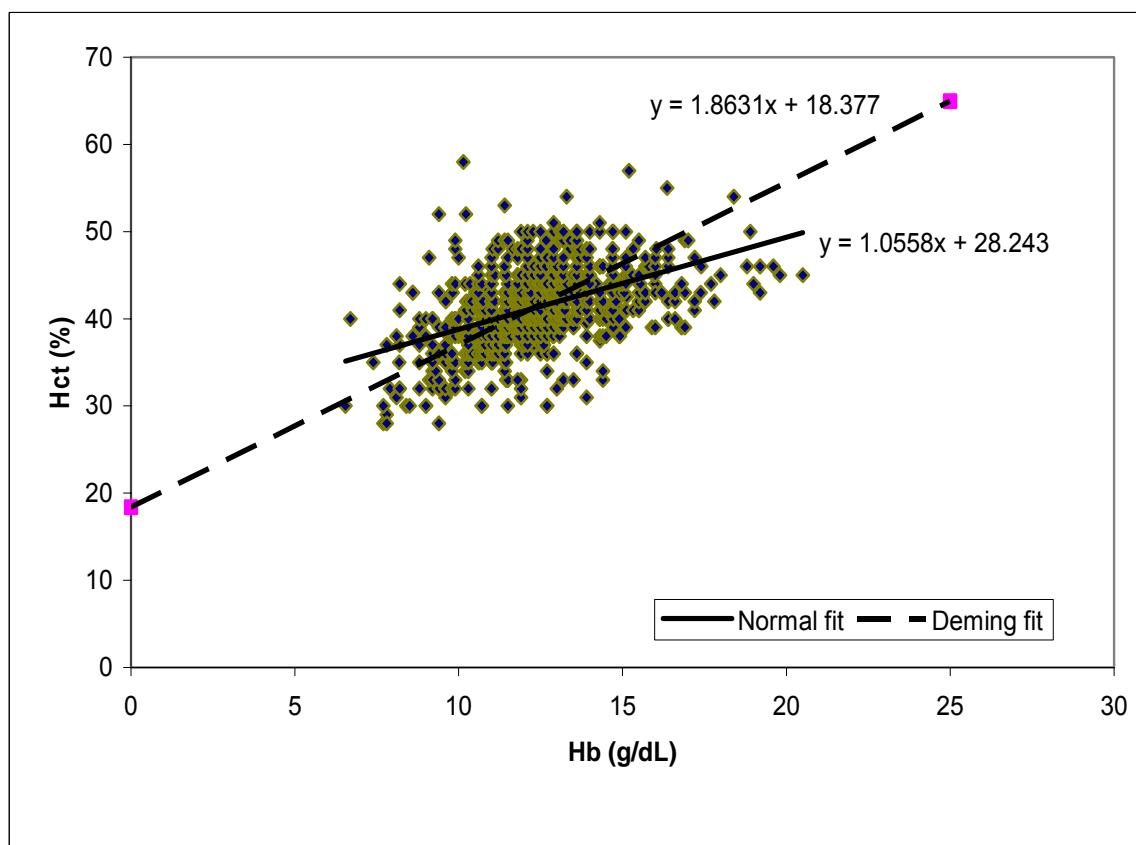
**Figure 5.4a: Scatter plot for [Hb] against Hct with regular and Deming regression fit lines for the whole study population**



**Figure 5.4b: Scatter plot for [Hb] against Hct with regular and Deming regression fit lines for males in the study population**



**Figure 5.4c: Scatter plot for [Hb] against Hct with regular and Deming regression fit lines for females in the study population**



From a [Hb] <13 g/dL for males, the estimated Hct cut-off of <44.33 was obtained, whilst [Hb] <12 g/dL for females corresponded with an Hct cut-off value of <40.72. These estimated cut-offs were then used as the bases for re-determining prevalences of anaemia in the study population.

In the table below (Tab. 5.3) anaemia prevalences defined by Deming derived Hct cut-offs have been compared between the sero-status groups and gender sub-group alongside prevalences estimated from [Hb] cut-offs presented earlier (Tab. 5.2).

**Table 5.3: Prevalence of anaemia using Deming derived Haematocrit cut-offs compared with estimates from [Hb] cut-offs**

Category	Prevalence of anaemia							
	[Hb] as indicator				Deming derived Hct as indicator			
	HIV+	HIV-	All	P value	HIV+	HIV-	All	P value
Males and females	51.4	45.8	46.4	0.123	53.5	41.5	42.9	0.001
Males	44.2	41.9	42.1	0.663	50.0	39.5	40.8	0.024
Females	57.1	48.7	49.7	0.066	56.4	42.9	44.4	<0.001

Statistical significance is denoted by  $p < 0.05$ .

Evidently, the prevalences estimated from the DR analysis show that levels of anaemia with Hct cut-offs are closer and in most cases slightly higher in the HIV infected persons as compared with anaemia defined by [Hb]. On the contrary, uninfected persons tended to have rather lower levels of anaemia estimated by the derived Hct cut-offs than [Hb] cut-offs. The differences between the 2 sero-status groups were however highly significant. Generally, HIV infected persons had anaemia prevalences that were still higher than those of their uninfected counterparts.

### 5.2.2.3 Prevalence of anaemia associated with iron status indicators

In Table 5.4 below, the proportions of individuals classified as anaemic by the use of various cut-offs for iron status markers are shown. The table shows that a marginally higher proportion of HIV infected persons compared with their uninfected peers could be regarded as having lower serum levels of iron (19.5% cf. 16.7%), TIBC (65.6% cf. 61.8%), % Saturation (25.4% cf. 20.7%) and ferritin (14.8% cf. 12.4%), though the differences in proportions were not significant. This implies that HIV sero-positives in this study population were as likely to be associated with iron deficiency related anaemia as sero-negatives. Sero-positive persons were proportionately more likely to be iron depleted (ferritin <15 µg/L) and less likely to be iron overloaded (ferritin >100 µg/L) though the differences were not statistically significant. Using ferritin and [Hb] cut-offs together, both sero-positive and sero-negative persons were about equally likely to be categorised with having IDA or ACI.

**Table 5.4: Characteristics of anaemia in the study population using iron status markers and their combinations**

Parameter	Cut-off point	Percentage below cut-off point			
		HIV-	HIV+	All	p value
[Hb] (g/dL)	<12 (♀)/13 (♂)	45.8	51.4	46.5	0.358
Hct (%) ; WHO cut-off	<36 (♀)/39 (♂)	11.2	20.0	12.3	<0.001
Hct (%) ; DR cut-off	<40.7 (♀)/44.3 (♂)	41.5	53.5	42.9	0.001
Serum iron (μmol/L)	<9.0	16.7	19.5	17.1	0.302
TIBC (μmol/L)	<70	61.8	65.6	62.3	0.295
Saturation (%)	<15	20.7	25.4	21.3	0.126
Iron deficiency	Ferritin <15 (μg/L)	12.4	14.8	12.6	0.307
Iron overload	Ferritin >100 (μg/L)	38.6	36.6	38.3	0.572
Iron deficiency anaemia	Hb<12/13(g/dL) & Ferritin<15(μg/L)	8.4	10.6	8.5	0.261
Anaemia of chronic inflammation	Hb<12/13(g/dL) & Ferritin>60(μg/L)	20.9	25.6	22.3	0.376

Hct – haematocrit; DR – Deming regression; TIBC - total iron binding capacity, ♀ - female, ♂ - male. Statistical significance is denoted by  $p<0.05$ .

However sero-positives had a slightly higher prevalence compared to their sero-negative peers with respect to both IDA (10.6 cf. 8.4%) and ACI (25.6% cf. 20.9%), even though the differences were not statistically significant. Generally, when the high levels of anaemia, as defined by [Hb], are considered side by side with other iron status parameters depicting iron depletion (low serum iron, low % transferrin saturation and low ferritin) and those depicting inflammatory presence (high ferritin, low TIBC), it is quite possible to observe that ACI is likely to be more common than IDA in the study population although the results do not confirm this.

### Summary of section 5.2

The results in the section above show that:

1. Comparing the anaemia defining markers between the sero-status groups showed that Hct was significantly lower in the HIV sero-positives and anaemics and effects of both HIV infection and anaemia on this parameter was very significant, although the interactive effect was not. For [Hb], serum iron, TIBC, % saturation and ferritin, differences between sero-positives and sero-negatives were marginal and not significant. There was also no significant effect of HIV on these, but anaemia had a significant effect on all except ferritin. With regards to interaction, there were no significant effects except [Hb] which showed a borderline significance that was lost after the adjustment.
2. Anaemia prevalences in the general study population, defined by both [Hb] and Hct cut-offs, were quite high. However, prevalence in the HIV sero-positive group was rather higher irrespective of gender and age grouping for either definition. However, using [Hb] definition, HIV sero-positive urban settlers, non-smokers as

well as non-consumers of alcohol were more likely to be anaemic, whilst rural settlers, smokers and consumers of alcohol were less likely to be so compared to their sero-negative peers. Hct definition gave contrasting results as rural settlers, smokers and consumers of alcohol were more likely to be anaemic than their uninfected peers. Only the difference in prevalence between the groups for non alcohol consumers was significant, but with Hct all but the differences for males, ≤40 year grouping, rural settlers, smokers and consumers of alcohol were highly significant. Only Hct defined anaemia prevalences were significantly higher. In addition, HIV sero-positives were more likely to be mildly and moderately anaemic and significantly more likely to be severely anaemic compared to their uninfected peers.

3. In terms of iron status, HIV persons tended to have marginally lower levels of serum Fe, serum ferritin and TIBC and marginally higher % saturation than their uninfected counterparts. Risk levels were, however, not significantly different, comparing the two groups for low serum Fe, serum ferritin and % saturation, but was higher and significant for lower TIBC in favour of the infected population.
4. Cut-offs for serum iron and ferritin levels revealed that a higher proportion of HIV sero-positive persons compared to their uninfected peers were more likely to be hypoferaemic (i.e. with low serum Fe) or hypoferritinaemic (i.e. with low serum ferritin levels), which made them more likely to be iron deficient. HIV sero-positive persons were also more likely to be associated with hyperferritinaemia (i.e. ferritin levels above 100 µg/L), IDA or ACI, even though in all cases the risk levels were not significantly different between the two groups. A closer look at the trends in the iron status markers revealed that ACI was most likely to be common than IDA in the study population.

The implications of the above findings are that HIV infected persons in the study population can be considered to be more anaemic than uninfected persons, which could mean that infection with HIV, even at an early stage, can be independently associated with anaemia.

The next section would begin the exploration of some factors considered as important and, within the constraints of the data set, could explain anaemia in the study population.

### 5.3 REDUCTIVE ADAPTATION AS A POSSIBLE EXPLANATION FOR THE ANAEMIA IN THE STUDY POPULATION

In this section the tendency for HIV infection to cause changes in body composition with time as a result of metabolic and other physiological changes that may be associated with the infection, is explored. The adaptive changes that may occur with HIV infection often affects energy balance and could have far reaching implications on the development of anaemia. Changes in body composition may manifest as loss of weight or lean body mass and/or an energy imbalance, which by and large affects red cell metabolism.

Table 5.5 below shows the comparative differences in body compositional measurements between sero-status groups in the study population. Sero-positives were proportionately more likely to be lighter but taller, which also meant that they were more likely to have lower BMI compared to sero-negatives. LBM and LMI were, however, closely similar between the two sero-status groups. With regards to skin fold measurements, sero-positives tended to have lower triceps and subscapular but higher iliac crest, supraspinale, abdominal, thigh and calf as compared to sero-negatives. Clearly, the marginal differences in weight, height, and derivatives of BMI, LBM and LMI as well as the skin fold measurements were not significantly different between the two sero-status groups even when the socio-demographic confounders were adjusted for. This shows that there were no significant effects of HIV infection on these parameters.

Comparing anaemics with non-anaemics, on the other hand, showed that anaemia had some significant effects on BMI, LBM and some skin fold measurements. Anaemics had higher BMI than non-anaemics, whilst LBM was rather higher in non anaemics compared with anaemics. The effect of anaemia on BMI and LBM were rather significant initially, but this was lost after the adjustment. There was also no significant effect of anaemia on LMI. With regards to skin fold thicknesses, some (subscapular, abdominal, thigh and calf) were higher in the anaemics than the non-anaemics and anaemia had a significant effect, before and after socio-demographic confounders were adjusted for. In terms of interactions between HIV infection and anaemia, significant effects were only apparent in supraspinale and abdominal skin folds only after adjusting for the socio-demographic confounders.

The difference in BMI between anaemics and non-anaemics may be explained by anaemics being heavier but of similar stature as their non-anaemic peers, together with their comparatively lower LBM. However, a lot of their heaviness may be attributed to

greater fat deposits depicted by comparatively higher skin fold thicknesses in most parts of the body.

From the above findings it is possible to infer that, although there are no significant effects of HIV infection on body compositional measures, Anaemia as well as its interaction with HIV infection may be related with a certain level of fat deposition, especially, in mid sections of the body.

When the two sero-status groups are compared, alterations in body compositional measures do not show clearly the effects of HIV in inducing reductive adaptation in the sero-positives. This may possibly manifest only over a long period and for that reason the sero-positives may be appropriately confirmed as being at an asymptomatic or early stage of infection. However, since anaemia has been associated with a significant alteration in some body compositional measures, it is very likely that in the long term anaemia in the study population could be determined in part by such body compositional changes.

**Table 5.5: Anthropometric indicators for measuring adaptational changes in the study population. Data values are means (95% confidence interval – CI)**

Anthropometric measurements	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (842)	Anaemic (705)	Non-anaemic (102)	Anaemic (110)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
Weight (kg)	63.06 (62.02-64.11)	65.01 (63.81-66.21)	61.64 (59.22-64.06)	63.85 (61.15-66.54)	0.257	0.272	0.069	0.763	0.908	0.689
Height (cm)	162.48 (161.88 - 163.07)	161.52 (160.91-162.13)	162.57 (160.82-164.31)	162.68 (161.23-164.14)	0.313	0.785	0.500	0.439	0.388	0.076
BMI (kg/m <sup>2</sup> )	24.02 (23.60-24.44)	25.13 (24.61-25.65)	23.47 (22.44-24.50)	24.17 (23.14-25.19)	0.110	0.235	0.051	0.985	0.660	0.210
Lean body mass (kg)	44.95 (43.79-46.10)	43.20 (41.76-44.64)	45.57 (42.84-48.30)	42.76 (39.02-46.49)	0.943	0.884	0.043	0.322	0.319	0.215
Lean mass index (kg/m <sup>2</sup> )	16.66 (16.28-17.05)	16.26 (15.85-16.66)	16.71 (15.93-17.48)	15.90 (14.91-16.90)	0.668	0.699	0.095	0.470	0.585	0.472
<b>Skin folds</b>										
Triceps SF (mm)	14.51 (13.83-15.19)	15.76 (14.93-16.58)	13.31 (11.53-15.09)	14.38 (12.40-16.36)	0.096	0.185	0.135	0.258	0.906	0.110
Subscapular SF (mm)	15.66 (14.92-16.39)	17.92 (16.98-18.86)	15.12 (13.11-17.12)	16.44 (14.17-18.72)	0.241	0.398	0.037	0.810	0.585	0.093
Iliac-crest SF (mm)	14.16 (13.20-15.11)	15.95 (14.75-17.15)	15.44 (12.64-18.25)	16.94 (13.94-19.93)	0.251	0.287	0.098	0.934	0.878	0.285
Supraspinale SF (mm)	10.23 (9.49-10.98)	11.74 (10.70-12.78)	10.68 (8.58-12.78)	11.77 (9.15-14.40)	0.767	0.763	0.108	0.905	0.801	0.043
Abdominal SF (mm)	16.95 (15.83-18.06)	20.14 (18.66-21.61)	17.97 (15.08-20.85)	20.19 (16.22-24.17)	0.648	0.859	0.022	0.073	0.682	0.008
Thigh SF (mm)	19.44 (17.93-20.95)	23.95 (21.97-25.92)	18.26 (14.58-21.93)	28.15 (22.87-33.42)	0.338	0.427	<0.001	0.011	0.088	0.889
Calf SF (mm)	13.88 (12.79-14.97)	11.99 (11.1 -12.84)	11.36 (9.22-13.49)	14.84 (12.27-17.40)	0.853	0.720	0.002	0.461	0.360	0.575

BMI – body mass index; SF – skinfold. Statistical significance is denoted by p<0.05. \*Adjusted for gender, age grouping, settlement type, smoking and alcohol consumption.

### **Summary of section 5.3**

This section can be summarised as follows:

1. HIV sero-positives tended to be lighter and relatively shorter than their sero-negative peers. They also had body compositional measurements that indicated that they were more likely to be associated with higher fat deposits in especially the lower body parts.
2. Anaemics were more likely to have lower LBM and higher BMI because they tended to be heavier and also had levels of skin fold fats that were not only high but quite significant for lower body parts.
3. Interaction between HIV infection and anaemia had a significant effect only on abdominal skinfold.

In conclusion, anaemia and to a lesser degree, its interaction with HIV infection may lead to alterations in body composition, which may manifest as changes in fat deposition in the mid section of the body or perhaps fat redistribution in, especially, the sero-positive population. Associating such adaptational changes in body compositional measures mainly with anaemia may suggest their consideration as explanatory factors of anaemia in the study population.

The next section would explore energy imbalances and nutrient deficiencies as possible causes of anaemia in the study population.

### **5.4 ENERGY IMBALANCE AND NUTRIENT DEFICIENCIES AS POSSIBLE EXPLANATION FOR THE ANAEMIA IN THE STUDY POPULATION**

HIV infection, as it progresses, can lead to poor or reduced energy and nutrient intakes. Infection with the virus may induce anorexia and other patho-physiological changes that may subsequently lead to a reduction in food intake. HIV/AIDS is also associated with increased metabolic energy expenditure. However, in spite of the increased metabolic expenditure, victims may eventually reduce their physical activity levels (PAL) in an attempt at lowering total energy expenditure, which may subsequently result in a reduction in food energy and nutrient intake. The state of affairs in such a situation is the likely emergence of an energy imbalance and/or a nutrient deficiency, which by and large may result in poor or ineffective RBC production as victims may be deprived of essential haematinics.

#### **5.4.1 ENERGY STATUS**

In this study, total energy intake, derived from carbohydrates, proteins and fats as main food sources, with or without alcohol, are presented. In table 5.6a below, the mean

total energy intake (including alcohol) tended to be significantly higher in the HIV sero-positive group compared with that of their sero-negative peers, though this significance was lost after adjusting for socio-demographic confounders.

With alcohol excluded in the estimation, there was no significant difference in energy intake between the two sero-status groups even after the adjustment. This means that HIV infection, in this study population, has no significant effect on energy intake irrespective of whether alcohol is included or excluded. Similarly anaemia did not have any significant effect on energy intake, even though sero-positive anaemics had marginally higher intakes than non-anaemics. There was also no significant interaction between HIV infection and anaemia on energy intakes, which implied that sero-positive anaemics were not likely to have significantly higher intakes compared to their sero-negative anaemic peers.

Although proportions of food energy sources seemed marginally different between the two sero-status groups, these differences were statistically significant after socio-demographic confounders were adjusted for. This may imply an effect of HIV infection being confounded by socio-demographic variables. However, there were no significant effects of anaemia as well as interaction between anaemia and HIV infection on the differences in food sources. In terms of proportions of energy intake, carbohydrate food sources provided the highest in both groups and also contributed the greatest amount of energy (~5700KJ cf. approx. 2200KJ from fat) in their diets.

Despite about 57% of the population being non-consumers of alcohol, HIV sero-positive consumers tended to have intakes that were higher than their sero-negative peers, though the difference was not significant. On the other hand anaemics had intakes that were significantly lower compared to non-anaemics even after socio-demographic confounders were corrected for. There was also a significant interactive effect between HIV infection and anaemia on alcohol consumption, which depicted that sero-positive anaemics were significantly more likely to consume lower amounts of alcohol.

**Table 5.6a: Measures related to energy intake in the study population. Data values are means (95% confidence interval – CI)**

Characteristics	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (821)	Anaemic (700)	Non-anaemic (104)	Anaemic (108)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
Total energy (+alcohol) (kJ)	8706.28 (8460.49-8952.07)	8452.65 (8204.35-8700.95)	9123.13 (8263.78-9982.47)	9149.92 (8522.38-9777.45)	0.032	0.272	0.661	0.198	0.588	0.079
Total energy (-alcohol) (kJ)	8408.94 (8180.40-8637.48)	8216.95 (7981.43-8452.46)	8542.74 (7818.07-9267.41)	8963.39 (8365.96-9560.83)	0.068	0.272	0.635	0.198	0.204	0.079
Food energy from protein (%)	11.86 (11.71-12.01)	12.02 (11.86-12.19)	11.89 (11.48-12.29)	11.47 (11.06-11.87)	0.091	0.023	0.065	0.243	0.416	0.393
Food energy from fat (%)	25.16 (24.65-25.67)	25.90 (25.33-26.46)	24.99 (23.61-26.38)	25.08 (23.74-26.43)	0.378	0.029	0.455	0.283	0.553	0.354
Food energy from carbohydrate (%)	64.72 (64.04-65.39)	63.71 (62.97-64.46)	65.45 (63.66-67.25)	64.74 (62.93-66.55)	0.224	0.014	0.237	0.610	0.841	0.653
Alcohol (g/day)	9.98 (7.98-11.97)	7.91 (6.20-9.62)	19.48 (9.02-29.94)	6.26 (2.88-9.64)	0.060	0.099	0.000	0.004	0.008	0.026

Statistical significance is denoted by p<0.05. \*Adjusted for gender, age grouping, settlement type, smoking and alcohol consumption.

**Table 5.6b: Estimates for energy requirements and expenditure in the study population. Data values are means (95% confidence interval – CI)**

Characteristics	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (850)	Anaemic (716)	Non-anaemic (104)	Anaemic (110)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
PAL	1.59 (1.58-1.60)	1.61 (1.59-1.62)	1.58 (1.57-1.59)	1.57 (1.55-1.59)	0.026	0.007	0.120	0.646	0.448	0.348
BMR (from Schofield equation) (MJ)	6.16 (6.10-6.21)	6.15 (6.10-6.21)	6.17 (6.03-6.32)	6.21 (6.05-6.36)	0.530	0.644	0.821	0.696	0.729	0.643
EAER (MJ)	9.75 (9.65-9.85)	9.90 (9.79-10.01)	9.72 (9.44-10.00)	9.81 (9.54-10.07)	0.553	0.056	0.276	0.489	0.784	0.726

PAL – physical activity level; BMR – basal metabolic rate; EAER – average estimated energy requirements. Data values are means (95% confidence interval – CI). Statistical significance is denoted by p<0.05.

\*Adjusted for gender, age grouping, settlement type, smoking and alcohol consumption.

In table 5.6b above mean estimates for BMR using gender specific Schofield equations, together with PAL estimated from activity factors ascribed to subjects in the study population (see appendix B) have also been shown. Despite the marginal differences with PAL estimates being lower in sero-positives, there was a significant effect of HIV infection on this variable, even after the adjustment. On the other hand, there were no differences in BMR between the two sero-status groups and the effect of HIV infection was not significant. On both PAL and BMR, there were no significant effects of anaemia as well as effects from its interaction with HIV infection.

Estimated average energy requirement (EAER), obtained as a product of PAL and BMR for each individual subject (see also Appendix B), are compared between the groups (Tab. 5.6b). Despite showing a marginal difference between sero-status groups, they emerged as significant after adjusting for confounding socio-demographic variables. However, effects of anaemia or its interaction with HIV infection were not significant on EAER.

Demarcations were made for persons who were most likely to have either a poor intake or an intake that provided adequate energy or perhaps excess, using the gender derived EAER. In table 5.7 below proportions for these demarcations are compared between sero-status groups as well as anaemic subgroups.

