THE EFFECT OF HIV ON THE NUTRIENT COMPOSITION OF BREAST MILK

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DECLARATION OF INDEPENDENT WORK

I, Moira Hattingh, do hereby declare that this research project submitted to for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work, complies with the Code of Academic Integrity as well as other relevant policies, procedures, rules and regulations of the Central University of Technology; and has not been submitted before to any institution by myself or any other person in fulfillment of the requirements for the attainment of any qualification.

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Josie Field

SUMMARY

Thirty-one years after the discovery and isolation of the human immunodeficiency virus (HIV) by French and American scientists, much progress has been made in basic research, clinical treatment, and public heath prevention. Although, much evidence of mother-to-child-transmission (MTCT) of HIV has been amassed since then, not much of it describes the effects of HIV on the nutrient composition of breast milk.

The aim of this study was to determine the effects of HIV on the nutrient composition of breast milk, by studying two groups of adult lactating respondents from the same socio-economic background, who were chosen randomly and participated voluntarily. The study population consisted of 60 breastfeeding mothers, divided into two groups of 30 mothers each. Group one represented the control group of HIV non-infected mothers whereas group two consisted of HIV-infected mothers who did not receive any treatment.

After a registered medical nurse took blood and breast milk samples, analysis was done on ethylenediamine tetra-acetic acid (EDTA) whole blood to determine the haematological and immunological parameters and breast milk was analyzed for nutrient composition. Standard laboratory operating procedures (SOP) were followed, throughout, to determine the parameters of the blood and breast milk samples.

Results showed that associations between the socio-economic statuses (SES) of the two respondent groups could be established. Albeit differences were not significant, some were, however, detected in the number of people contributing to the household income of the respondents ($p = 0.0051$), their employment status ($p < 0.0001$) and the availability of water sources ($p = 0.1124$). It is believed that

factors, such as the prevalence of HIV, if related to the different levels of SES may play an important role in the outcome of the health statuses of