**Table 5.7: Proportions with poor or adequate energy intake compared between groups and subgroups in the study population**

Total energy intake		HIV-		HIV+		p-value (Chi square)			
		Non-anaemic (a)	Anaemic (b)	Non-anaemic (c)	Anaemic (d)	a*b	c*d	a*c	b*d
Including alcohol	Poor	446 (54.3%)	416 (59.4%)	60 (68.3%)	54 (50.0%)	0.045	0.274	0.516	0.064
	Adequate /Excess	375 (45.7%)	284 (40.6%)	44 (31.7%)	54 (50.0%)				
Excluding alcohol	Poor	470 (57.2%)	435 (62.1%)	64 (56.7%)	54 (50.0%)	0.053	0.099	0.404	0.016
	Adequate /Excess	351 (42.8%)	265 (37.9%)	40 (43.3%)	54 (50.0%)				

Statistical significance is denoted by  $p < 0.05$ .

Clearly, table 5.7 above shows that 50% or more of the study population were likely to be classified as having a poor energy intake. Nevertheless, sero-negative anaemics were significantly more likely to be classified as having poor energy intakes as compared to their non-anaemic peers, irrespective of whether alcohol is included in the estimation or not. On the other hand seropositive anaemics were rather more likely to fall within the adequate /excess intake category, but this was not significantly so.

Comparing sero-positive non-anaemics with their sero-negative peers, it was evident that they had similar chances of being classified as having poor intakes. In contrast sero-positive anaemics were significantly less likely to fall in that category especially when alcohol was excluded from the estimation.

In sum, HIV sero-positives have marginally higher energy intakes compared with their sero-negative peers. Sero-positive anaemics are less likely whilst their sero-negative peers are more likely to be classified as having poor energy intakes compared to non-anaemics. More than 50% of the study population may be categorised as having poor energy intake.

#### **5.4.2 MICRONUTRIENT STATUS**

Micronutrients (i.e. vitamins and minerals) of major significance, especially to RBC synthesis, were compared between the groups (Tab. 5.8). It is evident from table 5.8 below that differences in micronutrient intakes between the sero-status groups were marginal and there were no significant effects of HIV infection. Though sero-positives tended to have intakes that were only slightly higher for some micronutrients, intakes in both groups were closely similar even after adjusting for socio-demographic confounders. Nevertheless, anaemics had intakes that were significantly higher for all except nicotinic acid, vitamin E, folate and copper. However, the significant effect of anaemia on B<sub>6</sub> and ascorbic acid were lost, whilst the effect on nicotinic acid and copper became significant after the adjustment. The interactive effect of anaemia and HIV infection on thiamin, iron, copper and zinc before the adjustment were significant, and this significant effect was maintained together with vitamin B<sub>12</sub> and nicotinic acid after the adjustment.

Whether micronutrient intakes were adequate or not was considered to depend on individual requirements. In view of this, mean levels of micronutrient intakes have been compared with Dietary reference intakes (DRI). DRIs are estimates of nutrient intakes that are supposed to meet the requirements of a healthy population with varying lifestyles (Gibney et al., 2002).

Tables 5.9a and 5.9b below compare micronutrient intakes with DRIs for sero-status groups and anaemic sub-groupings respectively. From table 5.9a it is evident that mean intakes of Vitamins A for HIV sero-positive subjects were higher and highly significant ( $p<0.001$ ) compared with DRIs, whilst intake of folate was higher but not significant. On the other hand, intakes of vitamins B<sub>1</sub>, B<sub>3</sub> and vitamin C, though marginally lower were not significantly different compared to the DRIs. In a similar

trend, mean intakes for sero-negative subjects with respect to vitamins A, E, B<sub>2</sub>, and B<sub>12</sub> were also significantly higher than DRIs. However, unlike their sero-positive counterparts, intakes for folate, vitamins B<sub>1</sub>, B<sub>3</sub> and vitamin C fell significantly below the DRIs. For the mineral micronutrients, which had different DRIs for males and females, mean intakes for iron were marginally but significantly higher for males irrespective of sero-status, whilst their female counterparts had average intakes that were significantly lower than the DRI. Females in either group, on the other hand, had intakes of copper and zinc higher than their DRIs, which like that for iron were lower compared to DRIs for males. However, HIV sero-positive females had intakes that could be considered to be slightly high than those of their sero-negative counterparts (Table 5.9a).

**Table 5.8: Intake of selected micronutrients compared between the two groups in the study population. Data values are means (95% confidence interval – CI).**

	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (718)	Anaemic (689)	Non-anaemic (98)	Anaemic (104)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
vitamin A (RE/day)	693.38 (650.89-735.87)	787.40 (730.92-843.89)	631.51 (526.88-736.14)	866.27 (741.80-990.73)	0.864	0.557	0.001	0.028	0.158	0.171
Thiamin (mg/day)	1.12 (1.09-1.16)	1.10 (1.07-1.14)	1.07 (0.97-1.16)	1.24 (1.14-1.34)	0.255	0.507	0.035	0.007	0.010	0.003
Riboflavin (mg/day)	1.37 (1.33-1.42)	1.43 (1.38-1.49)	1.32 (1.18-1.46)	1.49 (1.34-1.64)	0.965	0.327	0.030	0.003	0.289	0.065
Nicotinic acid (mg/day)	13.33 (12.83-13.83)	13.64 (13.15-14.13)	13.77 (11.82-15.71)	14.76 (13.42-16.10)	0.138	0.840	0.214	0.008	0.517	0.039
Vitamin B <sub>6</sub> (mg/day)	4.57 (4.27-4.88)	5.30 (4.87-5.72)	4.30 (3.47-5.13)	5.55 (4.60-6.51)	0.316	0.443	0.007	0.088	0.061	0.503
Vitamin B <sub>12</sub> (µg/day)	4.57 (4.27-4.88)	5.30 (4.87-5.72)	4.30 (3.47-5.13)	5.55 (4.59-6.51)	0.977	0.355	0.008	0.022	0.477	0.012
Ascorbic acid (mg/day)	35.72 (33.37-38.08)	39.08 (35.74-42.43)	32.19 (26.09-38.30)	41.93 (32.77-51.08)	0.906	0.275	0.025	0.373	0.275	0.393
Vitamin E (αTE/day)	10.90 (10.49-11.30)	10.61 (10.15-11.07)	11.74 (9.81-13.68)	11.10 (10.03-12.16)	0.152	0.366	0.314	0.224	0.698	0.646
Folate (µg/day)	206.27 (199.86-212.68)	202.28 (195.74-208.83)	210.53 (184.97-236.09)	218.31 (202.24-234.37)	0.140	0.701	0.782	0.282	0.392	0.082
Iron (mg/day)	8.88 (8.57-9.18)	8.66 (8.37-8.96)	8.05 (7.34-8.76)	9.91 (8.99-10.82)	0.499	0.957	0.008	0.007	0.001	<0.001
Zinc (mg/day)	8.32 (8.07-8.57)	8.37 (8.11-8.64)	7.95 (7.19-8.71)	9.14 (8.43-9.85)	0.464	0.760	0.021	0.017	0.034	0.013
Copper (mg/day)	1.16 (1.12-1.19)	1.15 (1.11-1.18)	1.15 (1.04-1.27)	1.30 (1.19-1.40)	0.062	0.408	0.084	0.052	0.042	0.011

\*ANOVA statistics adjusted for gender, age grouping, settlement type, smoking and alcohol consumption status. Statistical significance is denoted by p<0.05.

Data values are means (95% confidence interval – CI)

**Table 5.9a: Intakes of micronutrients for sero-status groups compared with dietary reference intakes (DRIs)**

Micronutrient	HIV status	n	Mean intake	DRI	Mean – DRI	95% CI	p value
Vitamin A (RE/day)	HIV+	214	751.10	600	151.10	68.63 – 233.57	<0.001
	HIV-	1578	735.53		135.53	101.33 – 169.74	<0.001
Vitamin E (αTE/day)	HIV+	214	11.41	10	1.41	0.33 – 2.50	<0.001
	HIV-	1578	10.76		0.74	0.44 – 1.04	<0.001
Vitamin B <sub>12</sub> (μg/day)	HIV+	214	4.94	2.4	2.54	1.90 – 3.18	<0.001
	HIV-	1578	4.88		2.48	2.23 – 2.74	<0.001
Thiamin (B <sub>1</sub> ) (mg/day)	HIV+	214	1.15	1.2	-0.05	-0.12 – 0.03	0.212
	HIV-	1578	1.11		-0.09	-0.11 – -0.06	<0.001
Riboflavin (B <sub>2</sub> ) (mg/day)	HIV+	214	1.41	1.0	0.41	0.30 – 0.51	<0.001
	HIV-	1578	1.40		0.40	0.36 – 0.43	<0.001
Nicotinic acid (B <sub>3</sub> ) (mg/day)	HIV+	214	14.27	15.0	-0.73	-1.89 – 0.44	0.220
	HIV-	1578	13.48		-1.55	-1.89 – -1.20	<0.001
Vitamin B <sub>6</sub> (mg/day)	HIV+	214	1.09	1.5	-0.41	-0.49 – -0.32	<0.001
	HIV-	1578	1.09		-0.45	-0.48 – -0.42	<0.001
Ascorbic acid (C) (mg/day)	HIV+	214	37.25	40	-2.85	-8.39 – 2.69	0.312
	HIV-	1578	37.15		-2.86	-4.82 – -0.89	0.004
Folate (μg/day)	HIV+	214	214.49	210	4.49	-10.37 – 19.35	0.552
	HIV-	1578	204.52		-5.78	-10.30 – -1.26	0.012
Iron (mg/day)	HIV+ ♂	95	9.16	8	1.16	0.22 – 2.06	0.016
	HIV+ ♀	119	8.51	18	-9.49	-9.90 – -8.33	<0.001
	HIV- ♂	669	9.14	8	1.14	0.83 – 1.50	<0.001
	HIV- ♀	889	8.89	18	-9.11	-9.76 – -9.22	<0.001
Copper (mg/day)	HIV+ ♂	95	1.22	1.5	-0.21	-0.34 – -0.08	0.002
	HIV+ ♀	119	1.10	1.2	0.03	-0.12 – 0.07	0.562
	HIV- ♂	669	1.29	1.5	-0.28	-0.32 – -0.23	<0.001
	HIV- ♀	889	1.17	1.2	-0.10	-0.13 – -0.07	0.102
Zinc (mg/day)	HIV+ ♂	95	8.86	11	-1.94	-2.83 – -1.06	<0.001
	HIV+ ♀	119	7.98	8	-0.03	-0.46 – 0.78	0.832
	HIV- ♂	669	9.06	11	-2.14	-2.43 – -1.84	<0.001
	HIV- ♀	889	8.16	8	0.16	-0.25 – 0.20	0.611

♂ - male, ♀ -females, DRI –Dietary reference intakes (source: Gibney et al., (Eds), 2002). Statistical significance is denoted by p<0.05.

**Table 5.9b: Comparing average Intakes of micronutrients with dietary reference intakes (DRIs) for anaemic status sub-groups**

Micronutrient	HIV status	Anaemic status	n	Mean intake	DRI	Mean – DRI (95% CI)	p value
Vitamin A (RE/day)	HIV-	N-anaemic	849	694.89	600	93.38 (50.87-135.89)	0.552
		Anaemic	715	788.48	600	187.40 (130.91-243.81)	<0.001
	HIV+	N-anaemic	104	631.51	600	31.51 (-73.12-136.14)	<0.001
		Anaemic	110	866.27	600	266.27 (141.80-390.73)	<0.001
Vitamin E (αTE/day)	HIV-	N-anaemic	829	10.91	10	0.90 (0.49-1.30)	0.077
		Anaemic	705	10.63	10	0.61 (0.03-2.16)	0.044
	HIV+	N-anaemic	104	11.74	10	1.74 (-0.19-3.68)	<0.001
		Anaemic	110	11.10	10	1.10 (0.15-1.07)	0.010
Vitamin B <sub>12</sub> (μg/day)	HIV-	N-anaemic	843	4.58	2.4	2.17 (1.87-2.48)	<0.001
		Anaemic	705	5.30	2.4	2.90 (2.47-3.32)	<0.001
	HIV+	N-anaemic	104	4.30	2.4	1.90 (1.07-2.73)	<0.001
		Anaemic	110	5.55	2.4	3.15 (2.19-4.11)	<0.001
Thiamin (B <sub>1</sub> ) (mg/day)	HIV-	N-anaemic	849	1.12	1.2	-0.08 (-0.11- -0.05)	<0.001
		Anaemic	715	1.11	1.2	-0.10 (0.15-1.07)	<0.001
	HIV+	N-anaemic	104	1.07	1.2	-0.13 (-0.23- -0.04)	0.008
		Anaemic	110	1.24	1.2	0.04 (0.02-2.16)	0.459
Riboflavin (B <sub>2</sub> ) (mg/day)	HIV-	N-anaemic	854	1.38	1.0	0.37 (0.33-0.42)	<0.001
		Anaemic	732	1.43	1.0	0.43 (0.38-0.49)	<0.001
	HIV+	N-anaemic	104	1.32	1.0	0.32 (0.18-0.46)	<0.001
		Anaemic	110	1.49	1.0	0.49 (0.34-0.64)	<0.001
Nicotinic acid (B <sub>3</sub> ) (mg/day)	HIV-	N-anaemic	849	13.35	15.0	-1.67 (-2.17- -1.18)	<0.001
		Anaemic	715	13.67	15.0	-1.36 (-1.85- -0.87)	0.723
	HIV+	N-anaemic	104	13.77	15.0	-1.23 (-3.18-0.71)	<0.001
		Anaemic	110	14.76	15.0	-0.24 (-1.50-1.10)	0.211
Vitamin B <sub>6</sub> (mg/day)	HIV-	N-anaemic	849	1.04	1.5	-0.47 (-0.51- -0.43)	<0.001
		Anaemic	715	1.07	1.5	-0.44 (-0.47- -0.39)	<0.001
	HIV+	N-anaemic	103	1.00	1.5	-0.50 (-0.62- -0.39)	<0.001
		Anaemic	110	1.19	1.5	-0.31 (-0.43- -0.19)	<0.001
Ascorbic acid (C) (mg/day)	HIV-	N-anaemic	849	35.72	40	-4.28 (-6.63- -1.92)	<0.001
		Anaemic	715	39.11	40	-0.92 (-4.23-2.43)	0.677
	HIV+	N-anaemic	103	32.19	40	-7.81 (-13.91- -1.70)	0.013
		Anaemic	110	41.93	40	1.92 (-7.23-11.08)	0.591
Folate (μg/day)	HIV-	N-anaemic	843	206.51	210	-3.73 (-10.14-2.68)	0.253
		Anaemic	710	202.64	210	-7.72 (-14.26- -1.17)	0.308
	HIV+	N-anaemic	101	210.53	210	0.53 (-25.03-26.09)	0.967
		Anaemic	109	218.31	210	8.31 (-7.76-24.37)	0.021

DRI –Dietary reference intakes (source: Gibney et al., (Eds), 2002). Statistical significance is denoted by p<0.05.

Comparisons of micronutrient intakes with DRIs are also shown for the anaemia sub-groupings in Table 5.9b above. Generally, anaemics had significantly higher intakes of vitamins A, B<sub>2</sub> and B<sub>12</sub>, but sero-positives had intakes that were slightly higher than for anaemics were much lower than that for anaemics, but were however significantly higher compared to DRIs

For vitamin E, the difference between intakes and DRI was marginal higher in all groups, but more significant in sero-positives compared to sero-negatives. Only sero-positive anaemics had comparable intakes to DRI for B<sub>1</sub> and ascorbic acid, all the other groups had intakes that were lower and in most instances significantly so. Intakes of folate compared to DRI were only marginally higher in the sero-positives, but intakes in anaemics were comparably higher. On the other hand both sero-negative anaemics and non-anaemics had intakes lower than DRI levels. Generally, levels of intake for B<sub>3</sub> and B<sub>6</sub> were all marginally lower than DRI for all groups, but whilst the differences were all significant for B<sub>6</sub>, only intakes in non-anaemics were so for B<sub>3</sub> intakes.

Even though sero-status and anaemia status groupings showed higher intakes of some micronutrients compared to DRIs, these could well be described as marginal. Furthermore, with some micronutrient intake levels falling below requirements, the likelihood that this could lead to deficiency states that could compromise RBC production is suggested.

Since the Micronutrient status of the population is also dependent upon their metabolic state, a measure of micronutrient level in blood was also considered. From the results shown in table 5.10 below, it is evident that serum vitamin A and iron levels were marginally lower whilst vitamin E levels were similar in the sero-positives compared to the sero-negatives. The differences in serum micronutrient levels between the two sero-status groups were not significant, and HIV infection did not have any significant effect on serum vitamins A and E as well as iron. However, vitamins A and E and also serum iron levels were lower in anaemics compared to non-anaemics and the effect of anaemia on the levels of these micronutrients were very significant, especially after confounding socio-demographic variables were adjusted for. In contrast, the interaction between HIV and anaemia did not yield any significant effect on these serum vitamins and mineral even after the adjustment. This may imply that HIV infection does not have a significant influence on the levels of these micronutrients in persons who are anaemic.

**Table 5.10: Comparing serum micronutrient levels in the study population. Data values are means (95% confidence interval – CI).**

	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (804)	Anaemic (627)	Non-anaemic (98)	Anaemic (102)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Serum Vitamin A ( $\mu$ g/L)	47.34 (46.24-48.44)	44.90 (43.65-46.14)	45.95 (43.31-48.60)	42.78 (40.05-45.50)	0.138	0.081	0.018	0.022	0.757	0.998
Serum Vitamin E ( $\mu$ g/L)	8.99 (8.78-9.19)	8.81 (8.55-9.08)	9.24 (8.73-9.75)	8.55 (8.07-9.03)	0.971	0.970	0.066	0.003	0.270	0.146
Serum Fe ( $\mu$ mol/L)	18.08 (17.51-18.66)	15.35 (14.76-15.93)	18.00 (16.22-19.78)	15.29 (13.81-16.78)	0.911	0.392	<0.001	<0.001	0.984	0.718

\*ANOVA statistics adjusted for gender, age grouping, settlement type, smoking and alcohol consumption status. Statistical significance is denoted by  $p < 0.05$ . Data values are means (95% confidence interval – CI)

The marginally lower levels of serum vitamins A and iron, in particular, observed in sero-positives and anaemics when compared to a tendency for higher intakes, especially in sero-positive anaemics (Tab. 5.9b), may suggest the influence of a metabolic or inflammatory presence that could lead to a deficiency state.

#### **Summary of section 5.4**

In this section the results have shown that:

1. HIV sero-positives in general as well as sero-positive anaemics have energy intakes that may be considered as marginally higher than that of their sero-negative peers. In addition, sero-positive anaemics are less likely to be categorised as having poor energy intakes, though over half of the study population were in this category.
2. Intakes of a number of micronutrients including vitamins, E, B<sub>2</sub> and B<sub>12</sub> were marginally higher in HIV sero-positives compared with their sero-negative peers. However, HIV did not have any significant effect on these intakes. When intakes were compared to DRIs, they were found to be marginal, with some being higher and others lower. But generally sero-positive anaemics tended to have higher intakes compare to the other groups. HIV sero-positives also had marginally lower serum vitamin A and serum iron, as well as vitamin E levels that were similar to that of their uninfected counterparts. However, there were no significant effects of HIV infection on serum micronutrients. Anaemics also had lower serum iron, vitamins A and E levels compared to non-anaemics, and there was a significant effect of anaemia both before and after adjusting for confounders. The relative higher levels of vitamin A and iron intakes for sero-positive anaemics, when compared with their comparatively lower serum levels may suggest a deficiency trait most probably brought about by metabolic and/or inflammatory causes.

In conclusion, it is not possible to show any significant interactions between HIV and anaemia on energy and micronutrient status in the study population. However, effects of anaemia in serum lowering vitamin A and iron levels, especially in sero-positives, may suggest a subtle influence of metabolic or inflammatory processes, especially when dietary intakes may be considered as adequate or marginal. This lowering effect on the two micronutrients may have implications for anaemia in the study population.

#### **5.5 INFLAMMATION AS A POSSIBLE EXPLANATION FOR THE ANAEMIA IN THE STUDY POPULATION**

HIV/AIDS may not only be a chronic inflammatory disease but may also permit other

inflammatory influences on infected persons. Inflammation and/or infection leads to the release of pro-inflammatory cytokines and a concomitant high protein turnover. Inflammation may also alter nutrient availability which may lead to reduced cellular synthesis.

Cytokines tend to mark more precisely the inflammatory process. However, in the absence of cytokine measurements, this study has relied on measurements of acute phase proteins (APP) that often parallel or relate to the release of cytokines, as well as measures that may be considered to indicate liver inflammation or insidious damage (liver enzymes and a by-product).

In table 5.11 below, mean serum levels of APPs, liver enzymes as well as a specific by-product are compared between sero-status and anaemia groups. With regards to the APPs, sero-positives were likely to have lower ferritin levels, whilst plasma fibrinogen levels were likely to be similar to that of sero-negatives. However there were no significant effects of HIV infection on these biomarkers. On the other hand, the effects of HIV infection on serum total proteins, albumin and 'globulins' were highly significant both before and after adjusting for socio-demographic confounders. Whilst serum total proteins and 'globulins' were likely to be higher serum albumin was likely to be lower in the sero-positives as compared with sero-negatives.

Between anaemics and non-anaemics, ferritin, plasma fibrinogen, total proteins and albumin were likely to be lower, whilst 'globulins' were likely to be higher in the anaemics. However, there were no significant effects of anaemia on ferritin and 'globulins'. With plasma fibrinogen a significant effect emerged after adjusting for socio-demographic confounders. Effects of anaemia on total proteins and albumin were, however very significant both before and after the adjustment. In terms of interaction between HIV infection and anaemia, this effect was only significant on plasma fibrinogen levels after the adjustment.

With regards to the liver enzymes and by-product, HIV sero-positives had lower total bilirubin, higher direct bilirubin and higher serum levels of ALP, GGT, ALT and AST as compared to sero-negatives. However, whilst there were no significant effects of HIV infection on bilirubin, ALP and GGT, effects on ALT and AST were very significant both before and after adjusting for the confounding socio-demographic variables. Anaemics were also likely to have lower bilirubin (Total and Direct) and some liver enzymes (ALP, ALT and AST) compared to their non-anaemic peers. GGT was however, higher in sero-negative anaemics and lower in sero-positive anaemics compared with their non-

anaemic peers. Except ALP and GGT levels, the effects of anaemia were very significant on Bilirubin, ALT and AST levels both before and after the adjustment. Interactive effects of HIV infection and anaemia were highly significant only on ALT and AST levels both before and after adjusting for the confounding socio-demographic factors.

**Table 5.11: Serum markers of inflammatory response in the study population. Data values are means (95% confidence interval – CI).**

Marker	HIV-		HIV+		ANOVA (p-value)					
	Non-anaemic (809)	Anaemic (609)	Non-anaemic (98)	Anaemic (102)	Effect of HIV		Effect of anaemia		Interaction	
					Crude	*Adjusted	Crude	*Adjusted	Crude	*Adjusted
Serum Ferritin (µg/L)	114.07 (103.54-124.61)	100.71 (91.51-109.91)	110.15 (87.95-132.35)	87.61 (68.45-106.78)	0.396	0.620	0.073	0.384	0.647	0.928
Plasma fibrinogen (g/L)	3.40 (3.32-3.48)	3.47 (3.39-3.56)	3.49 (3.23-3.74)	3.25 (3.02-3.47)	0.421	0.326	0.319	0.054	0.063	0.040
Serum total Protein (g/L)	74.33 (73.89-74.76)	73.13 (72.69-73.57)	78.25 (76.54-79.96)	77.62 (75.78-79.45)	<0.001	<0.001	0.059	0.018	0.562	0.513
Serum Albumin (g/L)	44.25 (43.96-44.54)	42.82 (42.49-43.16)	42.28 (41.11-43.25)	40.54 (39.44-41.65)	<0.001	<0.001	<0.001	<0.001	0.642	0.725
Serum Globulin (g/L)	30.25 (29.89-30.62)	30.44 (30.04-30.83)	35.98 (33.92-38.04)	37.07 (34.73-39.41)	<0.001	<0.001	0.177	0.469	0.335	0.341
<b>Liver enzyme or by-product</b>										
Serum Total Bilirubin (µmol/L)	6.51 (6.16-6.86)	5.75 (5.43-6.06)	6.07 (4.97-7.16)	5.38 (4.67-6.09)	0.249	0.069	0.039	0.026	0.910	0.923
Serum Direct Bilirubin (µmol/L)	3.05 (2.91-3.19)	2.51 (2.38-2.64)	3.20 (2.56-3.84)	2.56 (2.16-2.95)	0.501	0.813	<0.001	<0.001	0.733	0.696
Serum ALP (IU/L)	77.59 (75.42-79.75)	76.75 (73.61-79.89)	84.45 (75.64-93.26)	76.83 (68.25-85.41)	0.214	0.214	0.130	0.260	0.224	0.227
Serum GGT (IU/L)	38.94 (35.12-42.77)	44.36 (33.39-55.32)	51.89 (29.76-74.01)	36.65 (27.03-46.26)	0.737	0.811	0.528	0.717	0.184	0.267
Serum ALT (IU/L)	12.59 (11.73-13.46)	11.82 (11.06-12.57)	18.38 (12.44-24.33)	13.44 (9.78-17.10)	<0.001	<0.001	0.005	0.013	0.042	0.056
Serum AST (IU/L)	22.12 (20.98-23.27)	22.09 (20.72-23.46)	39.03 (14.85-63.21)	24.71 (21.92-27.50)	<0.001	<0.001	0.005	0.014	0.005	0.008

\*ANOVA statistics adjusted for gender, age grouping, settlement type, smoking and alcohol consumption status. Statistical significance is denoted by p<0.05.

Data values are means (95% confidence interval – CI)

In the next section the risk levels for associating high inflammatory biomarker levels with HIV infection in the study population would be explored using a binary logistic regression analysis.

### **Summary of section 5.5**

The results in this section could be summed up as follows:

1. HIV sero-positive persons in the study population are significantly more likely to be associated with higher serum levels of total proteins and 'globulins' as well as lower levels of serum albumin than sero-negative persons. Sero-positive persons are also more likely but less significantly to have lower levels of serum ferritin, plasma fibrinogen and total bilirubin than their sero-negative counterparts. Additionally, they were more inclined to having higher levels, especially, for direct bilirubin, ALT and AST, as compared to their sero-negative counterparts. There was a significant effect of HIV infection on serum ALT and AST.
2. Anaemics, irrespective of sero-status, were also significantly more likely to have lower total proteins and albumin as well as lower bilirubin (Total and Direct) and liver enzymes (serum ALP, GGT, ALT and AST). However, whilst sero-positive anaemics had lower plasma fibrinogen, the sero-negative counterparts had higher plasma levels of the APP. Anaemics also had lower liver enzymes compared to non-anaemics. However, apart from GGT which was lower in the sero-positive anaemics, all the other liver enzymes were higher compare to that of their sero-negative peers. Effects of anaemia on bilirubin (Total and Direct) as well as ALT and AST levels were highly significant.
3. In terms of interaction, HIV sero-positive anaemics had marginal but not significant differences between them and their sero-negative counterparts for all but plasma fibrinogen serum ALT and AST, which were highly significant.

The higher tendency for an increased level of total proteins and a decreased level of albumin, as demonstrated for sero-positives in this study population may indicate the influence of inflammatory stress. Higher and significant tendencies for increased serum ALT and AST in both sero-positives and sero-positive anaemics may also signify inflammatory stress on the liver or insidious liver cell damage in a population that can conveniently be described as asymptomatic. Whilst this may be as a result of the infection or inflammation its association with anaemia as well as its interaction with HIV may have implications for anaemia in the study population. On the other hand, the relatively high ferritin levels in both sero-negatives and sero-positives also suggest inflammatory tendencies in both groups.

## 5.6 KEY FINDINGS - CHAPTER FIVE

The following are the overall summaries for the results obtained in this chapter:

- HIV sero-positives had lower [Hb] and Hct levels compared to their sero-negative peers. However only the differences in Hct was significant. The effects of both HIV infection and anaemia on Hct were also very significant, although the interactive effect was not. DR showed Hct was not be related to 3 times Hb.
- Prevalence of anaemia was generally high and most of it could be categorised as mild, but sero-positive prevalences were higher than those of sero-negatives. Differences in prevalence were only highly significant when anaemia was defined by Hct. HIV sero-positive urban settlers, non-smokers as well as non-consumers of alcohol were more likely to be anaemic. HIV sero-positives were more likely to be mildly and moderately anaemic and significantly more likely to be severely anaemic compared to their sero-negative peers.
- With iron status markers, HIV sero-positive persons were more likely but not significantly so to be hypoferaemic (i.e. with low serum Fe) or relatively hypoferritinaemic (i.e. with low serum ferritin levels) compared to their sero-negative peers. Sero-positives were also more likely to be classified with IDA and ACI, but generally ACI was more likely to be common than IDA in the study population.
- HIV infection, just like anaemia, may be associated with changes in body fat measures in mid body parts. HIV infection and anaemia had a significant interactive effect on abdominal skin fold fat.
- Energy intakes were marginally higher in sero-positives, and anaemic sero-positives also had higher intakes than their sero-negative peers. They were also less likely to be categorised as having poor energy intakes, albeit over 50% of the study population could be classified as such.
- Micronutrient intakes were also marginally higher in sero-positives and anaemic sero-positives compared to their sero-negative peers. Intakes were also marginal compared to DRI for a large number of micronutrients especially in sero-positives. Regarding serum micronutrients, HIV sero-positives and anaemics were more likely to have lower serum levels of iron and vitamin A, whilst vitamin E levels were only lower in anaemics.
- HIV sero-positives were significantly more likely to be associated with higher serum levels of total proteins and 'globulins' as well as a lower serum levels of albumin. They were also likely to have levels of ferritin, plasma fibrinogen and total bilirubin that were lower but not significantly different from that of their sero-negative counterparts. Levels of Direct bilirubin, ALT and ST levels were higher in sero-positives, but the effects of HIV infection was significant on ALT and AST levels.

- Anaemics, irrespective of sero-status, were also significantly more likely to have lower total proteins and albumin as well as lower bilirubin (Total and Direct) and liver enzymes (serum ALP, GGT, ALT and AST). However, effects of anaemia only on bilirubin (Total and Direct) as well as ALT and AST levels were highly significant.

The effects of anaemia and HIV infection on various parameters as explored in this chapter reveals the complex interactions that may exist between HIV and anaemia in this study population. Thus far, subtle changes in body composition, poor energy and marginal micronutrient intakes, apparent serum micronutrient deficiencies coupled with altered APP metabolism and increasing levels of liver enzymes that may suggest ominous liver damage or stress, may constitute the minimum array of factors to explain anaemia in the study population. However, the higher prevalence of anaemia and the tendency for it to be severer in sero-positives, may suggest that the effects of HIV, whether directly or indirectly, could have an interactive influence on the development of anaemia in this population.

## 5.7 DISCUSSION OF KEY CHAPTER FIVE FINDINGS

The hypothesis in this study presupposes that HIV infection may, directly or indirectly, potentiate or multiply the factors that predispose many victims to anaemia. The aim of this chapter has been to resolve this postulation using various factors within the limits of the data available as possible explanatory factors. The framework for the study, nevertheless recognises many other added factors that may be prevalent in the population but may not be accounted for in explaining the presence of anaemia.

That HIV may directly induce anaemia has been suggested by a number of in vitro studies (Levine, 1999; Redd et al, 2007). However, evidence for this direct induction of anaemia by the virus in individuals and populations are difficult to come by or rather non-existent. Anaemia in HIV infection has also been associated with many pathophysiological and metabolic effects of the virus (Moyle, 2002) and other co-infections (Volberding et al., 2004). In asymptomatic HIV infection, it is likely that such indirect anaemia inducing effects are subtle and less severe, and could account for only a small part of anaemia. This may probably leave the direct effect of the virus as a major factor in explaining much of the anaemia. It is, perhaps, this direct effect of HIV that makes its impact on anaemia great (Northrop-Clewes, 2008).

Thus, the mere presence of HIV in a victim could be a major triggering factor for anaemia, and could make the difference between anaemia in sero-positives and sero-negatives. Notwithstanding, the effects of HIV in inducing anaemia can also be viewed

as a cascade since the many indirect and direct effects may interplay in a way that may enhance the development of anaemia in this population.

So far findings in this chapter may suggest a role for HIV infection and its effects in the magnitude of anaemia in the study population. Apart from the lower levels of [Hb] and Hct, which indicate a higher tendency for anaemia in HIV sero-positives, prevalence of anaemia, as defined by both indicators were also higher than those of their sero-negative peers. In addition, grades of anaemia were shown to be marginally higher in sero-positives coupled with a significant association of HIV infection with severe anaemia despite the low comparative numbers. For a sero-positive population, which is essentially asymptomatic, this may strongly suggest an influence of the virus either directly or interacting with other factors.

Anaemia induced by the HIV especially in asymptomatic persons could be mostly mild and subtle. The subtlety of the anaemia induced by HIV infection in this study may be inferred from the marginal differences in prevalence levels between infected and uninfected persons, which were not significant, especially when [Hb] definitions were used. This may imply that the contributions of HIV to the anaemia in the sero-positive group are minimal and perhaps other HIV-unrelated factors may be at play to account for the large part of the anaemia. The similarly high anaemia prevalences in both sero-status groups coupled with the lack of any significant differences may support this observation.

Representing the control group in this study, the high anaemia prevalence recorded for sero-negatives could only be due to factors other than HIV infection. This clearly shows that apart from HIV infection there may be other factors that are perhaps generally predisposing to anaemia in both sero-status populations. Conditions such as malaria, helminthiasis and other infectious diseases, often associated with anaemia, are known to be rife in the study region (Carneiro et al., 2007) and may add to the burden of anaemia in the population.

Another pointer in this study is the possible exacerbating effect of HIV infection, as shown by its significant association with severe anaemia. This particular effect of HIV may be ascribed to its ability to potentiate other anaemia inducing effects; which could also be described as interactive. For instance the chronic but rather mild inflammatory effects of HIV infection (Jahoor et al., 1999), could potentiate the general sub-clinical inflammatory effects from other stressors prevalent in the population, to induce anaemia. This may well explain why anaemia could be an early sign of HIV infection

(Fleming, 1989; Street and Milliken, 1993) or why anaemia prevalences in HIV infection tends to increase with disease progression (Sullivan et al., 1998; Belperio and Rhew, 2004).

Many studies have shown that anaemia prevalences are often higher in infected than uninfected persons irrespective of the stage of infection or whether it is assessed by [Hb] or Hct (Bain, 1999; Volberding, 2004; Thurnham and Northrop-Clewes, 2007; Nneli and Egene, 2007; Jemikalajah and Okogun 2009). However, prevalences that rely on Hct definitions are often lower than those that are defined by [Hb]. This is in spite of the assumed linear relationship between [Hb] and Hct, which is supposed to be a ratio of 3:1 (Bain and Bates, 2001). This ratio is accepted in normal clinical practice and is also a basis for the WHO definitions of anaemia (WHO/CDC, 2004).

In this study, the significant differences obtained for anaemia prevalence between sero-positives and sero-negatives with regards to Hct definitions, may suggest that the linear relationship between [Hb] and Hct deviates from the assumed one. This finding is consistent with studies by Quinto et al. (2006) and Carneiro et al. (2007), who separately showed that the relationship did not hold for children in malarious regions. The use of DR fits, in this study, to derive or predict better Hct cut-offs tended to buttress this apparent deviation of the linear relationship between [Hb] and Hct from what has been assumed. The implication is that HIV infection may have a significant effect on Hct which could perhaps be explained by the tendency for HIV to cause a decrease in RBC numbers over and above its normal production.

In HIV infection absolute iron deficiency due to dietary lack or blockage and relative deficiency due to inflammatory effects has been observed to cause erythropoietic failure and eventually microcytic anaemia. Indeed, erythropoietic failure has been considered as a major mechanism in the development of anaemia in HIV/AIDS (Volberding et al., 2004). The HIV may not only invade and destroy erythrocyte progenitor cells (Levine, 1999), but its presence in the body may alter the internal nutrient environment and enhance the demise or destruction of RBC (Jackson, 2007; Thurnham and Northrop-Clewes, 2007). In the early stages of HIV infection these effects may lead to a reduction in RBC numbers even as RBC production is not very much affected.

Perhaps, another important reason for a significantly lower Hct in sero-positives may be because of a higher tendency for HIV infected persons to develop ACI. Although no significant tendency for ACI is observed in HIV sero-positives in this study, ACI is

associated with an impaired utilisation of iron and often in its early course the anaemia is mild and normo-chromic resulting from the impaired iron cycling (Butensky, 2004). Generally, most anaemia in the study population has been mild but that in sero-positives have been marginally higher, much like ACI. Nevertheless, the lack of significant differences between the sero-status groups means that these may not explain the lower Hct in the sero-positives. This perhaps leaves the direct effects of HIV on erythrocytes or erythrocyte progenitor cells as the most plausible explanation for the lowered Hct.

With regards to prevalence of anaemia in asymptomatic HIV infection, a number of studies corroborate the increasing trend with advancing disease, being lower in asymptomatic infection and higher in AIDS (Spivak et al., 1989; Abrams et al., 2000; Sullivan et al., 1998). However, most of the studies undertaken outside sub-Saharan Africa show comparatively lower prevalences in the asymptomatic population, whilst studies undertaken in sub-Saharan African populations show higher prevalences (Belperio and Rhew, 2004). A considerably high anaemia prevalence as observed in this study, which is essentially asymptomatic and African, corroborates the observation in the study by Belperio and Rhew (2004).

Although reference to the infected population in this study as asymptomatic may be based primarily on the design and criteria for selecting the subjects, there is reason to suggest that, HIV infection is at an early stage in this population. The effects of early HIV infection may be contributing partly to this high level of anaemia, but other population factors perhaps unrelated to HIV infection may also be adding to the anaemia. This may be inferred from the fact that prevalence of anaemia was equally high in the sero-negative population. Indeed, the prevalence of anaemia in the study population as well as in each separate sero-status group suggests that anaemia in the study population is at a level that, by any standard, constitutes a serious public health problem (UNICEF/UNU/WHO, 2001).

Whether HIV infection is what drives anaemia in this study population to attain public health relevance (McLean et al., 2007) may however be contended as the sero-negative group also have similarly high levels of anaemia (over 40%) despite the absence of the infection. Nevertheless, in a population where the prevalence of HIV infection has the tendency to escalate, and where the infection is likely to be synergised by other potential anaemia inducing factors, this minimal contribution to anaemia may add to the increasing magnitude of anaemia in the population.

Other than the direct effects of the HIV, the evidence that other factors, such as concurrent infections and population characteristics, may be contributing greatly to the high prevalence of anaemia in the sero-positive population are depicted by a number of epidemiological studies, which have shown significant associations between various factors and anaemia in the HIV/AIDS population (Sullivan et al., 1998; Volberding et al., 2004). From this study, an equally high anaemia prevalence in the sero-negative group can only suggest the importance of factors, apart from HIV infection, to the development of anaemia in the study population.

In as much as a large number of factors have been suggested in the framework for addressing the hypothesis, data constraints pertaining to its secondary source, restricts the elucidation of certain factors as part of the causal web between HIV infection and anaemia as they have not been gathered. However, in order to understand the reasons behind the high prevalence of anaemia in the sero-positive population and to place the role of HIV in perspective, the involvement of some factors, considered important, have been elucidated whilst others may only be conjectured based on their inter-relationships.

The differences between the levels and characteristics of anaemia in sero-positives and sero-negatives in this study may not entirely be due to chance as certain respects have been very significant. In trying to explain the possible reasons for the observed differences, the phenomenon of reductive adaptation has been invoked together with other plausible factors. Reductive adaptation, which may result in significant changes in body composition, is most likely to occur in the latter stages of HIV infection or AIDS. It may manifest as weight loss, a reduction in lean body mass or changes in the internal milieu that could affect other bodily functions. The effects of such changes can drastically reduce red cell mass as RBC production is depressed and destruction is enhanced (Jackson, 2007). So far, this phenomenon may not be an obvious explanatory factor for anaemia in the study population since there were no significant differences between the sero-positives and sero-negatives with regards to a large number of the parameters that may depict these changes. However, a significant effect of anaemia and HIV infection on fat deposition in parts of the body may suggest some effect of body compositional changes on anaemia even at an early stage of HIV infection.

The effects of HIV infection could also result in food or energy and nutrient imbalances. Infections and inflammation can cause inadequate energy and nutrient intakes through anorexia and other gastrointestinal disturbances (Thurnham and Northrop-Clewes,

2007). However, these often manifest at the latter stages of the infection or when inflammation has been persistent or chronic. There is no doubt that energy imbalances and nutrient deficiencies can predispose to malnutrition (Jackson, 2007), which may lead to anaemia. However, considering the asymptomatic stage of sero-positives and the apparently healthy state of their sero-negative peers, these nutritional considerations could not be taken as significant explanatory factors for anaemia in the study population. In fact, sero-positive individuals as well as anaemics showed tendencies for being adequately nourished. Nevertheless, lack of significant differences between the sero-status and anaemia groups for mean energy and micronutrients intakes were probably indicative of the fact that these intakes were not very far from normal.

Though average energy and micronutrient intakes seemed close to normal requirements, individual intakes for a large number of persons in the study were rather marginal or even lower. For sero-positives, such marginal intakes may rather express an inadequate intake since infection has a tendency to increase requirements (WHO, 2003). However, because of the widespread nature of the marginal or inadequate intake tendencies in the study population, other factors related to the socio-demography and nutritional or dietary practices within the population, rather than the effects of HIV infection or inflammation may be plausible explanations for the poor intakes.

The effects of poor socio-demographic characteristics on energy and nutrient intakes has been demonstrated in a study by Peña and Bacallao (2002), who showed a significant inter-relationship between poor socio-demographic factors and nutrition. It has also been recognised that poor socio-economic characteristics generally relate to food and nutrition insecurity (Gillespie and Kadiyala, 2005). In this study, a poor socio-demography may be inferred from levels of earnings and educational attainments in this study in a large section of the study population, that can only be described as poor. Even though these have not been related to the nutrition of the study population, it is quite possible that such poor socio-economic tendencies could have a strong influence on food intake and other dietary practices (Peña and Bacallao, 2002). As earlier intimated in the previous chapter, the existence of such socio-demographic tendencies could be the trigger that would heighten the vulnerability of persons to nutritional problems and to infections such as HIV/AIDS.

In this study population, poor socio-demographic conditions may have their origin in the wave of rapid urbanisation blowing across the region (Vorster et al., 2005). The effects

of a rapid urbanisation is often accompanied by transitional changes in nutritional and socio-demographic structures inimical to the health of the population (Vorster et al., 2000). During this process, smoking, consumption of alcohol, commercial sex and violence are some of the increasing tendencies that heighten the vulnerability of individuals to nutritional problems and infectious diseases.

In spite of marginal energy and nutrient intake tendencies in the study population, the fact that these tendencies were slightly higher in HIV sero-positives generally and sero-positive anaemics in particular raised some important questions. However, because the differences were not significant they were probably not real or due to chance. Nevertheless, a marginally higher dietary energy and micronutrient intake in sero-positives could be due in part to the consumption of a significantly higher proportion of carbohydrate rich food sources or, perhaps, to a greater tendency for consuming alcohol, of which intake in moderation has been shown to boost food intake (Yeomans, 2004). But in the case of sero-positive anaemics, alcohol intakes may not explain the marginal tendencies for higher intakes over their non-anaemic peers as they were less likely to consume it.

Apart from the above explanations, the phenomenon of hyperphagia, which may be common in asymptomatic HIV infection (Crenn et al., 2004), but may also occur at all stages of HIV infection, especially, in those with an elevated BMR (Melchoir et al., 1993, Grunfield et al., 1996), could be invoked as part of the explanation for higher intake tendencies in the sero-positive population in particular. What makes hyperphagia a plausible explanation the higher BMR tendencies in sero-positives and seropositive anaemics compared to their peers. This finding is buttressed by another study that suggested the occurrence of hyperphagia in an HIV infected cohort whose BMR were marginally elevated (Macallan, 1998). The basis for a marginally elevated BMR in asymptomatic HIV infection has been partly ascribed to an increased protein turnover (Crenn et. al., 2004) which may be linked to the release of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 (Roubenoff et al., 2002). In effect, inflammation rather than causing anorexia, could be at the core of an increased food intake, especially at an early stage of the infection, and sero-positive anaemics could be more predisposed.

On the other hand, a critical examination of the differences in intakes between the sero-status groups, and the fact that they were marginal and not significant, may suggest a chance occurrence. In view of the problematic methodology surrounding the assessment of diets (Margetts and Nelson, 2008), the differences in intakes between

the two groups could therefore be ascribed to methodological limitations. Firstly there is the issue of measuring or estimating energy and nutrients from a largely variable intake of foods that may not be constituted entirely of a single form of energy or nutrient. Secondly, intakes of foods, and especially alcohol can be highly variable, and especially when estimated would be an obvious source of bias (Margetts and Nelson, 2008). It is also possible that bias was introduced during the reporting of dietary intake, which is very common in vulnerable groups. When the individuals know their status, which could be HIV or other, they could be prompted to over-report or under-report their food intakes. Various other physical and psycho-social issues, such as being overweight, tend to affect the reporting of food intake. In the case of subjects in this particular study they did not know their HIV status at the time of the study, but it is possible that these other issues could affect their reporting.

For persons infected with HIV the need to maintain energy and nutrient intakes above what is normally required must be intended and pursued, especially, as alterations in metabolic and inflammatory processes brought about by the infection, create an increased nutritional demand. An increase in energy and nutrient requirements may become even more imperative should anaemia set in. It would therefore be expected that asymptomatic HIV infected persons, especially anaemics, would strive or be encouraged to maintain intake levels that are not less than 10% above the normal requirements of a healthy population, in accordance with WHO recommendations (WHO, 2003). Despite the comparatively higher intakes of energy and nutrients observed in the sero-positive population thus does not seem to even satisfy the normal requirements let alone meet recommended requirements. This poor state of energy and nutrient intake may therefore partly account for the anaemia in the sero-positive population.

Reasons for a higher prevalence and severity of anaemia in sub-Saharan African populations have been placed on the presence of specific population characteristics (Ssali et al., 2006). Some epidemiological evidence points to the racial characteristic of black or African descent as a significant associate with anaemia in HIV/AIDS (Sullivan et al., 1998, Volberding et al., 2004). This racial characteristic may probably explain the high prevalence of anaemia in the study population. However, it is very likely that this racial predisposition to anaemia is based on other factors that have been suggested as responsible for the increased susceptibility of this group to anaemia, and some of which could be at play within the study population. In the black African race there are certain genetic population characteristics often associated with prevalent or endemic environmental conditions that may confer a higher survival of the race. For

instance, hereditary traits, such as G-6-PD (Calis et al., 2008) and sickle cell disease (Tolentino and Friedman, 2007), as well as other rare haemoglobinopathies, may have protective value. Sickle cell trait, in particular, protects against specific malaria parasites and therefore serves as a survival strategy for most of the black race who reside in malaria holoendemic regions. All these genetic traits have been associated with anaemia in HIV infected black persons (Volberding et al., 2004). Additionally, there are aspects of nutritional and socio-cultural practises in black people that often reflects in their dietary preferences and choices, which may present significant risks to anaemia (Antelman et al., 2000, Semba, 2003).

Aside from these hereditary and dietary factors, there are also socio-demographic and geographical circumstances, common to black Africans in particular, which may significantly influence the health and development of the population. While these factors may have a general effect on the entire population, their presence may create vulnerabilities in the population or may pose a treat to particular vulnerable groups. Some of these socio-demographic factors, which are manifest in this study population, could hamper good nutrition and health of the population and invariably contribute to the high levels of anaemia.

Black people predominantly reside in sub-Saharan Africa where malaria and other common infectious diseases are highly prevalent. Although the incidence of malaria in the study area is quite low, it is known that high levels of other infectious diseases affect the nutrition and health of the population (Vorster et al., 2005). It is the presence of these conditions, which prevail in most developing countries, that enhances the risk to anaemia (Thurnham and Northrop-Clewes, 2007). These conditions are worsened by the ominous presence of poverty (Brabin et al., 2004). Whilst poverty together with inadequate health care and pervading unsanitary conditions, common in much of the developing world, encourage the spread of diseases and expose individuals to anaemia and other co-morbidities (Calder and Jackson, 2000; Thurnham and Northrop-Clewes, 2007). Thus, the prevalence of hereditary and environmental factors favourable to the cause of anaemia in the black population may be compounded by circumstances that allow anaemia to thrive. The causes of anaemia in such populations may therefore be complex and intricately interwoven.

Although the factors responsible for anaemia in the study population can be said to be expansive, HIV infection and its effects, either directly or indirectly, remain crucial in the causation of anaemia. The impact of HIV and its associated effects on anaemia may stem from the fact that it does not only directly cause a reduction of RBC mass, but can

also, through its associated inflammatory effects cause disturbances in iron metabolism (Butensky et al., 2004), which could subsequently lead to erythropoietic failure.

For quite some time now, the idea of HIV impacting on haemopoiesis and anaemia through this immunological effect on iron metabolism and not necessarily through a genuine iron deficiency has been a point of discussion (Kreuzer and Rockstroh, 1997; Bain, 1999). Both the virus and the characteristic inflammation associated with the infection have been implicated (Butensky et al., 2004). The ensuing ACI, that favours iron accumulation, has gained recognition as a more frequent occurrence. Nevertheless, a genuine iron deficiency and iron accumulation form the basis of two main types of anaemia often associated with HIV (Eley et al., 2002; Butensky et al., 2004).

This study has revealed that, a tendency for hypoferaemia or low levels of serum iron, consistent with the hypoferaemia that tends to occur at the onset of inflammation, even when iron stores seem to be normal (Thurnham and Northrop-Clewes, 2007), may be present. The higher likelihood for elevated serum ferritin levels in both sero-status groups, buttressed by the higher proportions with ACI over IDA further supports the likelihood of inflammatory influences and perhaps body iron redistribution tendencies (Butensky et al., 2004). Indeed, elevation of ferritin have been strongly associated with immune activation (Sarcletti et al., 2003), with pro-inflammatory cytokines most likely to be the cause of this elevation.

However, whether inflammatory effects on iron can contribute to anaemia in the study population may depend on the expression of metabolic iron deficiency and the ability of individuals to maintain iron stores, which are known to be critically determined by absorption, loss or dietary bio-availability of iron (Thurnham and Northrop-Clewes, 2007).

For those individuals with ferritin levels below 15 µg/dL, a metabolic iron deficiency is not far fetched. However, for an apparently healthy or asymptomatic population whose intake of iron remains close to normal requirements, it is perhaps a high intake of dietary blockers or the effect of an inflammatory curtain that can explain such metabolic iron deficiency tendencies. Nevertheless, the higher likelihood for elevated ferritin levels as well as alterations in APP that are reminiscent of the inflammatory process strongly suggest inflammation as a significant explanatory factor to the anaemia in the study population. Furthermore, the predominant prevalence of mild anaemia and ACI all add to the suggestion that inflammation may contribute to the cause of anaemia in

the study population. This is supported by evidence from other studies that suggests that hypoferaemia and hyperferritinaemia induced by inflammation may be associated with anaemia (Thurnham and Northrop-Clewes, 2007).

Changes observed in the turnover of APP as well as shifts in nutrients that often accompany the inflammatory process may represent inflammatory influence in the study population. With the onset of an infection, injury or stress, the body normally evokes a rapid immune response (Kushner, 1982; Calder and Jackson, 2000), which is generally associated with the release of pro-inflammatory cytokines, concomitant with increases in positive APPs such as CRP, globulins, ceruloplasmin, fibrinogen and ferritin and decreases in negative APPs such as albumin, transferrin, retinol binding protein, lipoproteins and pre-albumin (Kushner, 1982; Schreiber, 1988; Tomkins, 2003; Jackson, 2007). In this study, the tendencies for increased serum total proteins and decreased serum albumin may be consistent with reports that have shown the occurrence of similar changes in protein turnover in humans with symptomless HIV/AIDS (Macallan et al., 1995; Selberg et al., 1995).

Such alterations in protein metabolism are not restricted to HIV infected populations only, but even in the absence of HIV infection, these sub-clinical markers of inflammation can be observed in apparently healthy populations (Thurnham et al., 2003). The alterations in protein metabolism may also be associated with anaemia irrespective of whether infection with HIV is present or not as shown by the significant effect of anaemia on the APP. However, the significantly lower levels of serum total proteins and albumin associated with anaemia irrespective of HIV status rather indicates the limits to which inflammation may be influenced by anaemia.

The significantly higher total proteins may suggest an increased synthesis of positive APP in response to an ever present threat of the virus. However, this phenomenon may be a common occurrence at the onset of most infections (Meenan et al., 1992). A study by Jahoor and colleagues (Jahoor et al., 1999) suggest that symptomless HIV infected subjects compared to uninfected controls, are able to maintain higher plasma concentrations and rates of syntheses of most positive APPs without a corresponding decrease in plasma concentration of most negative APPs, which, according to the authors, may represent a beneficial adaptation by the individual subjected to infection by the virus. The authors also contended, based on their evidence, that subclinical infections, including asymptomatic HIV infection, may not be capable of eliciting a full-blown APR (Jahoor et al., 1999). This is notwithstanding the fact that hypoalbuminaemia occurred in the symptomless HIV infected persons (Jahoor et al., 1999).

and also in children with other sub-clinical infections (Morlese et al., 1996) despite increased rates of synthesis.

The significance of lower albumin levels observed in the HIV sero-positive group may be similar to the hypo-albuminaemia observed in the two studies by Jahoor et al. (1999). Certainly with synthesis rates not lowered any decreases or drop in the levels of albumin and perhaps most other negative APPs may be ascribed to something more than protein turnover. Although it is possible that positive APP may be produced at the expense of albumin (Jackson, 2007), it is more likely that an increased trans-capillary escape (Fleck et al., 1985; Tomkins, 2003) is responsible for the drop. This may presuppose that the effect of the inflammatory process on albumin, even if mild, is exerted more at increasing trans-capillary membrane losses than at altering metabolic or synthesis rates. In addition, such significant lowering of albumin levels during a mild APR might only occur when the APR is either persistent or chronic or perhaps when other inflammatory stressors add on synergistically.

Ferritin serves as an indicator of iron stores and also as an acute phase protein. However, the elevated tendencies for serum ferritin levels as seen in the study population could be ascribed to increased rates of synthesis as pertains during an inflammatory response as the levels tended to be averagely higher than normal storage levels. Nevertheless, the degree to which it rises in the blood can be limited by underlying iron status (Northrop-Clewes, 2008) as well as the presence of inflammation. Thus, the higher likelihood for ferritin levels to increase, especially, in the sero-negative population only shows that although the population is apparently healthy, sub-clinical inflammatory influences may be present.

The occurrence of inflammation in the study population, as suggested by the evidence of altered APP, may have deleterious effects on the synthesis of haem or RBC. This is especially so, where nutrients useful for RBC or haem synthesis are restricted, used up or lost during the process and the alterations lead to the creation of an environment that enhances the destruction of RBC (Jackson, 2007). Because levels of albumin and other transport protein carriers, which are mostly negative APPs, are depressed during inflammation the levels of micronutrients (especially those carried by the transport proteins) are also likely to fall. Lower levels in serum vitamins A and E observed in this study may be depicting this inflammatory influence, which may seem to be greatly exerted in the HIV sero-positives as they have a higher tendency for lower serum levels of these vitamins.

A further elucidation of inflammatory effects on anaemia in the study population was revealed by the levels of liver enzymes and a particular by-product, that otherwise would indicate an ominous presence of liver tissue damage. The measurement of bilirubin (Total and direct) gave some indication of the way this haem by-product was handled by the RES. A marginally lower total bilirubin and higher direct bilirubin levels as shown for the sero-positives and sero-positive anaemics when compared with levels in sero-negatives and non-anaemics might be indicative of a lower likelihood for HIV infection to be associated with haemolytic anaemia in this population. In haemolytic anaemia, RBC are broken down and total bilirubin levels rise together with the unconjugated bilirubin (i.e. Total minus Direct). The tendency for haemolytic anaemia to be a contributor to the anaemia in the study population would be explained by the possible high prevalence of haemoglobinopathies. The significant tendencies for the serum liver enzymes, ALT and AST, to be elevated in sero-positive as well as in sero-positive anaemics compared to their peers, may also elucidate inflammatory effects. Both HIV and anaemia can alter the metabolic properties of the liver and other related cells, and considering the significant interactive effect between HIV and anaemia, it stands to reason that inflammatory stress could lead to damage of liver cells and spill over of enzymes. An indication of ominous hepato-cellular damage in HIV infected subjects who are essentially asymptomatic portrays HIV infection as capable of interacting with other effects to exert a detrimental influence even at an early stage.

Though HIV infection alone can significantly increase the tendency for liver damage (Richardson et al, 1994; Sulkowski, 2008), the likelihood that it does so by interacting with or potentiating other stressors is high. In HIV infected populations, a close occurrence of HIV and other viral opportunistic infections, including viral hepatitis (Richardson et al, 1994; Sulkowski, 2008) as well as ARV toxicities (Sulkowski and Benhamou, 2007), form the bases for most liver damage. Generally, excessive intake of alcohol, especially at levels considered to be hazardous, has been linked to liver damage (Bellantani et al., 1997). Such excessive intakes have also been associated with an increased risk of liver damage in HIV infected persons (Chaudhry et al., 2009). Furthermore, the effects of alcohol in damaging the liver may be enhanced in black persons (Stranges et al., 2004). In this study, HIV sero-positives, who were black, were more likely to consume alcohol but in quantities that may be considered less hazardous. Despite the absence of information on viral hepatitis and ARV toxicity statuses for this population, the presence of some predisposing factors coupled with their HIV sero-positivity may be considered as important explanatory factors for the increased tendencies of serum ALT and AST.

In sum, HIV infection and its many eliciting effects can be very important in evoking changes within the body of its victim that may be associated with body composition, nutritional status, or the body's immunological response in relation to metabolism of proteins and nutrients. External or environmental stresses unrelated to the infection may also exert their influence on the effects of the virus. Together, all these factors may eventually determine the development of anaemia in the infected population. However, in asymptomatic infected persons, the viral effects may be mild, and yet the persistence of these effects may prime other potential anaemia-inducing effects to significantly alter RBC metabolism and predispose to anaemia. The effects of HIV and anaemia on many of these factors have been shown as independent or interactive. However the inter-relationships between all these factors together without necessarily considering the level at which they may be acting to cause anaemia is what the next chapter seeks to explore.

## CHAPTER SIX

### 6.0 EXPLORATIVE EVALUATION OF FACTORS THAT MAY INTERPLAY WITH HIV OR PREDICT ANAEMIA IN THE STUDY POPULATION

So far, results in the preceding chapters can lead to the conclusion that though anaemia in the study population is high, it is higher in HIV sero-positives compared to sero-negatives. In addition, the causes of high anaemia prevalences may be attributed to a number of factors that may include the virus as well as possible changes brought about by the infection and/or inflammation, alterations in body composition, energy or nutrient deficiencies, and perhaps a number of other factors not identified in this study. The association of HIV infection with a higher prevalence and severer grade of anaemia may suggest an independent effect of HIV or its presence to potentiate or interplay in the cause of anaemia by other factors.

It is in view of this possible interactive effects of the HIV with factors that could predict anaemia in the study population that this chapter seeks to elucidate.

#### 6.1 INTRODUCTION

In this chapter, the hypothesis which states that, 'HIV infected persons are more anaemic in comparison with uninfected persons' would be explored by partial correlations between the possible explanatory factors and markers of anaemia, whilst adjusting for confounding socio-demographic variables. Furthermore, factors that are more likely to predict anaemia in the study population would be identified using various multivariable logistic regression models.

The objectives set to achieve the above goals are as follows:

- To show the extent to which HIV infection may interplay with markers of body composition, energy and micronutrient intakes, serum micronutrients and levels of inflammatory bio-markers that may possibly explain anaemia in the study population
- To identify significant factors or effects that may predict anaemia in the study population using binary regression modelling

The next sections would explore the extent to which individual factors identified in the previous chapters could interactively constitute significant determinants of anaemia in the study population. Partial correlations would be used to explore for any relationships between HIV and anaemia using the variables independently against indicators of anaemia whilst adjusting for confounding socio-demographic variables. This would be followed by the use of various logistic regression (LR) models to explore

for significant predictors of anaemia within each sero-status group and also within each strata of the significant socio-demographic variables.

## **6.2 EXPLORING THE RELATIONSHIP BETWEEN SIGNIFICANT FACTORS AND MARKERS OF ANAEMIA IN THE STUDY POPULATION BY PARTIAL CORRELATIONS**

Tables 6.1a, b and c below show summarised outcomes for the partial correlations between factors that are likely to be associated with anaemia (using [Hb] and haematocrit definitions) in the study population.

Considering anthropometric variables (Tab. 6.1a), abdominal weight and calf skin folds measurements showed significant negative or inverse correlation coefficients ( $r$ ) with [Hb] only in sero-negatives. However,  $r$  with abdominal skinfold and weight were rather weak. On the other hand correlations with other anthropometric variables were either marginal or not significant. An inverse correlation can be interpreted as a relationship where a variable increases as the other on which it may be dependent decreases. For instance, in this case weight as well as abdominal and calf skinfolds would increase with a decreasing [Hb], i.e. with progressing anaemia. The implication here may be that, in sero-negatives, anaemic persons are more likely to have higher weights or abdominal or calf skin folds and *vice versa*.

With dietary energy and micronutrients (Tab 6.1b), correlation coefficients for thiamin, riboflavin and folate intakes against [Hb] in sero-positives were also inverse and very significant. Most others were however very weak and also not significant. This also implies that a lower [Hb] level is most likely to be associated with higher intakes of these micronutrients in this study population.

From table 6.1c, correlation coefficients ( $r$ ), for serum vitamin A was positive and significant with [Hb] in sero-positives, whilst in sero-negatives it was only marginally significant with Hct. Serum vitamin E levels were also significantly and positively correlated with [Hb] and Hct in HIV sero-positives, and with [Hb] in HIV sero-negatives. Similarly, correlations between serum iron and either [Hb] or Hct were positive and highly significant in HIV sero-positives, but in the sero-negatives though  $r$  was positive it was only significant against Hct. Positive and significant  $r$  values were also revealed for TIBC against [Hb] only in sero-positives, whilst  $r$  values for % transferrin saturation against [Hb] and Hct were also positive in sero-positives.

**Table 6.1a: Partial correlations between anthropometric variables against [Hb] and haematocrit as possible determinants of anaemia in the study population**

Variables	Haemoglobin		Haematocrit		
	HIV-	HIV+	HIV-	HIV+	
Weight	r	-0.087	-0.078	0.041	0.046
	p	0.032	0.414	0.313	0.629
	n-1	608	109	608	109
Height	r	-0.035	-0.156	0.037	0.060
	p	0.389	0.102	0.358	0.529
	n-1	608	109	608	109
Triceps skin fold	r	-0.060	0.019	0.060	-0.060
	p	0.139	0.846	0.142	0.535
	n-1	608	109	608	109
Subscapular skin fold	r	-0.078	0.090	0.069	-0.045
	p	0.055	0.348	0.090	0.636
	n-1	608	109	608	109
Iliac crest skin fold	r	-0.059	0.012	0.072	-0.036
	p	0.147	0.903	0.076	0.710
	n-1	608	109	608	109
Supraspinale skin fold	r	-0.040	0.083	0.074	0.063
	p	0.327	0.387	0.068	0.510
	n-1	608	109	608	109
Abdominal skin fold	r	-0.088	0.034	0.071	0.059
	p	0.029	0.727	0.078	0.540
	n-1	608	109	608	109
Thigh skin fold	r	-0.120	-0.030	0.027	-0.007
	p	0.003	0.754	0.509	0.941
	n-1	608	109	608	109
Total energy intake	r	-0.002	-0.190	0.017	0.036
	p	0.953	0.046	0.671	0.706
	n-1	608	109	608	109
Calf skin fold	r	-0.117	-0.039	0.008	0.069
	p	0.004	0.685	0.843	0.472
	n-1	608	109	608	109

Correlation statistics corrected for sex, age grouping and settlement type. r = correlation coefficient, p = statistical probability and n-1 is degrees of freedom

**Table 6.1b: Partial correlations between dietary intake variables against [Hb] and haematocrit as possible determinants of anaemia in the study population**

Variables	Haemoglobin		Haematocrit		
	HIV-	HIV+	HIV-	HIV+	
Dietary Vitamin A	r	-0.029	-0.113	-0.016	-0.061
	p	0.275	0.108	0.536	0.390
	n-1	1454	201	1454	201
Dietary Vitamin E	r	0.046	-0.036	0.015	-0.043
	p	0.077	0.609	0.578	0.544
	n-1	1454	201	1454	201
Dietary Vitamin B <sub>12</sub>	r	-0.002	-0.102	0.018	0.037
	p	0.945	0.146	0.482	0.596
	n-1	1454	201	1454	201
Dietary Thiamin (B <sub>1</sub> )	r	-0.024	-0.144	-0.017	-0.052
	p	0.362	0.040	0.522	0.463
	n-1	1454	201	1454	201
Dietary Riboflavin (B <sub>2</sub> )	r	-0.032	-0.137	0.019	-0.051
	p	0.229	0.051	0.460	0.472
	n-1	1454	201	1454	201
Nicotinic acid (B <sub>3</sub> )	r	-0.017	-0.086	0.015	0.053
	p	0.512	0.223	0.560	0.454
	n-1	1454	201	1454	201
Dietary Vitamin B <sub>6</sub>	r	-0.046	-0.036	-0.019	-0.018
	p	0.080	0.607	0.467	0.797
	n-1	1454	201	1454	201
Dietary Ascorbic acid	r	-0.010	-0.055	0.056	0.034
	p	0.694	0.435	0.033	0.633
	n-1	1454	201	1454	201
Dietary Folate	r	0.015	-0.138	0.039	-0.064
	p	0.567	0.049	0.137	0.366
	n-1	1454	201	1454	201
Dietary Iron	r	0.011	-0.119	-0.007	-0.017
	p	0.684	0.091	0.802	0.805
	n-1	1454	201	1454	201
Dietary Copper	r	0.017	-0.075	-0.017	-0.005
	p	0.529	0.285	0.514	0.944
	n-1	1454	201	1454	201
Dietary Zinc	r	-0.019	-0.113	0.020	0.060
	p	0.463	0.108	0.442	0.392
	n-1	1454	201	1454	201

Correlation statistics corrected for sex, age grouping and settlement type. r = correlation coefficient, p = statistical probability and n-1 is degrees of freedom

**Table 6.1c: Partial correlations for biochemical variables against [Hb] and haematocrit to show predictive value for anaemia in the study population**

Variables	Haemoglobin		Haematocrit		
	HIV-	HIV+	HIV-	HIV+	
Serum Vitamin A	r	0.140	0.090	0.152	0.133
	p	0.075	0.003	0.053	<0.001
	n-1	161	1127	161	1127
Serum Vitamin E	r	0.299	0.119	0.044	0.093
	p	<0.001	<0.001	0.577	0.002
	n-1	161	1127	161	1127
Serum Fe	r	0.101	0.189	0.194	0.245
	p	0.200	<0.001	0.013	<0.001
	n-1	161	1127	161	1127
TIBC	r	-.132	-0.083	-0.031	0.019
	p	0.094	0.005	0.696	0.522
	n-1	161	1127	161	1127
% Transferrin saturation	r	0.100	0.186	0.118	0.200
	p	0.203	<0.001	0.133	<0.001
	n-1	161	1127	161	1127
Serum ferritin	r	-.045	0.016	0.036	0.077
	p	0.566	0.601	0.646	0.009
	n-1	161	1127	161	1127
Serum total Protein	r	-0.088	0.154	-0.124	0.213
	p	0.266	<0.001	0.116	<0.001
	n-1	161	1127	161	1127
Serum Albumin	r	0.161	0.167	0.203	0.237
	p	0.040	<0.001	0.009	<0.001
	n-1	161	1127	161	1127
Plasma Fibrinogen	r	0.145	0.041	0.075	0.069
	p	0.066	0.167	0.339	0.021
	n-1	161	1127	161	1127
Total bilirubin	r	0.054	0.083	0.174	0.128
	p	0.495	0.005	0.027	<0.001
	n-1	161	1127	161	1127
Direct bilirubin	r	0.128	0.200	0.139	0.150
	p	0.102	<0.001	0.077	<0.001
	n-1	161	1127	161	1127
Serum ALP	r	0.143	0.009	0.055	-0.012
	p	0.044	0.739	0.439	0.652
	n-1	196	1388	196	1388
Serum GGT	r	-0.006	-0.005	-0.069	0.020
	p	0.931	0.858	0.332	0.467
	n-1	196	1388	196	1388
Serum ALT	r	0.062	0.034	0.063	0.118
	p	0.389	0.209	0.376	<0.001
	n-1	196	1388	196	1388
Serum AST	r	0.016	-0.001	0.022	0.030
	p	0.824	0.982	0.760	0.261
	n-1	196	1388	196	1388

Correlation statistics corrected for sex, age grouping and settlement type. r = correlation coefficient, p = statistical probability and n-1 is degrees of freedom

With regards to the APPs, a correlation coefficient for ferritin, even though was weak, was positive and significant against Hct in only the sero-positives. There emerged a strong positive correlation between total proteins against both [Hb] and Hct only in the sero-positives, whilst serum albumin showed strong positive r as well against both indicators for both sero-negatives and sero-positives. With plasma fibrinogen a weak

positive but significant  $r$  was shown against Hct only in the sero-positives. When the liver enzymes and bilirubin were analysed, both total and direct bilirubin showed a direct significant correlation with [Hb] and Hct in sero-positives whilst in the sero-negatives there was a significant direct correlation only with Hct. For the liver enzymes, apart from the positive significant correlation between ALP and [Hb] in sero-negatives and also between ALT and Hct in sero-positives, all others were not significantly correlated.

### **Summary of section 6.2**

This section may be summarised as follows:

1. Weight, abdominal or calf skin folds may be considered as negative significant determinants of anaemia in the sero-negative population or in other words sero-negative anaemic persons are more likely to have higher weights, abdominal or calf skin folds.
2. High thiamin, riboflavin and folate intakes may be positive significant determinants of anaemia in sero-positives in this study population
3. Serum vitamins A and E may be significant positive determinants of anaemia in the sero-positive and to some extent in the sero-negative population. Similarly, serum iron, TIBC and % transferrin saturation were considered as positive determinants of anaemia in the sero-positive population. Whilst serum ferritin was weak and positive, total proteins and albumin were rather strong and positive determinants in the sero-positives, whilst albumin was a positive and significant determinant in the sero-negatives.
4. Total and direct bilirubin were considered significant positive determinants of anaemia in the sero-positives, much like serum ALT, whilst serum ALP was a determinant of anaemia in the sero-negatives

In the next section, various multivariable logistic regression models would be applied to explore for significant factors that may interactively predict anaemia in the study population.

### **6.3 PREDICTORS OR DETERMINANTS OF ANAEMIA BY MULTIVARIABLE LOGISTIC REGRESSION MODELLING**

To identify variables that were more likely to interplay between HIV and anaemia and which could significantly predict anaemia in the study population, backward stepwise likelihood ratio (LR) logistic regression models were applied to each of the groups and also to the significant socio-demographic strata. Each model, except for the strata,

incorporated all variables considered as possible explanatory factors for anaemia together with significant socio-demographic variables that served as covariates.

### 6.3.1 PREDICTORS OF ANAEMIA IN THE STUDY POPULATION

Table 6.2 below is a summary of the outcomes from the regression models for factors within the study as well as the sero-positive and sero-negative populations respectively.

The numbers included in each respective adjusted model were 1365 out of 1845 (79.4%) in the first, 179 out of 216 (86.6%) in the second and 1187 out of 1605 (69.3%) in the third. Cases that had missing values for any of the variables in the model were excluded. Also, variables included in each model were all categorical and included socio-demographic variables that served as co-variates for adjustment. Furthermore, a Nagelkerke R squared value was included to show how much of the variability in each model was accounted for by the interacting variables – the closer the value is to 1 the more the model is explained by the interaction. Variables at each step of the analyses were excluded in order of significance with those removed earlier tending to have lower significance compared to those removed later, which represented an estimate of the degree to which the variable was likely to be significant as a predictor of anaemia.

The table (Tab.6.2) also lists the variables that depicted higher probabilities and emerged as significant in predicting anaemia. In the general study population these were; Urbanisation, Serum albumin, Serum vitamin E, Plasma fibrinogen, Serum GGT, TIBC, Direct bilirubin and Abdominal skinfold. In the model applied to the infected group, the significant factors emerging were; Serum albumin, Serum globulins, Serum vitamin E, Serum AST, Smoking status, BMI, Mass, Stature, Supraspinale skin fold and LMI. Whilst the model for the uninfected group yielded Urbanisation, Serum total proteins, Serum vitamin E, Serum GGT and Abdominal skinfold as the most significant determinants.

**Table 6.2: Summary of significant predictors of anaemia in the study population by logistic regression modelling**

Stratified Model	No in model/total	Significant variables in model	Nagelkerke R square values
Study population	1365/1845	Settlement type, Serum albumin, Serum vitamin E, Plasma fibrinogen, Serum GGT, TIBC, Direct bilirubin, Abdominal skinfold	0.256
HIV sero-positive group	179/216	Serum albumin, Serum globulins, Serum vitamin E, Serum AST, Smoking status, BMI, Mass, Stature, Supraspinale skin fold, LMI	0.350
HIV sero-negative group	1187/1605	Settlement type, Serum total proteins, Serum vitamin E, Serum GGT, Abdominal skinfold	0.257

Tables 6.3, 6.4 and 6.5 below respectively present crude odds ratios (OR) and adjusted OR (aOR) for the significant variables which emerged for models applied to the study population, sero-positive group and sero-negative group respectively. Whilst the crude OR accounted for the risk levels for each variable independently, aOR accounted for the interactive effect of all the variables in the model and their relationship with each other. Odds ratios greater than 1 denoted a greater likelihood or probability for association with the outcome and *vice versa*.

**Table 6.3: Significant predictors of anaemia in study population by logistic regression modelling**

Variable		No of persons in adjusted model	Crude OR (p value)	Adjusted OR (p value)	95% CI of aOR
Serum albumin	low	715	0.589 (0.002)	0.635 (0.016)	0.439–0.918
	high	650			
Settlement type	Rural	539	1.658 (0.001)	1.708 (0.004)	1.182–2.466
	Urban	826			
TIBC	low	706	1.356 (0.002)	1.506 (0.028)	1.044–2.173
	high	659			
Vitamin E	low	664	0.715 (0.002)	0.603 (0.006)	0.419–0.864
	high	701			
serum GGT	low	705	0.896 (0.008)	0.613 (0.007)	0.430–0.875
	high	660			
Plasma fibrinogen	low	876	0.697 (0.065)	0.607 (0.010)	0.415–0.886
	high	489			
Direct bilirubin	low	856	0.831 (0.041)	0.695 (0.046)	0.486–0.994
	high	509			
Abdominal skinfold	low	673	1.753 (<0.001)	1.768 (0.002)	1.235–2.531
	high	692			

Backwards-stepwise logistic regression analyses with anaemia (dependent variable). Odds ratios (OR) have been adjusted for other variables in the model P values less than 0.05 denoted statistical significance.

Table 6.3 above shows that settlement type, TIBC and abdominal skin folds are significant indirect determinants with aOR that are greater than 1. This means that Urban settlement type, high TIBC and abdominal skin folds are strong predictors of anaemia in the study population. It can also be inferred from the table that low serum albumin, vitamin E, serum GGT, plasma fibrinogen and direct bilirubin are strong predictors of anaemia as well in the study population.

From table 6.4 below, female sex, non-smoking, high serum 'globulins', serum AST, BMI, stature and LMI as well as low serum albumin, vitamin E, weight and supraspinale skin fold all predicted anaemia in the sero-positive group.

With regards to the significant predictors of anaemia in the sero-negative population, table 6.5 below has shown emerging variables as urban settlement type, high abdominal skin fold as well as low serum total proteins, serum vitamin E and serum GGT.

**Table 6.4: Significant predictors of anaemia in the HIV sero-positive group by logistic regression modelling**

Variable		No of persons in adjusted model	Crude OR (p value)	Adjusted OR (p value)	95% CI of aOR
Sex	Male	74	4.053 (0.001)	3.557 (0.001)	1.410–9.412
	female	104			
Serum albumin	low	128	0.706 (0.006)	0.753 (0.051)	0.037–24.107
	high	50			
Serum globulins	low	70	5.449 (0.033)	7.402 (0.001)	5.857–87.486
	high	108			
Vitamin E	low	117	0.532 (0.135)	0.051 (0.001)	0.009–0.284
	high	61			
Serum AST	low	83	1.971 (0.023)	0.017 (0.001)	0.002–0.179
	high	95			
Smoking status	yes	76	1.246 (0.432)	12.034 (0.004)	2.174–66.607
	No	102			
Body mass index	low	81	5.344 (0.067)	22.092 (0.018)	1.693–288.216
	high	97			
Weight	low	117	0.044 (0.007)	0.058 (0.018)	0.006–0.610
	high	61			
Height	low	54	7.445 (0.008)	42.894 (0.004)	3.227–570.152
	high	124			
Supraspinale skin fold	low	97	0.612 (0.061)	0.116 (0.044)	0.014–0.943
	high	81			
Lean mass index	low	77	2.603 (0.002)	12.898 (0.015)	1.659–100.261
	high	101			

Backwards-stepwise logistic regression analyses with anaemia (dependent variable). Odds ratios (OR) have been adjusted for other variables in the model. P<0.05 denoted statistical significance. AST – aspartate transaminase

**Table 6.5: Significant predictors of anaemia in the sero-negative group - logistic regression modelling**

Variable		No of persons in adjusted model	Crude OR (p value)	Adjusted OR (p value)	95% CI of aOR
Settlement type	Urban	695	2.086 (<0.001)	1.852 (0.002)	1.249–2.747
	Rural	492			
Serum total proteins	low	581	0.791 (0.017)	0.585 (0.007)	0.396–0.865
	high	606			
Serum vitamin E	low	571	0.624 (0.034)	0.670 (0.044)	0.453–0.990
	high	616			
Serum GGT	low	561	0.770 (0.061)	0.667 (0.036)	0.456–0.974
	high	626			
Abdominal skin fold	low	587	1.768 (<0.001)	1.771 (0.003)	1.212–2.589
	high	600			

Backwards-stepwise logistic regression analyses with anaemia (dependent variable). Odds ratios (OR) have been adjusted for other variables in the model. P<0.05 denoted statistical significance. GGT – gamma glutamyl transaminase

A comparative binary regression analysis was also used to identify predictors of anaemia between sero-positive and sero-negative anaemics using all variables as before. (Tab. 6.6). Out of 828 cases, 612 (73.9%) were included in the model whilst the rest were excluded for missing values. The significant predictive variables, from table 6.6 below, when the two anaemic groups were taken into consideration were; Age

grouping ≤ 40 years together with high serum total proteins, serum 'globulins', and iliac crest skin fold as well as low serum albumin, plasma fibrinogen and supraspinale skin fold.

**Table 6.6: Significant predictors of anaemia comparing seropositive and seronegative anaemics using logistic regression modelling**

Variable		No of persons in adjusted model	Crude OR (p value)	Adjusted OR (p value)	95% CI of aOR
Age grouping	≤40 yrs	438	0.434 (0.002)	0.362 (0.039)	0.138–0.950
	>40 yrs	174			
Serum total proteins	low	313	5.665 (<0.001)	7.203 (0.002)	2.058–25.159
	high	219			
Serum albumin	low	437	0.342 (0.006)	0.217 (0.005)	0.075–0.625
	high	175			
Serum globulins	low	210	3.775 (0.007)	4.942 (0.022)	1.253–19.488
	high	332			
Plasma fibrinogen	low	447	0.410 (0.045)	0.294 (0.024)	0.102–0.853
	high	165			
Iliac crest skin fold	low	217	3.987 (0.003)	4.482 (0.019)	1.274–15.775
	high	315			
Supraspinale skin fold	low	449	0.355 (0.146)	0.177 (0.007)	0.050–0.630
	high	163			

Backwards-stepwise logistic regression analyses with anaemia (dependent variable). Odds ratios have been adjusted for other variables in the model. P<0.05 denoted statistical significance.

In the next section, significant predictors of anaemia within the strata of the significant socio-demographic variables would be explored.

### 6.3.2 PREDICTORS OF ANAEMIA WITHIN SIGNIFICANT SOCIO-DEMOGRAPHIC STRATA

To further identify interacting variables that could predict anaemia within each strata of the significant socio-demographic predictors in the study population, a stratified forced entry logistic regression analysis was undertaken. The forced entry strategy was adopted because the backward LR model yielded smaller sample sizes at the end of the stratified adjustment, which would make the statistical analysis highly underpowered.

Table 6.7 below represents the summary for the forced entry binary logistic model for the stratified significant socio-demographic variables of sex, settlement type, age grouping, smoking status and alcohol consumption status..

Many important and significant predictors of anaemia are common within each sero-status group as the analyses has shown. In view of this, the variables for predicting anaemia within the socio-demographic strata were considered for the entire study population without considering HIV status. For instance in males, high energy intake,

abdominal skinfold and serum ferritin as well as low serum vitamin E, plasma fibrinogen, serum GGT, and direct bilirubin were significant in predicting anaemia, whilst for females, urban dwelling type, non smoking, high TIBC together with a low serum albumin constituted significant predictors of anaemia.

Within the settlement type strata, there were no significant predictors for rural dwellers. However, for their urban counterparts, anaemia was predicted by high serum iron and TIBC as well as low serum albumin, serum vitamin E, plasma fibrinogen and direct bilirubin.

The age grouping strata also showed urban settlement type, non-smoking, high serum ferritin, TIBC and abdominal skin fold together with low serum vitamin E and direct bilirubin as major predictors of anaemia in persons who were 40 years and below. For those who were above 40 years the significant predictors were urban settlement type, high stature as well as low serum total proteins, plasma fibrinogen and serum GGT.

For smokers significant predicting variables included high stature, abdominal skin fold and LMI as well as low serum vitamin E plasma fibrinogen and direct bilirubin whilst for non-smokers they were urban settlement type together with low serum albumin and serum iron.

With regards to alcohol consumers there were a large number of predictive variables for anaemia. These included; high energy intake, TIBC, % transferrin saturation, serum ferritin and abdominal skin fold as well as low serum vitamin E, plasma fibrinogen, serum GGT, serum iron and direct bilirubin. On the other hand non-consumers of alcohol had significant predictors of anaemia which included, urban settlement type, high mass or weight and a low serum GGT.

Within the various significant socio-demographic strata, anaemia was most likely to be predicted by urban settlement type, low serum vitamin E, low plasma fibrinogen and low direct bilirubin in 5; low serum GGT, high TIBC and high abdominal skin fold in 4; low serum albumin, high serum ferritin, serum iron (both high and low) and smoking status (both smoker and non-smokers) in 3. The rest of the factors predicted anaemia in either 2 strata or 1 stratum.

Table 6.7: Predictors of anaemia in significant socio-demographic strata (aOR with probabilities in parentheses)

Significant predictors	Socio-demographic strata									
	Sex		Settlement type		Age grouping		Smoking status		Alcohol consumption status	
	male	female	rural	Urban	40 years and below	Older than 40 years	smokers	Non - smokers	Consumers	Non consumers
Urbanisation	-	2.005 (0.015)	-	-	2.210 (0.003)	2.008 (0.015)	-	2.434 (0.002)	-	2.026 (0.023)
Serum vitamin E	0.580 (0.026)	-	-	0.565 (0.011)	0.474 (0.003)	-	0.439 (0.008)	-	0.451 (0.004)	-
Plasma fibrinogen	0.436 (0.004)	-	-	0.585 (0.020)	-	0.405 (0.003)	0.375 (0.006)	-	0.523 (0.028)	-
Direct bilirubin	0.475 (0.002)	-	-	0.546 (0.024)	0.554 (0.002)	-	0.538 (0.042)	-	0.378 (0.033)	-
Serum Gamma glutamyl transaminase	0.463 (0.006)	-	-	-	-	0.482 (0.004)	-	-	0.396 (0.005)	0.560 (0.032)
Abdominal skinfold	2.532 (0.001)	-	-	-	2.106 (0.039)	-	2.120 (0.026)	-	3.383 (0.050)	-
Serum ferritin	1.905 (0.025)	-	-	-	2.330 (0.015)	-	-	-	2.645 (0.018)	-
Serum albumin	-	0.444 (0.006)	-	0.546 (0.025)	-	-	-	0.488 (0.016)	-	-
Total iron binding capacity	-	1.915 (0.023)	-	2.039 (0.003)	2.136 (0.004)	-	-	-	1.764 (0.001)	-
Smoking status	-	3.737 (0.011)	-	0.533 (0.008)	1.840 (0.002)	-	-	-	-	-
Serum iron	-	-	-	1.714 (0.011)	-	-	-	0.571 (0.001)	0.293 (0.005)	-
Energy intake	2.472 (0.012)	-	-	-	-	-	-	-	2.393 (0.029)	-
Height	-	-	-	-	-	1.869 (0.022)	2.374 (0.024)	-	-	-
Serum total proteins	-	-	-	-	-	0.410 (0.032)	-	-	-	-
Lean mass index	-	-	-	-	-	-	2.306 (0.041)	-	-	-
% saturation	-	-	-	-	-	-	-	-	4.098 (0.001)	-
Weight	-	-	-	-	-	-	-	-	-	1.845 (0.006)

Forced entry binary logistic regression analyses with anaemia as dependent variable. Odds ratios have been adjusted for other variables in the model. P<0.05 denoted statistical significance.

### **Summary of section 6.3**

The results in the above section have shown that:

1. Within the study population urban settlement type, high TIBC and abdominal skin folds as well as low serum albumin, vitamin E, serum GGT, plasma fibrinogen and direct bilirubin were significant predictors of anaemia. In the sero-positive group, female sex, non-smoking, high serum 'globulins', serum AST, BMI, stature and LMI as well as low serum albumin, vitamin E, weight and supraspinale skin fold all predicted anaemia. In the sero-negative population, urban settlement type, high abdominal skin fold as well as low serum total proteins, serum vitamin E and serum GGT were the significant predictors. However, between the HIV infected and their uninfected anaemic peers, significant predictors were; age grouping  $\leq 40$  years together with high serum total proteins, serum 'globulins', and iliac crest skin fold as well as low serum albumin, plasma fibrinogen and supraspinale skin fold.
2. Within the male socio-demographic strata, high energy intake, abdominal skinfold and serum ferritin as well as low serum vitamin E, plasma fibrinogen, serum GGT, and direct bilirubin were significant in predicting anaemia, whilst for females, urban dwelling type, non smoking, high TIBC together with a low serum albumin constituted significant predictors of anaemia. In the rural settlers there were no significant predictors. However, for their urban counterparts, anaemia was predicted by high serum iron and TIBC as well as low serum albumin, serum vitamin E, plasma fibrinogen and direct bilirubin. When age grouping was considered, urban settlement type, non-smoking, high serum ferritin, TIBC and abdominal skin fold together with low serum vitamin E and direct bilirubin predicted anaemia, whilst for those who were aged above 40 years, significant predictors were urban settlement type, high stature as well as low serum total proteins, plasma fibrinogen and serum GGT. For smokers, significant predicting variables included high stature, abdominal skin fold and LMI as well as low serum vitamin E plasma fibrinogen and direct bilirubin whilst for non-smokers they were urban settlement type together with low serum albumin and serum iron. In alcohol consumers predictive variables included; high energy intake, TIBC, % transferrin saturation serum ferritin and abdominal skin fold as well as low serum vitamin E, plasma fibrinogen, serum GGT, serum iron and direct bilirubin. For non-consumers of alcohol significant predictors of anaemia included, urban settlement type, high weight and a low serum GGT.

The results in this section demonstrate the complexity of factors that may be responsible for anaemia within the study population and especially for persons infected with HIV. Whilst some of the factors are not uniquely ascribable to HIV infection there

are, however, those that can be related to an increase in metabolic and/or inflammatory response, which may then be given consideration as more likely to interactively predict anaemia in the HIV infected population in this study.

#### **6.4 KEY FINDINGS - CHAPTER SIX**

In this results chapter the key findings were as follows:

1. High weight, abdominal and calf skin folds as well as high thiamin, riboflavin and folate intakes are significant determinants of anaemia in the sero-positive population. In addition low serum vitamins A and E may be significant determinants of anaemia in the sero-positive and to some extent in the sero-negative population. Similarly, serum low iron, TIBC and % transferrin saturation are positive determinants of anaemia in the sero-positive population, whilst high serum ferritin is a weak and high total proteins and low albumin are rather strong determinants in the sero-positives. Low albumin is also a significant determinant in the sero-negatives. Low total and direct bilirubin were also considered significant determinants of anaemia in the sero-positives, much like serum ALT, whilst serum ALP was a determinant of anaemia in the sero-negatives.
2. Within the study population, some variables commonly predicted anaemia, whilst a few were restricted to particular groups. Among the significant but more common predictors were urban settlement type, high serum total proteins, high abdominal skin fold as well as low albumin and low vitamin E. Between sero-positives and sero-negatives, age grouping  $\leq 40$  years together with high serum total proteins, serum 'globulins', and iliac crest skin fold as well as low serum albumin, plasma fibrinogen and supraspinale skin fold were more likely to predict anaemia. Within significant socio-demographic anaemia was most likely to be predicted by urban settlement type, low serum vitamin E, low plasma fibrinogen and low direct bilirubin.

In sum, the results presented in this chapter demonstrate that determinants of anaemia may be common between sero-positives and sero-negatives as well as between different socio-demographic strata within the study population. However, the unique predictive strengths of other variables in particular groups may suggest other unrelated factors that have potential to add to or significantly interplay in determining anaemia in the study population.

#### **6.5 DISCUSSION OF KEY CHAPTER SIX FINDINGS**

In this chapter, the significant factors that may determine the inter-relationships between HIV infection and anaemia and significantly predict anaemia in the study population have been explored using various multivariable logistic regression models.

The models used in the various analyses may closely demonstrate interplay between various factors including HIV infection as well as other potentially anaemia-eliciting factors, including those that may not be associated with the infection, but may pose a significant risk to the development of anaemia in infected persons.

The previous chapters highlighted various factors as likely to explain anaemia in the study population. From the analyses in this chapter it can be observed that the commonalities of factors between the sero-status groups and the various socio-demographic strata, in causing or contributing to the cause of anaemia are vast.

Although HIV infection can be linked to anaemia (Sullivan et al., 1998; Volberding, 2000; Moyle, 2002), in this study in particular, there are many factors that seem to suggest that this linkage is quite weak. Apart from the asymptomatic character proffered on the sero-positive group, it is also very likely that many other factors unrelated to HIV infection but prevalent within the population could significantly contribute to the cause of anaemia.

Even though it remains speculative at this point, there are a number of factors other than HIV infection peculiar to sub-Saharan Africa and other resource-poor settings that may tend to predispose to or exacerbate anaemia (McLean et al., 2007). Many of these endemic conditions, such as poverty and malnutrition, have been known to heighten the burden of HIV/AIDS (UNAIDS, 2006; 2008).

It may be argued that, even at an apparently asymptomatic population, inflammatory responses to HIV infection and perhaps to other stressors can be crucial in determining the balance between RBC synthesis and destruction or loss. Such inflammatory tendencies may be identified in the significant relationships between increased total serum proteins, high serum ferritin and low serum albumin and [Hb] and the strengths of their prediction of anaemia in various study population groups.

One may also allude to the overwhelming predictiveness of urban settlement as more likely to buttress the inflammatory tendencies. The rapidity at which urbanisation is occurring in the study region coupled with the transitional changes that accompany it, may create conditions that could be associated with an increase in inflammatory stress. The creation of urban slums is likely to breed poverty and malnutrition. Insanitary conditions and infectious disease may also be rife and looming and may, perhaps, be aggravated by other conditions such as limited access to health care that together would heighten the vulnerability of persons to both HIV/AIDS and anaemia.

The emergence of low serum albumin, low serum vitamin E levels and perhaps low TIBC as significant determinants of anaemia in the study population and especially in the sero-positives, may be reflecting an imbalance in nutrients similar to what happens during the inflammatory process. In this study such tendencies in sero-positives, could have been caused by the presence of the HIV and perhaps also primed by the presence of other inflammatory stressors. The fact that this may be happening in an asymptomatic HIV seropositive population who are most likely to be at the early stages of the infection shows that either inflammatory stress at this stage is high or an apparently mild HIV effect potentiates the effects of other inflammatory stressors.

The predictive significance of low vitamin E and TIBC may also reflect an enhanced inflammatory response or oxidative stress. It has been shown that immunological changes associated with HIV and other infections may tend to decrease micronutrient levels as their transport protein levels are depressed (Tomkins, 2003). However, the significant effects of HIV on haematopoiesis and RBC metabolism could as well increase the utilisation and metabolic loss of these micronutrients, as confirmed by significant associations between HIV infection and micronutrient deficiencies reported in some studies (Tang et al., 1997a; 1997b; Tang and Smit, 1998; Tang et al., 2005; Friis, 2005). Irrespective of cause, a tendency for vitamin E deficiency (Traber and Kamal-Eldin, 2007) can be associated with anaemia as it may be associated with oxidative stress.

Significantly, urbanised settlement type and low vitamin E could be considered as more likely to predict anaemia in the study population, whilst females, low serum albumin, and high serum total proteins reflect the HIV sero-positive predictors. The implications for these predictive factors could be very relevant in public health terms, considering that both HIV and anaemia could have serious public health consequences in this population. Though, generally accepted as very vulnerable to HIV infection, females are highly likely to be anaemic because of socio-economic and other biological reasons. With both HIV and anaemia presenting as serious threats to females, it would be prudent to give a serious attention to this gender group as far as policies in addressing issues surrounding these threats are concerned. It is also a recognised fact that urbanisation comes with some socio-demographic and nutritional changes that more often than not are inimical to the health and survival of the most vulnerable. Vulnerability to HIV and anaemia can be heightened by poverty and inadequate and inappropriate education, which may commonly be associated with transitional (squatter camps) or residence in urban slums.

Low vitamin E as a significant predictor of anaemia may simply be highlighting the general importance of the pervasive inflammatory tendencies, which is likely to precipitate oxidative stress both within the infected and uninfected populations. Although oxidative stress has not been actually determined in this study, higher tendencies and predictive value of low vitamin E levels with anaemia within the study population may be considered as significantly reflecting oxidative stress levels within the population.

It may be inferred from the findings that anaemia in this asymptomatic HIV sero-positive population is more likely be associated with alterations in serum protein levels that may signify inflammatory influence, and most likely determined by the influence of an ever present virus interacting with other potential stressors.

## CHAPTER SEVEN

### 7.0 GENERAL DISCUSSION

The results from various analyses employed to address the hypothesis of this study has been outlined and discussed in the preceding three chapters. The first part elucidates the baseline socio-demographic characteristics that could serve as explanatory factors in the relationship between HIV infection and anaemia, which is followed by the examination of various factors as possible explanatory factors for anaemia in the study population. Lastly, factors that may interplay with HIV infection and could significantly predict anaemia in the study population have been explored.

In this general discussion chapter findings that are considered as key in this study, which may be pertinent in addressing the main hypothesis will be discussed in the light of the evidence from literature relating HIV/AIDS to anaemia. From this, relevant conclusions drawn would be outlined. The limitations of the study will also be highlighted, whilst implications of the findings for public health will be derived and suggestions or recommendations made for future work in this study area.

### 7.1 KEY STUDY FINDINGS

The key findings in this study are summarised as follows:

- Prevalence of HIV infection in the study population was 11.8%.
- HIV sero-positive persons were significantly more likely to be  $\leq 40$  years, i.e. younger: sero-positive females were more likely to be younger than males and, anaemics, irrespective of HIV sero-status, were also more likely to be younger.
- HIV sero-positive persons, irrespective of gender and anaemia status, were significantly more likely to be urbanised.
- Over half of the study population, irrespective of HIV sero-status, gender and anaemia status, were more likely to have lower educational attainment, earn lower monthly income and speak Tswana as first language.
- Females, irrespective of sero-status, were significantly more likely to be lower income earners and more likely to be anaemic.
- Males were significantly more likely to smoke or indulge in alcohol consumption. Anaemics generally were significantly less likely to smoke, whilst seropositive anaemics in particular were less likely to consume alcohol compared to their non-anaemic counterparts.
- HIV sero-positives had lower [Hb] and Hct levels compared to their sero-negative peers. The effects of both HIV infection and anaemia on Hct were very significant, but the interactive effect was not.
- Prevalence of anaemia was generally high and most of it could be categorised as

mild, but sero-positive prevalences were higher and significant with Hct defining anaemia, than those of sero-negatives. HIV sero-positives were more likely to be mildly and moderately anaemic and significantly more likely to be severely anaemic. HIV sero-positive urban settlers, non-smokers as well as non-consumers of alcohol were more likely to be anaemic.

- HIV sero-positive persons were more likely to be hypoferaemic (i.e. with lower serum Fe) or relatively hypoferritinnaemic (i.e. with lower serum ferritin levels) compared to their sero-negative peers. Sero-positives were also more likely to be classified with IDA and ACI, though ACI was more likely to be common than IDA in the study population.
- HIV infection, much like anaemia, may be associated with changes in body fat measures in mid body parts, independently and interactively especially on abdominal skin fold fat.
- Energy and micronutrient intakes were marginally higher in sero-positives and anaemic sero-positives than their peers. Over 50% of the study population could be classified as having poor energy intakes. Intakes were generally marginal compared to DRI. HIV sero-positives and anaemics were more likely to have lower serum levels of iron and vitamin A, whilst vitamin E levels were only lower in anaemics.
- HIV sero-positives had significantly higher serum levels of total proteins and 'globulins' as well as a lower serum levels of albumin. Levels of Direct bilirubin, ALT and AST levels were higher in sero-positives, but the effects of HIV infection was significant on ALT and AST levels.
- Anaemics, irrespective of sero-status, were also significantly more likely to have lower total proteins and albumin as well as lower bilirubin (Total and Direct) and liver enzymes (serum ALP, GGT, ALT and AST). However, effects of anaemia only on bilirubin (Total and Direct) as well as ALT and AST levels were highly significant.

## **7.2 DISCUSSION AND GENERAL IMPLICATIONS OF KEY FINDINGS**

In most regions of the world, especially in places that have been hardly hit by HIV/AIDS, efforts to stem the tide of the pandemic has met with a lot of difficulties (UNAIDS, 2006; 2008). Similarly, in places where anaemia is a serious public health problem, prevalences have hardly declined despite the numerous programmes aimed at reducing it. Because of the way they influence each other, there is a need to address these two global problems with a multidimensional approach as their causation and effects may not only be related but also interactive. Both HIV/AIDS and anaemia have important nutritional implications for victims and the population at large, which makes it imperative to place nutrition at the core of addressing them.

HIV/AIDS is inextricably linked with the nutrition of the victim, and problems associated with nutrient losses and increased nutrient utilisation mostly emanate from an increased metabolism and/or inflammation, inadequate food intake and oxidative stress (Babamento and Kotler, 1997; Bogden et al., 2000) due to the infection. The interaction between HIV/AIDS and nutrition is of particular significance, especially in many sub-Saharan African countries where malnutrition and infectious diseases may occur simultaneously, sequentially or repeatedly in infected persons, and may contribute towards the acceleration of HIV disease (Semba and Tang, 1999; Anabwani and Navario, 2005). The cycling between malnutrition and endemic infectious diseases in most of such regions, which are often resource-limited, may also be reinforced by population characteristics in the development of anaemia in the population (Ssali et al, 2006).

In asymptomatic HIV infection, the effects of the virus on the nutritional status and metabolic alterations in victims may be mild. Nevertheless, the occurrence of anaemia in asymptomatic or symptomless HIV infected populations is just not mythical, but levels are more often higher than in the uninfected populations (Spivak et al., 1984; Costello, 1988; Hoxie, 1995; van Den Broek et al., 1998). In the infected population, prevalence of anaemia remains much higher in sub-Saharan African countries than in developed countries (Belperio and Rhew, 2004), and levels within asymptomatic populations follow a similar trend (van Den Broek et al., 1998; Ayisi et al., 2000; Mlisana et al., 2008). The high anaemia prevalences in this apparently asymptomatic population, which tends to be significantly higher than that of sero-negatives using Hct definitions may be consistent with the findings of the earlier studies.

In this study, prevalence of anaemia in both infected and uninfected populations of around 50% may have serious public health implications, however, it is perhaps the tendency for anaemia and its exacerbated forms to be associated with HIV infection which should draw attention to the contribution HIV infection could be making to anaemia in an environment where anaemia may already be a burden. In fact, the significantly higher anaemia prevalence between sero-positives and sero-negatives, when Hct definitions are considered coupled with the significant association of severe anaemia with HIV infection, albeit numbers were low, may suggest that, even in asymptomatic infection, HIV can exert a significant impact on the numbers of RBC. However, whether this impact of HIV is due to its direct influence or to its associated eliciting effects or to an interplay between these direct and indirect effects cannot be clarified because of the limitations of the data.

Whilst the comparative differences in levels of asymptomatic HIV related anaemia between developed countries and developing countries may suggest different mixes or potencies of aetiological factors, it also highlights the fact that the impact of the infection might be greater in developing populations. In this study, an equally high prevalence of mild and moderate anaemia is demonstrated in the sero-negative as in the sero-positive population, which indicates that factors other than HIV are also at play in inducing anaemia within the study population. It is therefore possible that these other factors may add to or interact with the effects of HIV to contribute to its greater impact on anaemia in the sero-positive population. Though the elucidation of these other factors are constrained by the limitations of the study data, the unique importance of HIV in either directly or indirectly enhancing the predisposition of infected persons to anaemia may be conjectured from a number of factors inferring from certain population characteristics in the study.

Among the many socio-demographic characteristics that have been demonstrated to be associated with anaemia in populations including PLWHA are sex (female), age (being older), race (black), use of ARV and antiviral myelosuppressive medication (e.g. AZT), CD4+ cell count (<200 cells/ $\mu$ l), viral load (increased), intravenous drugs (users) and many others (Sullivan et al., 1998; Volberding et al., 2004). Those factors that closely relate with HIV disease state or severity such as CD4+ cell count and viral load may constitute viral effects or HIV-related effects which could mark more precisely the direct involvement of the virus in causing anaemia in an infected population. On the other hand, the socio-demographic determinants may be regarded as HIV-unrelated and therefore more generally considered as explanatory factors of anaemia for the entire study population, unless they differ significantly between the two population groups. However, any significant link or association with HIV infection would enable socio-demographic and other HIV-unrelated determinants to be constituted as significant explanatory factors for differences in levels and characteristics of anaemia between infected and uninfected populations. Unfortunately, HIV-related characteristics like CD4+ cell count and viral load were not available for consideration as significant determinants of anaemia in the infected population because they were not measured. With regards to the HIV-unrelated factors, the socio-demographic characteristics of sex (female) and race colour (black), which may be associated with anaemia, were not significantly different between HIV ser-positive and sero-negative groups, and therefore may only be used in explaining part of the anaemia in both study population groups.

As significant predictors of anaemia in the entire study population, female sex and

urbanised residence type together with the predominant black racial characteristic may constitute important explanatory variables for the high levels of anaemia in the entire population. In a population where female proportions tend to be higher than that of males this population characteristic may assume some importance in partly explaining the high prevalence of anaemia within the population, especially, when it constitutes a significant determinant of anaemia (Sullivan et al., 1998; Volberding et al., 2004). Whilst the female characteristic is associated with anaemia because of biological predispositions, the black race has been associated with anaemia for other reasons that may be considered as either biological or social.

Associating anaemia with females may be largely attributed to menstrual blood loss and to the drains on iron stores that occur with pregnancy and delivery (Volberding et al., 2004). On the other hand, the black racial group, who predominantly reside in or have their ancestral roots in sub-Saharan Africa, have been associated with certain population specific characteristics, which may have survival significance, but may also be significantly predisposing to anaemia (Ssali et al., 2006). In populations where HIV infection is high these population specific characteristics may serve as important priming or potentiating factors for anaemia. These characteristics include endemic and rare genetic disorders like sickle cell disease, thalassaemias and G-6-P-D, (Sullivan et al., 1998; Volberding et al., 2004), which by themselves may predispose to anaemia. Although these characteristics were not assessed in the study population, a higher tendency for haemolytic anaemia, may be an indication of the presence of such characteristics.

As part of the characteristics of black populations that may explain anaemia in the population, dietary considerations associated with certain nutritional and/or cultural practices, which tend to limit the availability of iron for RBC production, have been suggested (Semba, 2003). The effects of such a population specific characteristic can often be compounded by the prevalence of environmental conditions that are inimical to the maintenance of good nutrition and health, which may further enhance the predisposition of individuals to anaemia. For instance, a poor socio-economic dispensation often associated with rapid urbanisation and changes in demography, could essentially depict levels of poverty and therefore a basis for the dietary alterations that are likely to lead to low RBC production. In this study such circumstances are reflected in the poor average income earnings and educational attainments in a large section of the study population. This tendency for poverty to prevail, which could most probably be ascribed to the rapid urbanisation process in the population (Vorster et al., 2000), may underlie the poor dietary energy and

micronutrient intakes observed in a large section of the population.

The potential for pervasive poverty to increase prevalence of HIV/AIDS in the region (UNAIDS, 2008) coupled with the presence of unrelenting infectious diseases (Vorster et al., 2000), may serve to explain the adverse changes in health and nutrition in the study population. The significant association of anaemia with urbanisation in the study population simply buttresses the role for these changing socio-demographic and associated factors in causing anaemia. This is against the background that residence in urban transition or slum is more likely to be associated with a high prevalence of unsanitary conditions and other vices which could encourage the spread of diseases including HIV and expose individuals to anaemia and other co-morbidities (Calder and Jackson, 2000; Thurnham and Northrop-Clewes, 2007). Obviously as a strong predictor of both HIV infection and anaemia, the urbanised stratum would be most appropriate for targeting as a public health intervention strategy.

Subclinical levels of endemic infectious diseases or helminthic infestation may constitute a potential cause of anaemia in the study population although indicators of such conditions were not assessed in this study. In sub-Saharan Africa and many developing countries, including the study location, the presence of endemic malaria, helminthic and respiratory infections have been suggested to contribute to the predisposition of persons to anaemia (van Eijk et al., 2001; Muhangi et al., 2007). It is possibly for these reasons that countries and regions that account for the very high global prevalences of anaemia, all fall within the zones where such infectious diseases and other causes of inflammatory stress abound (McLean et al., 2007).

Endemic infectious diseases, including HIV infection, may have specific ways by which they influence the development of anaemia, but nevertheless, they all generally tend to activate an inflammatory response which, more often than not, deprives victims of nutrients such as iron, whilst creating conditions within the body that may impair RBC production or increase its destruction (Jackson, 2007; Northrop-Clewes, 2008).

However, since the APR is a broad based response to many inflammatory stressors and not necessarily restricted to infections (Thurnham and Northrop-Clewes, 2007), it means that inflammatory influences in the study population may still be present without overt infection. Such sub-clinical inflammatory effects cannot be overlooked in explaining the anaemia in the study population, and especially in the uninfected group who were considered to be 'apparently healthy'. In fact, pro-inflammatory cytokines released during inflammation could significantly contribute to the initiation and the

continued presence of anaemia (Thurnham and Northrop-Clewes, 2007), more so in populations where exposure to inflammatory stress is especially high. Associated with the inflammatory response is a rapid fall in plasma concentrations of several nutrients (though a few may increase in concentration), which occurs irrespective of nutritional status (Thurnham and Northrop-Clewes, 2007). Additionally, the inflammatory response is also characterised by sequestration of iron and zinc and a reordering of the metabolic processes in the liver resulting in an increased formation of positive APPs at the expense of negative APPs (Jackson, 2007). During the inflammatory process serum ferritin levels tend to rise to levels that parallel that of CRP (Feeelders et al., 1998), an APP which has often been used to mark acute inflammation.

The evidence for the presence of inflammatory stressors in the study population, apart from HIV, may lie in the tendencies for hypoferaemia and, especially, hyperferritinaemia in both infected and uninfected populations. This may be buttressed by the attribution of most anaemia in the two populations to ACI, which also tends to have hypoferaemia and hyperferritinaemia as significant features (Thurnham and Northrop-Clewes, 2007). In addition, both populations have shown characteristics associated with hepatic metabolic protein and nutrient alterations that often accompany the inflammatory response. However, these inflammatory effects may seem to have been more pronounced in the sero-positives. Since such tendencies strongly suggest inflammatory influence, it may follow that the hypoferaemia and perhaps the lower serum micronutrients characterised in the study population are not necessarily genuine dietary iron or nutrient deficiencies, but most likely may represent iron redistribution or nutrient conservation tendencies associated with the inflammatory process (Tomkins, 2003; Thurnham and Northrop-Clewes, 2007).

More recently, the hypoferaemia and hyperferritinaemia associated with inflammation have been attributed to the effects of hepcidin, a small hepatic-secreting polypeptide, produced through induction by the inflammatory cytokine IL-6 on hepatocytes (Nemeth et al., 2003; 2004; Nemeth and Ganz, 2006). Hepcidin regulates iron metabolism by inhibiting iron adsorption from the intestines, iron recycling by macrophages and iron mobilisation from hepatic stores (Thurnham and Northrop-Clewes, 2007). The essence of the nutrient and iron redistribution during inflammation have been thought to be protective; first against the effects of infection either by conserving nutrients or creating an environment that is not conducive for the survival of the invading pathogen (Thurnham and Northrop-Clewes, 2007), and secondly as anti-inflammatory, protecting against the potential pro-oxidant effects of iron and the exacerbation of tissue damage at the sites of inflammation (Thurnham, 1990; Thurnham, 1997). There is also some

evidence to show that hypoferaemia might promote macrophage cytotoxicity (Rouault and Klaussner, 1996; Domachowske, 1997), though in iron deficient individuals it may be associated with a reduction in lymphocyte proliferation and activation (Brock and Mulero, 2000).

In the short term, changes in the levels of biomarkers of nutritional status during the inflammatory process may be considered detrimental though probably minimal (Beisel, 1998), but as a way of conserving nutrients or keeping them away from invading pathogens, it may be especially beneficial (Thurnham and Northrop-Clewes, 2007). However, in the long term hypoferaemia during a persistent infection or poor nourishment can further reduce nutrient levels and worsen an existing nutrient deficiency or impair tissue function (Thurnham and Northrop-Clewes, 2007). These changes create conditions within the body that may increase the risk of anaemia by impairing RBC synthesis and/or shortening their lifespan (Jackson, 2007).

The overall risk of anaemia during the inflammatory process may, however, be dependent on the presence of a metabolic iron deficiency as determined by the ability of the individual to maintain iron stores through iron absorption, iron loss and the dietary intake of bioavailable iron (Thurnham and Northrop-Clewes, 2007). Maintaining normal iron stores can be very difficult for persons under chronic inflammatory stress as the inflammatory process, and specifically hepcidin, is known to restrict iron through its main regulatory route of absorption (Cox and Halsall, 2003). Although assessing hepcidin levels may have been very useful in showing the effects of inflammation as well as the occurrence of iron maldistribution, the observed tendencies for shifts in APPs and nutrients that are characteristic of the inflammatory process may be good indicators of inflammation in the study population.

Nevertheless, apart from the inhibitory actions of hepcidin on iron absorption, other limitations on absorption and utilisation of iron could have been imposed either by riboflavin deficiency stemming from a poor dietary intake of diary products (Powers and Thurnham, 1981), or consumption of large components of substances (like phytates) in the diet that bind to iron (Halberg, 1981; 1982; Halberg et al., 1997). Although these dietary limitations have not been established for the study population, a previous study that analysed the dietary intakes in this population (Vorster et al., 2004) suggests the likelihood that such poor dietary practices with combined effects of infection and inflammation may be mediating the anaemia in the study population.

In many developing countries and poorly-resourced settings such combined influences

from infection, inflammation and poor dietary practices are known to contribute to the high prevalence of anaemia (WHO, 2001). In addition, the anaemia emanating from such influences often tend to be mild, but nevertheless remains a major public health problem in these poor settings (Stoltzfus, 1997). The largely mild anaemia in this study population coupled with the preponderance of ACI may therefore be considered as being consistent with the suggestion that infection and inflammation and perhaps poor dietary practices are important in the aetiology of anaemia in the study population.

Though the effects of a mild but persistent anaemia can carry some adverse consequences (such as impairing cognitive capacity in children as well as increasing delivery of pre-term babies and reducing capacity to work in all persons) (Stoltzfus, 1997; Black et al., 2004), the health risks associated with such mild anaemia are much lower compared to moderate and severe forms (Thurnham and Northrop-Clewes, 2007). Considering that mild anaemia comes with a comparatively lower health risk and has other beneficial effects may presuppose that most anaemia in this 'apparently healthy' study population could be an advantageous strategy (Thurnham and Northrop-Clewes, 2007) or perhaps an adaptive, beneficial response to an underlying disease state, especially, in the HIV infected population (Zarychanski and Houston, 2008). If these assertions are acceptable then a high prevalence of mild anaemia as is apparent in the study population or as may occur in any population with predominant inflammatory causes may have implications for iron therapy. This may be particularly important considering that the approach to addressing anaemia in many such settings, which are often poorly-resourced, have centred mostly on supplementation with iron or iron therapy. This approach at solving the problem of anaemia and iron deficiency has often been undertaken due to the misconception of equating iron deficiency to anaemia (Thurnham and Northrop-Clewes, 2007).

Administering iron as therapy in circumstances where inflammatory tendencies exist has been known to increase vulnerability to infections (Prentice, 2008). Much of the evidence for this helped in shaping the WHO Guidelines for Treatment of Severely Malnourished Children, which recommended the withholding of iron therapy to malnourished children until wide-spectrum antibiotics have been used to control bacterial infections, especially in malaria endemic areas (Prentice, 2008). More recently the Pemba trial (Sazawal, 2006) gave impetus for further caution in iron supplementation programmes to prevent iron deficiency in infants and young children in malaria-endemic areas (WHO, 2007).

Though it has been argued that the detrimental effects of iron therapy were confined to

iron replete subjects (Stoltzfus, 2007), the need to weigh the balance of effects before any such interventions, as suggested by Prentice (2008), is very pertinent. In HIV and other viral infections iron may play a number of roles including its ability to increase the proliferation of the viral agent (Drakesmith and Prentice, 2008). There is also evidence to suggest that elevated ferritin levels or iron stores strongly predict disease severity and earlier mortality in HIV infected persons (McDermid et al., 2007; Rawat et al., 2008). Together with studies that may relate the occurrence of iron accumulation in the tissues of infected persons to an increase in viral replication (Boelaert et al., 1996; Weiss, 1999), the need for caution in administering iron as therapy for infected persons especially with anaemia becomes imperative.

Another important consideration in this interrelationship between anaemia, iron metabolism and HIV infection is vitamin A deficiency. Vitamin A deficiencies have been associated with both HIV infection (Fawzi, 2003; Friis, 2005) and anaemia (Fishman et al., 2000; Dreyfuss et al., 2000; Gamble et al., 2004). Vitamin A deficiency may increase the risk of iron deficiency and anaemia particularly because it alters the absorption, storage and release or transport of iron to the marrow (Semba and Bloem, 2002) by enhancing the inflammation-induced sequestration of iron as well as other responses to infections that tend to increase the risk to anaemia. Epidemiological surveys show that the prevalence of anemia is high in populations affected by vitamin A deficiency in developing countries whilst improvement of vitamin A status has generally been shown to reduce anaemia (Semba and Bloem, 2002; Thurnham and Northrop-Clewes, 2007). However, the influence of vitamin A deficiency on anaemia can be complicated by concurrent chronic infectious disease as they may alter both the distribution of vitamin A and iron independently or impact directly on the vitamin A-iron pathway. The significant tendencies for anaemic HIV infected persons in the study population to be vitamin A deficient may therefore be attributed to the influence of perhaps the virus as well as the pervasive inflammatory tendencies conferred by the virus and other inflammatory stressors within the population. Clearly, if adequate supplementation of vitamin A can ameliorate iron deficiency anaemia in deficient individuals, especially, residing in places where causes of inflammation abound (Kolsteren et al., 1999; Semba et al., 2001) then there is a possibility that HIV infected persons who are iron deficient may benefit from such interventions.

Regardless of the magnitude of factors that may be collectively responsible for anaemia in the study population, the significant differences in anaemia prevalence between the infected and uninfected populations as well as the tendencies for exacerbated anaemia, a lower [Hb] and a significantly lower Hct, significantly higher

serum total proteins and globulin levels and lower serum albumin levels in the infected population, may buttress the greater impact of HIV, and perhaps its potential interactive effects, on anaemia. Even though it has been suggested earlier that the inflammatory effects of HIV can induce anaemia, it is perhaps its ability to directly destroy stromal or erythrocyte precursor cells (Davis, and Zauli, 1995; Levine, 1999; Redd et al., 2007) and also suppress the bone marrow (Hambleton, 1996) that is crucial for haematopoiesis. These direct effects may be distinguished from the concurrent effects of medication, OI and nutritional deficiency.

The inflammatory effects of HIV infection, much like most other infections, are mediated by inflammatory cytokines (Means, 1995; 2000). However, HIV, by virtue of its persistence and ability to proliferate rapidly makes it a perpetual or chronic inflammatory disease. Even with potent ARV the virus remains undefeated being able to mutate and remain quiescent in latent reservoirs. Researchers are coming to a general consensus that the formidable impact of HIV on a large number of health outcomes, including anaemia, in the infected population is due to inflammation (Wohl, 2009). Whilst inflammation in HIV infection is not a cause of disease, it may result from the HIV and other stressors like those related to co-infections and/or therapy. It is perhaps the collective influence from these stressors that would determine the total burden of inflammation in an individual and which may enhance the inflammatory effects of a mild HIV infection. Such inflammatory effects in HIV infection have been significantly linked with a reduction in erythropoietin synthesis and response (Spivak et al., 1989) and deficiencies of several vitamins (Volberding et al., 2004), which have all been associated with anaemia.

The combined effects of all the factors that can be ascribed to HIV constitute a great arsenal for the virus in inducing anaemia in infected persons but, more often than not, have been regarded as more likely to manifest in advancing HIV disease or AIDS. Nevertheless, associating asymptomatic HIV infection with significant levels of anaemia such as in this study and others (Hoxie, 1995; van Den Broek et al., 1998; Ayisi et al., 2000), may indicate the significant ability of the virus on its own to predispose victims to anaemia, more especially, when the effects of HIV are considered independently of concurrent inflammation and medications (van Den Broek et al., 1998). Nevertheless, the significant effects of the HIV on anaemia in a population may only be assumed to be independent of inflammatory influences since the mere presence and tenacity of the virus, even in apparently healthy victims, is accompanied by mild, atypical (Jahoor et al., 1999) and persistent inflammatory responses. Even if undetectable at times the inflammatory influences from HIV may still be pervasive, which is why the infection is

designated as chronic. The results in this current study, which has similarities in terms of the outcome of anaemia with the study by van Den Broek and colleagues, depicts a possibility of inflammatory influence on anaemia in asymptomatic HIV infection.

In both studies CD4+ cell counts and other indicators of disease progression were not available. Furthermore, clinical examinations to exclude cases that might fulfil the definitions of AIDS, as well as the apparently healthy state conferred by the selection criteria allowed subjects to be categorised as asymptomatic. On the other hand there were differences in the subjects and markers of inflammation. Whilst pregnant women were the subjects and CRP was the only inflammatory marker used in the van Den Broek study, the current study excluded pregnant women and children and relied on several other APPs (serum total proteins, ferritin, globulins, albumin and plasma fibrinogen) as well as proxy markers (serum GGT, ALP, ALT and AST) as indicators of inflammation.

Measuring CRP may be important for marking acute inflammation as it tends to rise and fall sharply with inflammation (Northrop-Clewes, 2008). This should make its use alone as highly unsuitable for marking chronic inflammation. In any 'apparently healthy' HIV infected population, the measurement of a chronic but considerably mild inflammation (Jahoor et al., 1999) with CRP may not yield a significantly noticeable difference between infected and uninfected groups as the van Den Broek study has confirmed. The APR of HIV infection, especially in asymptomatic persons, would require a marker that can measure the sustained presence of the stimulus or even long after the stimulus is removed. Because of the extensive influence of inflammation on iron status, the use of ferritin with other APPs has been recommended (WHO, 2004). Increases in ferritin levels are not only induced by cytokines (Kobune et al., 1994), but the rise in ferritin levels tend to parallel that of CRP (Feeelders et al., 1998), confirming it as an APP. However, levels of ferritin and some other APPs fluctuate much slower and may remain active during the APR (Northrop-Clewes, 2008). Marking inflammation with serum ferritin and the other APPs in the present study has revealed that, even in an apparently healthy population, inflammatory tendencies, which may not be obvious, can still be noted. The shifts in the APPs used in this study, i.e. serum ferritin, plasma fibrinogen, serum globulins and albumins, were consistent with changes in plasma proteins associated with the APR.

However, serum ferritin acts as an indicator of iron stores and so the degree to its rise is influenced by the underlying iron status of the individual (Northrop-Clewes, 2008). What is notable is that inflammatory tendencies were depicted in the uninfected

population as well inferring from the higher ferritin and plasma fibrinogen levels, but nevertheless, the overall effects of inflammation on anaemia seem to have been accentuated in the infected population. Though anaemia in infected persons was associated with comparatively lower serum ferritin and plasma fibrinogen levels albumin levels were much lower. In addition, anaemic infected persons had similar risks levels for liver enzymes as their uninfected peers. Since this may suggest a lowered tendency for insidious damage to the liver, it may be consistent with the suggestion that the most type of anaemia in this population is protective and perhaps an adaptive strategy. In addition, the lower ferritin and fibrinogen levels and the significantly lower albumins may presuppose that the body of infected persons may place a premium on producing materials for defending itself against the infection to the detriment of synthesising transport proteins (Jahoor et al., 1999) and perhaps haemoglobin resulting in the higher predisposition to anaemia.

It is more appropriate to suggest at this point that the lower ferritin levels in the infected anaemics could be due to the restrictions placed on its synthesis as a result of the low serum iron levels, which may have resulted from either, the inflammatory blockage of absorption or dietary restrictions or both. The implication here is that in HIV infection anaemia is more likely to occur in persons who are iron deficient irrespective of the cause of the iron deficiency. This ties in well with the greater tendencies for iron deficiency, IDA and ACI as defined in the study and more likely to be associated with the infected population.

Whatever the mechanism that lowers serum albumin levels, hypo-albuminaemia may have significance in the development of anaemia as albumin may constitute a major protein reserve usually available for meeting hepatic syntheses as well as haem and RBC production to meet the rapidly changing demands of the body for oxygen carriage and energy synthesis (Jackson, 2007), especially when the body's metabolism is altered. Although a poor nutritional status could account for the reduced levels of serum albumin, much of this lowered effect could have been due to cytokine regulation or leakage into the interstitial space during the inflammatory process (Tomkins, 2003). The link between hypo-albuminaemia and anaemia in HIV infected persons has been shown in a study by Shah and colleagues (Shah et al., 2007), which also suggested that both albumin and haemoglobin could serve as important markers of HIV disease progression.

In this comparative study it is important to recognise that a number of the factors compared between the infected and uninfected population, including exposure

variables used to assess inflammation, serum micronutrients as well as iron status and some outcome variables that classified anaemia, were not significantly different statistically, and as revealed by post hoc power calculations (Appendix C) were highly underpowered. Important considerations that may have determined this under-powering of comparative statistics were the effect sizes and the sample sizes. Having been predetermined from the use of a secondary data source, there is a high probability that an adequately powered study could have provided clearer and more conclusive answers to the propositions in this study. However, even for those which presented with statistically significant differences, there is an absolute need to be cautious in their interpretation as statistical significance does not always connote biological significance.

On the other hand, there is some possibility that outcomes with biological significance may not necessarily result in statistical significance. A biological significance is important and becomes imperative when differences in biological parameters relate to physiological or functional differences, and in cases where statistical significance is not obtained, a biological significance must be explored and results interpreted accordingly.

For instance, differences observed in serum levels of albumin and globulins between infected anaemics and their uninfected counterparts were very significant ( $p<0.001$ ). However, whether the real differences obtained between the two sero-status groups by comparing the mean values of albumin (2.28g/l) and globulins (6.63g/l) have any biological significance is another issue worth considering. Even though the mean values of serum albumin in both groups were within the lower fringes of normal, lower levels are associated with fatal risks (Goldwasser and Feldman, 1997) and in HIV infected persons it marks changes in early infection and disease progression (Feldman et al., 2003). Graham and colleagues (2007) showed that every 1 g/l reduction in serum albumin was accompanied by a 13% increase in disease progression. Thus in this study, the differences in serum albumin levels between the two groups can be said to have both statistical and biological significance. In effect, the grand impact of HIV and its attendant effects on the victim as well as its potential to interplay with other anaemia-inducing effects in the environment may be considered most plausible in explaining differences in the prevalence of anaemia and its exacerbated forms between the two groups in the study population. The significant differences between the infected and uninfected populations in inflammatory markers, coupled with that of anaemia as measured by Hct and exacerbated forms, strongly suggest that HIV infection may induce inflammation even in asymptomatic infection that is perhaps

potent enough to influence the development of anaemia in the infected population.

### **7.3 LIMITATIONS OF THE STUDY**

The study has been presented with a number of limitations some of which can be related to the limitations inherent in the design of the original THUSA survey from which secondary data was obtained. These limitations generally have determined, to a very large extent, the interpretations to the outcomes in this study and calls for caution in relating these findings to the wider country and provincial populations as well as to the general HIV/AIDS population.

The design of the THUSA study, which was a cross sectional collation of data on dietary intake, nutritional status and other variables of health, socio-demographic and lifestyle characteristics in an African population (precluding the identification of HIV status), made it appropriate for characterising or observing the study population with respect to the different parameters measured. It would therefore allow for the description of the study population in terms of how certain exposures may relate to outcomes without any control over any exposure of interest (Margetts and Nelson, 1997). Thus, it may provide a snapshot of the prevalence of a condition in the study population. Such a design also allows the exploration of relationships between exposures and outcomes but does not allow the determination of causality between them (Margetts and Nelson, 1997). Thus, the relationships proposed in the present study may not necessarily indicate causality.

Since the outcome and exposures are dynamic phenomena, it would have also been more appropriate to use a cohort or longitudinal study to establish a causal pathway between the exposures and anaemia in the study population. Nevertheless the outcome results from this study could serve as a basis for further work in a specially designed longitudinal study to establish causality.

Another important limitation was imposed by the exclusion criteria, also inherent from the THUSA study design. Since the original survey was not intended to address the current objective, many potential HIV infected subjects were excluded. The exclusion of pregnant women and children as well as individuals who may have signs associated with HIV is a cause for this limitation. According to the UNAIDS (2006 and 2008), pregnant women and children, especially in regions where the pandemic is unrelenting, are the most vulnerable population groups with very high prevalences of HIV infection. Thus, any measure of HIV prevalence in the study population may not truly represent prevalence in the entire Province or country, which therefore limits any generalisations based on study outcomes to the entire population. By extension, the under-

representation of infected persons compared to their uninfected peers is the consequence of this limitation, which is probably the basis for statistical under-powering in some of the comparative analyses and especially in those where stratification of data by socio-demographic characteristics were done. This has been confirmed in post hoc power calculations.

The cross-sectional quasi-random design also meant that potential confounders or effect modifiers such as age and sex could have been a source of bias, especially as they were not equally distributed between the two groups being compared, which could lead to spurious interpretations concerning the findings. However, to address the problem of bias, adjustment controls have been made for these potential confounders in the analyses of data.

The use of a prevalent cohort of HIV-seropositive persons whose duration of infection was not known, further limited the extent to which the interactions between HIV and anaemia could be explained. The difference in study objectives precluded the inclusion of variables that could be described as HIV related, such as date of initial infection, CD4+ cell counts, viral load (or antigen titres), opportunistic infection and medications and drugs, which have been shown to be associated with anaemia in the HIV/AIDS population (Volberding et al., 2004). Thus, knowing the sero-status of subjects was not enough to indicate duration or degree of exposure to the virus for subsequent adjustments in analyses. This limitation hampered the categorisation or staging of infected subjects according to the CDC criteria, and in according risks to these categories as well as for making adjustments during analyses. The importance of this limitation becomes apparent when HIV is related directly to anaemia, specifically because HIV has a direct involvement in the causation of anaemia and more so because higher levels and severer anaemia are associated with advanced HIV disease.

Since data for this study was based on a secondary source, there was a limit to the availability and use of relevant data to address the current hypothesis. Apart from the absence of data to assess duration and intensity of the infection, most variables were adapted for answering the various propositions in this study. This meant that whilst some data were inadequate some others were considered more as proxies. For instance, since HIV infection is recognised as a chronic inflammatory disease, and inflammation may be associated with anaemia, data on inflammatory cytokine levels (e.g. TNF- $\alpha$ , IL-1, IL-6 or INF- $\gamma$ ) would have been most appropriate to mark inflammation. On the other hand, since the use of markers that closely parallel the

release of inflammatory cytokine (such as CRP or  $\alpha$ -anti-chymotrypsin – ACT or AGP) are accepted they could have been used. This notwithstanding important acute phase proteins (serum ferritin, plasma fibrinogen, serum globulins and serum albumin) as well as serum liver enzymes (GGT, ALP, ALT, AST and LDH), which were close proxies, were selected as marking inflammation and/or increased metabolism in the study population. Results from the use of these variables may not necessarily be conclusive. In addition, some data for depicting micronutrient deficiencies were inadequate (serum vitamin B<sub>6</sub> and B<sub>12</sub>), leading to under-powering and constraining of statistical interpretations.

In general, the effects of HIV on energy and nutrient intake is based on the argument that the infected population are asymptomatic, and because HIV related factors that are likely to affect food intake such as anorexia, and other gastrointestinal disturbances have not been accounted for, a poor dietary energy and micronutrient intake could not be associated with the infection. There is also likelihood that other factors that are beyond the scope of this study, but which may have influences on the relationship between HIV infection and anaemia (such as physical activity levels) could limit the interpretations of the findings in this study.

#### **7.4 IMPLICATIONS OF THE STUDY FOR PUBLIC HEALTH**

There is no doubt that HIV/AIDS is an important public health problem especially in most developing countries and resource-poor settings where it continues to advance. In most such places anaemia prevalences are often high and HIV infection may be an important aetiological factor. The factors that relate HIV infection to the problem of anaemia are many and may be considered as either HIV-related or HIV-unrelated. However, nutrition and inflammation may be at the heart of these two problems, which calls for paying attention to both nutrition and the causes of inflammation in addressing the problems.

The findings in this study add to the suggestion that HIV has a significant and crucial role in the development and exacerbation of anaemia, HIV/AIDS may add to the problem of anaemia, especially, in regions where its prevalence is on the rise, and other factors that may prime both problems abound. It also adds to the suggestion that the aetiology of anaemia in HIV infection, and even in asymptomatic infection, may be multifactorial and chronic inflammation might be an important contributor. This calls for an 'all hands on deck' approach to addressing the problem. Essentially, for any public health effort to be effective in controlling anaemia in HIV/AIDS or anaemia and HIV/AIDS, it must be geared toward an integrated focus and tailored to meet the

particular needs and opportunities within the country or region where the problem is (Kraemer and Zimmermann, 2007).

Many public health programmes have failed to address the problem of anaemia because they have not only failed to recognise its multifactorial cause among other reasons, and often programmes are designed with the assumption that iron deficiency is the sole cause of anaemia.

The importance of inflammation as an aetiological factor of anaemia in HIV infection, as well as in a population, which may not only be resource poor, but may have undefeated infectious diseases and infestations that provoke inflammation, has been highlighted in this study. In many instances administration of iron as supplements or treatment for anaemia, in individuals or populations with inflammatory tendencies, has often met with adverse effects or unresponsiveness. Inflammation may even be the major factor restricting haematinics to the bone marrow and reducing erythropoiesis. The strategy for addressing anaemia in this context would be to identify the inflammatory causes and addressing them as a primer or an adjunct to the main treatment. The control of endemic malarial, helminthic infestations and other infectious diseases, especially in vulnerable groups, must be part of the integrated approach as well as ensuring that poverty, unsanitary conditions and inadequate health care, all of which encourage the spread of disease, are tackled appropriately.

As part of the integrated approach, it is important to recognise individuals or populations that may be particularly vulnerable to poor nutrition. Despite this study showing a large proportion of the study population as most likely undernourished, HIV infected persons, because the disease places a higher burden on their nutritional requirements, can be considered to be more vulnerable. If there is any need to provide supplements in this group for anaemia, then ameliorating inflammation as has been demonstrated with vitamin A administration (Thurnham and Northrop-Clewes, 2007) must be considered. Otherwise, food based approaches that aim at dietary diversification and meeting requirements for RBC production must be targeted.

In most of the developing world where HIV and anaemia coexist as major problems standard treatment and care of patients for, especially, these two conditions are restricted to just a few due mainly to poor resources. Even where treatment is available, the two conditions are considered independently. This study has shown that there is a very high possibility that being infected with HIV would result in the victim becoming anaemic or if anaemia was present prior to the infection it was likely to

worsen with infection. Indeed, anaemia may even be the first sign of the infection, which means that once a person has been diagnosed as being HIV positive an assessment of anaemia must be required. In such poor settings, a simple assessment of anaemia could also be used to monitor the progress of the disease since anaemia is a useful marker of disease in infected persons.

Considering that current efforts are geared toward scaling up of ART and care in resource-poor settings, it becomes imperative that anaemia is included in the central issues in initiating therapy as well as in choosing which medications to administer. Clearly, anaemia being an independent risk factor for disease progression and survival is potentially a good indicator of rapid clinical deterioration in HIV infected persons, especially in resource-poor settings. In times when efforts to eradicate the virus through various therapies is not yielding, it would be a prudent idea to consider the prevalence of anaemia in patients awaiting HAART particularly with regards to the choice of medication as certain ARVs have been known to induce or exacerbate underlying anaemia.

Anaemia has remained a common and prevalent public health problem in South Africa among women and children especially (Eley et al., 2002). With HIV and other infectious diseases remaining undefeated in the population, anaemia is more likely to become a bigger problem, which would invariably add to the increasing health burden. If the increasing trend should continue, then both HIV and anaemia are likely to have a detrimental impact on the socio-economic development of the country. The approach to controlling these problems, as has been reiterated would lie in an integrated approach, which places inflammation and nutrition at the core of the solution.

Although there may be many aetiological factors responsible for low iron status in HIV infection, the possible deleterious effects of iron administration as part of the treatment of iron deficiency and anaemia should be given critical consideration. While the relationship between infection/inflammation and iron status remain contentious, the association of iron overload in HIV infection with increased susceptibility to the virus and other OI (Boelaert et al., 1996) should invoke caution in the liberal administration of iron therapy or prophylaxis in infected populations.

## **7.5 RECOMMENDATIONS FOR FUTURE WORK**

The nature of this study provides many opportunities for research to further elucidate or clarify some of the issues raised. Primarily, there is a need for a longitudinally designed research that would address the causality of asymptomatic HIV infection and

anaemia. This should look at the direct effects of the virus on erythropoiesis, the HIV-related effects as well as the HIV-unrelated effects, both of which may include inflammation and other co-infections.

Where resources and time are important constraints, and there is a need for a cross-sectional study, then the design should include the collection of data on more appropriate measures of exposures and outcomes. The need to measure, for instance, HIV disease intensity by CD4+ and viral load as well as inflammation by more specific inflammatory markers including cytokines and/or APPs as well as hepcidin must be included in order to establish causality.

It is important to also measure the environmental stressors and take into consideration other potential and competing causes of anaemia in the entire population. These may include OI and endemic infections (Malaria, helminthic infestations, etc) as well as genetic disorders (sickle cell disease, thalassaemias, G-6-PD, etc) in order to account for their contributions or to include them in the causal inter-relationship between HIV and anaemia. The particular influence on anaemia provided by the inflammatory effects of the virus must not be underestimated. Similarly, the inflammatory stresses provided by sub-clinical endemic infections like malaria and helminthic infestations must be taking into account.

The ultimate aim for identifying persons at high risk for anaemia in a population would be to help in charting appropriate treatment pathways or institute appropriate interventions especially for those who may belong to the vulnerable population. Therefore, any future work must take into consideration steps at identifying the different sub-groupings within the population that may be highly vulnerable to anaemia. This may be particularly important for public health intervention and for a cost-effective intervention approach.

## CHAPTER EIGHT

### 8.0 CONCLUSIONS

In this study the inter-relationships between HIV infection and anaemia has been explored. The hypothesis was that 'HIV infected persons are more anaemic compared to their uninfected counterparts'. In considering that HIV infected persons were significantly more likely to be associated with anaemia (indicated by Hct) as well as mild, moderate and severe forms of anaemia demands the acceptance of the hypothesis. This would make HIV infection in asymptomatic individuals a very significant determinant of anaemia, especially, in the study population.

The high levels or prevalence of anaemia in both study population groups presupposes that HIV alone is not responsible for anaemia in the population, and considering the observed inflammatory tendencies, inflammation generally can be said to be contributory to the public health significance of anaemia in the study population. In drawing the framework for this study a large number of intermediary factors were considered as likely to be part of the inter-relationship between HIV infection and anaemia. The direct effect of the virus, though not measured, was considered crucial in the linkage as were effects associated with inflammation. Indeed, the combine effects of inflammation perhaps emanating from different inflammatory stressors (including the HIV) and producing changes in body nutrients and proteins that may be described as subtle but effective in the long run may be considered as significant determinants of anaemia in the study population.

Tendencies for nutrient deficiencies, especially, in iron and vitamin A, which were more likely in the infected population, could be considered as due to the effects of inflammation since inflammation is known to cause a redistribution or alteration in the production of transport proteins for these micronutrients. This further buttresses the significant influence of inflammation in determining anaemia in the study population.

The effects of HIV in this asymptomatic population were not significantly expressed as changes in food intake and body composition, perhaps because the infection was at its early stages. Thus, factors that may lead to a reductive adaption such as anorexia and a reduction in lean body mass were not clearly distinguishable. These were, therefore, not considered to significantly contribute to or determine anaemia as the study has shown. In spite of the limitations of this study, the evidence points to the fact that HIV infection, even in asymptomatic individuals, serves as a significant risk for developing anaemia.

## APENDICES

### APENDIX A

#### ASSESSMENT OF ANAEMIA

Since iron deficiency is the most important and widespread micronutrient deficiency associated with nutritional anaemia, assessing iron status is very valuable and most crucial for identifying nutritional anaemia (Biesalski and Erhardt, 2007). Generally, however, most of the methods represent an assessment of the functional capacity of the blood.

Although some clinical indicators for iron deficiency, such as chronic fatigue as well as dietary assessments of intake (haem and non-haem) have been used to assess anaemia, the most relied upon are, however, laboratory or biochemical measurements.

By far, the most common of these measurements are Hb and Hct determinations from erythrocytes and whole blood. These measurements have helped screen anaemia and putative iron deficiencies as they reflect the largest iron compartment in the body (Biesalski and Erhardt, 2007). They can be quite easy and inexpensive to run and very essential, especially, in the diagnosis of nutritional anaemia. However, the measurement of Hb and/or Hct is not very sensitive or specific, especially, for detecting iron deficiency (Cook, 2005). Therefore to better evaluate iron status and identify most forms of anaemia, it has been agreed to measure Hb, ferritin and sTfR, complemented with indicators of acute or chronic infections, which has rendered the procedure quite difficult and comparatively expensive (Biesalski and Erhardt, 2007). Descriptions of some measurements used to indicate anaemia in the population are presented below.

#### **ASSESSMENT OF ANAEMIA BY WHO CRITERIA**

**Haemoglobin (Hb):** Haemoglobin is the iron containing pigment in the RBC that carries oxygen from the lungs to the cells of the body for metabolism. Hb is composed of two pairs of globin chains and four haem groups containing ferrous iron. It is commonly measured by automated spectrophotometry in which the red cell is lysed and the Hb concentration is determined by measuring the iron-containing pigment. Hb thus provides a direct measurement of the oxygen carrying capacity of the blood.

Haemoglobin concentration is a relatively easy and inexpensive measurement involving the dilution of only a small amount (about 20 $\mu$ l) of whole blood in Drabkins' reagent and measuring the absorption at 540 nm with a spectrophotometer. The amount of Hb in the RBC is measured in grams per decilitre (g/L) of blood.

When the concentration of Hb in the blood falls to a level where oxygen carriage is compromised anaemia is said to occur. In healthy persons the Hb levels may show wide variations, but generally, adult men tend to have higher values than women and children (Sacher and McPherson, 2000). Based on conventional cut-off values (WHO/UNICEF/UNU, 2001), Hb levels have been used to distinguish between anaemic and non-anaemic persons, as well as between stages of anaemia, i.e. mild, moderate and severe, in the population (see Tables A1 & A2 below).

There are a number of conditions that influence Hb concentration. For instance, sex, age and pregnancy as well as race (being of African descent may lower the Hb levels by about 0.5 to 1.0 g/dL); smoking also can increase it by about 0.3 to 0.7 g/dL whilst living in an altitude may increase it for up to about 2.0 g/dL (Gibson, 2005).

**Haematocrit (Hct):** Also known as the packed cell volume (PCV), Hct is the ratio of the volume of packed RBCs to the total blood volume. When whole blood is centrifuged or spun it separates into two main layers, the blood cells and plasma. Between the RBCs and plasma is a layer, the buffy coat layer. This constitutes approx. 1% and consists of white blood cells and platelets and is therefore not calculated as part of the PCV. Thus the RBCs make up around 99% of the cells and the rest are white cells and platelets. The haematocrit is reported as a percentage or a ratio. Hct can be determined either by automated blood-counters or by microhaematocrit (or spun) readings. In the case of automated complete blood count equipment, the Hct is a calculated percentage derived from the product of two red cell indices: the red blood cell count and the average size of red blood cells (also referred to as the mean corpuscular volume, or MCV). In healthy adult individuals the red blood cells constitute approx. 40-48%, i.e. less than half of the blood, whereas newborns may have haematocrits of up to 60% (Soldin et al., 1995).

In contrast to Hb, Hct provides an indirect measurement of the body's oxygen-carrying ability. Cut-off values have been determined for defining anaemia in a healthy population (WHO/UNICEF/UNU, 2001) (see Table A1 below).

Haemoglobin and Hct continue to be the hallmark laboratory parameters for monitoring and managing anaemia in most patients. Because of a seemingly close correlation between Hb concentration and Hct in normal conditions, these two have been used interchangeably and, roughly, the Hct has been estimated as three times the Hb concentration (Sacher and McPherson, 2000). Despite the seemingly close correlation between Hct and Hb concentration in measuring anaemia it remains even less

sensitive compared to Hb (Biesalski and Erhardt, 2007). However, different studies have shown that the two parameters are not comparable, but that they have their separate applications (Grainger et al., 1981; Young et al., 1986).

The tables below (Tab. A1 & A2) depict the WHO recommended cut-offs for measuring anaemia and graded form respectively for different population groups. The cut-offs are based on measurements in a healthy population at sea level, and ranges from 110 g/L or 33 in pregnant women and children below 5 years to 130 g/d or 39 in adult men, for [Hb] and Hct respectively.

**Table A1: Normally used haemoglobin and haematocrit cut-offs to define anaemia in healthy persons living at sea level (WHO/UNICEF/UNU, 2001).**

Age or sex group	Haemoglobin below (g/dL)	Haematocrit below (%)
Children 6 to 59 months	11.0	33
Children 5 – 11 years	11.5	34
Children 12 – 14 years	12.0	36
Non-pregnant women	12.0	36
Pregnant women	11.0	33
Men	13.0	39

**Table A2: Normally used haemoglobin cut-offs to define stages of anaemia in healthy persons living at sea level (WHO/UNICEF/UNU, 2001).**

Age or sex group	Mild	Moderate	Severe
Children 6 to 59 months	10.0-10.9	7.0-9.9	<7.0
Children 5 – 11 years	10.0-11.4	7.0-9.9	<7.0
Children 12 – 14 years	10.0-11.9	7.0-9.9	<7.0
Non-pregnant women 15 years plus		7.0-9.9	<7.0
Pregnant women 15 years plus	10.0-11.9	7.0-9.9	<7.0
Men 15 years plus	12.0-12.9	9.0-11.9	<9.0

According to the WHO, since Hb values change significantly with altitude, formulas are available to make adjustments for these changes to define anaemia at different altitudes.

#### **OTHER MEASUREMENTS FROM ERYTHROCYTES (RBC)**

**RBC count:** This is the total number of erythrocytes or RBCs measured in a cubic

millimetre ( $\text{mm}^3$ ) of blood and often denoted by millions of RBCs per  $\text{mm}^3$ , which represents the density of blood cells in whole blood. A low RBC count indicates anaemia whilst a higher than normal count may indicate an overproduction or polycythaemia. Under normal circumstances RBC counts are higher in men than in women. Whereas overhydration can lower the RBC count dehydration is noted to have the opposite effect (Sacher and McPherson, 2000).

**Reticulocyte count:** This is a measure of the number of immature RBCs that have been released from the source of production (bone marrow) into circulation. After their release from the bone marrow, reticulocytes take between 4 to 5 days to mature. The reticulocyte count gives an indication of how well the bone marrow is functioning or responding to erythropoietin. A normal reticulocyte count is from 0.5% to 2.5% and a low count may signify bone marrow failure or depressed erythropoietin synthesis (Sacher and McPherson, 2000).

**Mean corpuscular volume (MCV):** This refers to the relative volume or size of the RBCs. When the MCV is low the RBCs are said to be microcytic or smaller than normal and when MCV is high then they are macrocytic or larger than normal. The normal volume of RBCs ranges from 80fL to 98fL (Sacher and McPherson, 2000).

**Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC):** The MCH is a measure of the average amount of Hb in the RBCs whilst the MCHC refers to that in a single RBC. Low MCH or MCHC signifies hypochromic (pale colour) RBCs whereas high MCH or MCHC suggests that the RBCs are hyperchromic. Normal MCH values range from 28 to 32 fL and MCHC values from 32 to 36 fL (Sacher and McPherson, 2000).

## **MEASUREMENTS OF IRON STATUS**

**Serum iron:** Iron levels measured in serum constitute an indirect assessment of iron status as most of the iron is in transit. Serum iron levels must therefore be evaluated side by side with serum transferrin, serum transferrin saturation and serum ferritin (Leclair, 1997). A low serum iron level may indicate iron deficiency whereas a high serum iron level may be indicative of iron toxicity (Sacher and McPherson, 2000)...

**Serum ferritin:** Ferritin is an iron-phosphate-protein complex made by the intestinal mucosa when iron combines with apoferritin (a protein produced by the liver). Thus, iron is accumulated as ferritin which is primarily stored in the reticulo-endothelial cells of the liver, spleen and bone marrow (Sacher and McPherson, 2000). Ferritin is

currently the most important indicator of iron status as serum concentrations tend to correlate well with iron stores. It is the most sensitive in determining iron status as it shows a decrease even in the first stage of iron deficiency (Biesalski and Erhardt, 2007). Whilst a low ferritin level indicates storage iron depletion, a high value is not necessarily a sign of good iron status as infections and, especially, inflammation increases its levels. Thus, it is very important to also measure parameters of acute and chronic infections in order to distinguish persons whose ferritin levels might be elevated by infection. Parameters for indicating acute or chronic infection have included the use of C-reactive protein as well as Alpha-1 glycoprotein (AGP) (Biesalski and Erhardt, 2007; Northrop-Clewes, 2008). Cut-off values for serum ferritin are not very clear cut but have been set around 10 to 30 µg/L and values below 10 or 15 µg/L are certainly more indicative of iron deficiency (Biesalski and Erhardt, 2007; Northrop-Clewes, 2008).

**Serum transferrin/transferrin saturation:** Serum transferrin measures the amount of transferrin, an iron-binding globulin in the blood that is responsible for binding and transporting iron. Transferrin levels increase as the plasma levels of iron drops (iron deficiency) and decreases during chronic illnesses and in hypoproteinaemic states (Sacher and McPherson, 2000). Serum transferrin saturation on the other hand is a measure or calculation to indicate how much iron is available for Hb synthesis. It is calculated from the measurements of serum iron and total iron binding capacity (TIBC), which is a measure of the available sites for iron binding in the blood. The calculation is obtained by dividing the iron concentration by the TIBC and multiplying by 100 to express as a percentage (i.e. serum transferrin saturation = serum iron/TIBC x 100). Values should be at least 20% to ensure that there is enough iron in the blood to meet the requirements of Hb synthesis. Any values that fall below this mark may indicate iron deficiency (Sacher and McPherson, 2000).

**Soluble transferrin receptors (sTfR):** Transferrin receptors (TfR) are transmembrane protein receptors that bind and internalise transferrin, thereby delivering iron to the cytosol (Beguin, 2003). Mostly found on erythroid progenitor cells, TfR expression is increased to facilitate iron uptake when a cell needs iron. Proteolysis of these membrane receptors leads to the soluble monomers that can be measured in plasma and serum and referred to as sTfR. STfR concentration which marks a need for iron or erythropoietic activity is elevated in iron deficiency. Unlike the other measures of iron status, sTfR is not affected by chronic disease or inflammation (Beguin, 2003). Because sTfR concentrations tend to increase in the second stage of iron deficiency, after the iron stores are exhausted and Hb concentration is still above the cut-off, it is a

less sensitive parameter than ferritin but more sensitive than Hb (Biesalski and Erhardt, 2007). Unfortunately it has no internationally standardised method of assessment and it is still very expensive.

**Zinc protoporphyrin (ZnPP):** During iron deficiency the iron in protoporphyrin can be replaced by zinc, which can be measured by specific methods (Biesalski and Erhardt, 2007). The replacement of iron by zinc happens in the second stage of iron deficiency even before Hb levels fall under the cut-off values, which makes ZnPP a more sensitive parameter than Hb (Biesalski and Erhardt, 2007). However, it is important to keep in mind when measuring ZnPP that lead can increase its levels in the blood (Biesalski and Erhardt, 2007).

## APPENDIX B

### **DEFINING AND ESTIMATING ENERGY REQUIREMENTS AND IMBALANCES IN THE STUDY POPULATION**

The recommended level of dietary energy and nutrient intake for a population group is the mean requirement of healthy, well-nourished individuals who constitute that group. For most nutrients, a certain excess of intake will not be harmful. Thus, when dietary recommendations are calculated for these nutrients, the variation among individuals in a population group is taken into account, and the recommended level of intake is then estimated as the amount that will meet or exceed the requirements of practically all individuals in the group. This recommended level of intake, usually stipulated as the mean intake value plus two standard deviations (Mean + 2SD), can be satisfied with an average balanced diet. However, for dietary energy, this approach is not applicable because intakes that exceed requirements will produce a positive balance, which may lead to overweight and obesity in the long term.

#### **DIETARY ENERGY INTAKE**

Experts have agreed that the best descriptor of the dietary energy intake that could be safely recommended for a population group is the *estimated average energy requirement* (EAER) of that group, whose derivation is illustrated in figure B1 below (WHO, 1985).

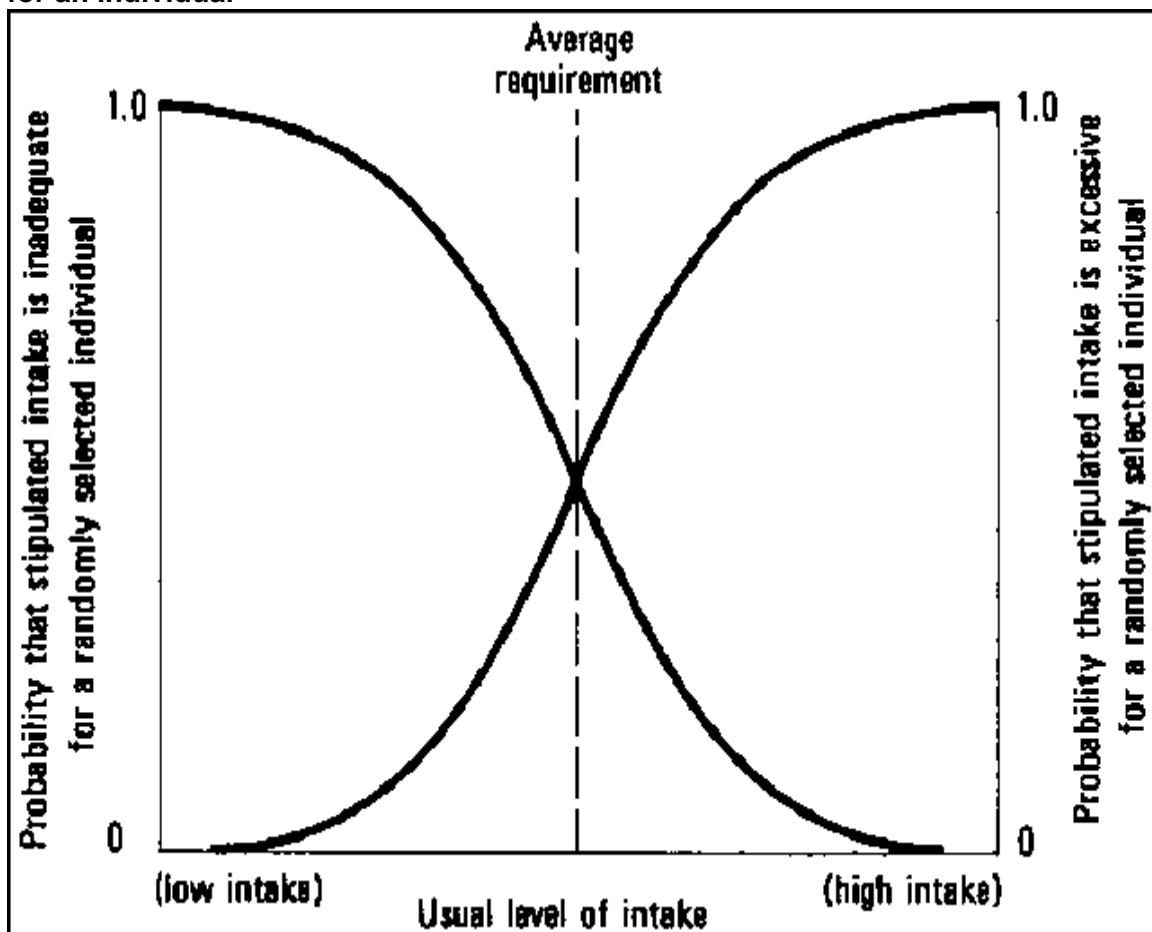
Within the range of energy and nutrient intakes in a population, any levels that fall below the estimated average requirements are not likely to meet the body's demands for these nutrients, which would make such intake levels inadequate. This would mean that, any persons having such intake levels may be considered as having poor intakes. In contrast, persons whose intakes fall above the EAER have a higher probability of excessive intakes which could have detrimental consequences if maintained with time.

#### **Estimating poor or inadequate intake – Usual energy intake versus requirements**

In free-living, healthy and well-nourished adults, energy requirements are equivalent to total energy expenditure (TEE), and these requirements are estimated for meeting the energy demands of the body for basal metabolism (BMR), metabolic response to food and physical activity (PA). In the absence of experimental data, energy requirements of adults can be calculated from factorial estimates of habitual TEE (FAO/WHO/UNU, 1985). This takes into account body size, body composition and habitual PA irrespective of the geographic, cultural and economic background of the population. Among population groups of a given age and gender, BMR is relatively constant. Consequently, habitual PA and body weight are the main determinants for the diversity

in energy requirements of adult populations with different lifestyles (James and Schofield, 1990).

**Figure B1: Probability that a particular energy intake is inadequate or excessive for an individual\***



\* Individuals are randomly selected among a class of people or a population group. The two probability curves overlap, so the level of energy intake that assures a low probability of dietary energy deficiency is the same level that implies a high probability of obesity owing to dietary energy excess. Source: WHO, 1985.

BMR estimated from age, gender-specific predictive equations (Schofield, 1985, see below) based on the average body weight of the population as well as PAL values calculated for individuals within the population based on their occupation or physical activity, and lifestyle characteristics were used to estimate TEE and subsequently the average energy requirement for the population.

### The Schofield Equation

This is a method of estimating the basal metabolic rate (BMR) in calories of adult men and women. It is commonly used by dieticians as a means of estimating the total calorie intake required to maintain current body mass. This figure can then be used to design a dietary regime that places the subject in calorie deficit or surplus, depending on whether weight loss or gain is the intended clinical outcome.

**Men:**

10 - 17 years BMR =  $17.7 \times W + 657$  SEE = 105

18 - 29 years BMR =  $15.1 \times W + 692$  SEE = 156

30 - 59 years BMR =  $11.5 \times W + 873$  SEE = 167

**Women:**

10 - 17 years BMR =  $13.4 \times W + 692$  SEE = 112

18 - 29 years BMR =  $14.8 \times W + 487$  SEE = 120

30 - 59 years BMR =  $8.3 \times W + 846$  SEE = 112

Key: W = Body weight in Kilogram; SEE = Standard error of estimation

The SEE value means the calculated BMR could be this number of calories out, in other words either too many or too little. As an example, if you are very muscular and possess more lean weight than an average person of the same height and weight, then you may have to add the SEE value to the BMR calculated. The simple reason is more lean weight means more calories needed.

Table B1 below is a summary of the estimated means for both PAL and BMR for the two groups, which also show disparities between the sexes.

**Table B1: Mean estimates for PAL and BMR compared between the HIV sero-status groups and sexes in the study population**

Variable	HIV status	n	Mean	95% CI	*t (p value)	*F (p value)	Eta squared
PAL (Estimated by lifestyle)	HIV+	216	1.594	1.575 – 1.613	0.235 (0.814)	1.218 (0.270)	0.001
	HIV-	1605	1.592	1.585 – 1.599			
	Males	779	1.605	1.595 – 1.615	3.451 (0.001)	14.085 (<0.001)	0.008
	Females	1040	1.583	1.574 – 1.591			
	Total	1819	1.592	1.586 – 1.599	-	-	-
BMR (Estimated by Schofield eqn.)	HIV+	214	6.19	6.08 – 6.29	0.546 (0.545)	0.375 (0.540)	0.000
	HIV-	1576	6.10	6.05 – 6.20			
	Males	766	6.52	6.47 – 6.57	18.118 (<0.001)	335.63 (<0.001)	0.158
	Females	1024	5.89	5.85 – 5.94			
	Total	1790	6.16	6.12 – 6.20	-	-	-

\*t – derived from Independent samples t-test statistics. \*F – derived from univariate ANOVA statistics which adjusted for gender, age grouping and settlement type. Statistical significance is denoted by p<0.05. Eta squared values represent the proportion of the variance explained by the exposure.

Although the estimated mean PAL difference between males and females was significant (p<0.001), mean estimates between the two groups, which took into consideration the gender disparity and proportions, showed that the infected subjects had a slightly higher value than their uninfected counterparts even though the

difference was not significant even after adjusting for the socio-demographic confounders. With regards to the mean BMR estimated for the groups, table B1 above shows also that males had a significantly higher BMR than females ( $p<0.001$ ). However, in comparing the two HIV groups, and considering gender disparities and proportions, infected persons tended to have a marginally higher estimated BMR than their counterparts, but again the difference was not significant after the adjustment for age grouping and settlement type were considered.

From the Schofield equation (TEE or EAER = BMR x PAL) energy requirements were calculated for males and females with gender disparities and disproportions in mind. The estimated EAERs for both males and females were derived as follows:

$$\text{EAER}_{(\text{males})} = 6.52 \times 1.605 = 10.465 \text{ MJ/day (i.e. 10465KJ/day)}, \text{ and}$$

$$\text{EAER}_{(\text{females})} = 5.89 \times 1.583 = 9.324 \text{ MJ/day (i.e. 9324 KJ/day)}$$

From these derived values, subjects for whom intakes fell below their respective gender specific EAER estimates were classified as having poor intakes, whilst those whose intakes were equal to or above the EAERs were classified as more likely to have adequate or excess energy intakes.

## APPENDIX C

### **CALCULATION OF POST HOC POWER FOR MAIN HYPOTHESIS**

The power calculations in this section are based on the observed effects and only comparative results that were not significant would be used. However, power calculations were ignored for effects that are based on very small sample sizes as these were more obviously underpowered.

The main hypothesis which states that 'HIV infected persons are more anaemic in comparison with uninfected persons' has several components. It presupposes that HIV infected persons are more likely to be anaemic as they are also more likely to have poor energy and micronutrient intakes, have an increased inflammatory response and metabolism leading to lower serum micronutrients and also marked by higher levels of positive APP and lower levels of negative APP. It is these attributes that are suggested, together with the direct effects of the virus, to interactively predispose infected persons more to anaemia, as the attributes can induce anaemia by themselves.

The continuous exposure variables compared between the two HIV groups were as follows:

- Energy intake
- Anthropometry (BMI and LMI)
- Serum micronutrients (Vitamins A and E)
- Serum/Plasma APP (Total proteins, albumin, globulins, fibrinogen, ferritin)
- Liver enzymes (s-ALP, s-GGT, s-ALT, and s-AST)

The equation used for the post hoc power calculation for the continuous variables has been derived from the equation for sample size calculation and is described in Margetts and Nelson (2008).

From;

$$n = 2 \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(d^*/\sigma)^2} = 2 \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{f^2} \quad (1)$$

Where  $f = d^*/\sigma$  ( $d^*$  is the difference between the groups to be detected and  $\sigma$  is the standard deviation)

By rearranging equation (1)

$$f = (Z_{1-\alpha/2} + Z_{1-\beta})\sqrt{n} \quad (2)$$

And then from equation (2) eqn. (3) is derived as follows:

$$Z_{1-\beta} = f\sqrt{n}/2 - Z_{1-\alpha/2} \quad (3)$$

Where;  $Z_{1-\beta}$  = Power component

$n$  = sample size and,

$$Z_{1-\alpha/2} = 1.96$$

The tables below (Tabs. C1-C3) summarises the derivation of post hoc power ( $1-\beta$ ) from a list of variables required for calculating the power for each variable that was not significantly different between the HIV infected and uninfected groups.

**Table C1**

Variables required for power derivation	Non-significant comparative variables			
	Energy intake (plus alcohol)	Energy intake (minus alcohol)	BMI	LMI
	212	212	212	212
$d^*$	648.38	437.64	0.10	0.01
$\Sigma$	3519.05	3274.62	1.00	1.00
$f(d^*/\sigma)$	0.184	0.135	0.100	0.01
$Z_{1-\alpha/2}$	1.96	1.96	1.96	1.96
$Z_{1-\beta}$	-0.07	-0.57	-0.93	-1.85
Power ( $1-\beta$ )	47.2	28.4	17.6	3.2

**Table C2**

Variables required for power derivation	Non-significant comparative variables			
	Serum Vitamin A	Serum Vitamin E	Plasma Fibrinogen	Serum Ferritin
N	202	201	210	216
d*	2.11	0.09	0.10	15.11
$\Sigma$	15.72	3.11	1.14	106.85
f ( $d^*/\sigma$ )	0.134	0.029	0.088	0.141
$Z_{1-\alpha/2}$	1.96	1.96	1.96	1.96
$Z_{1-\beta}$	-0.61	-1.67	-1.14	-0.50
Power (1- $\beta$ )	27.1	4.8	12.1	30.9

**Table C3**

Variables required for power derivation	Non-significant comparative variables			
	Haemoglobin	s-ALP	s-GGT	S-LDH
N	214	209	209	209
d*	0.32	3.24	3.60	6.57
$\Sigma$	2.17	37.58	104.62	33.60
f ( $d^*/\sigma$ )	0.148	0.086	0.034	0.196
$Z_{1-\alpha/2}$	1.96	1.96	1.96	1.96
$Z_{1-\beta}$	-0.43	-1.08	-1.61	0.04
Power (1- $\beta$ )	33.4	14.0	5.4	61.6

It is obvious from the post hoc power calculations shown in the tables above that the study was generally underpowered (below 80%) for showing any significant differences between the two groups for the variables considered. Given the effect sizes ( $d^*$ ) obtained for the various parameters, increasing the sample size (n) could have possibly increased the power for the differences to be significant, but as to whether the effect sizes were biological significant was also another issue to consider.

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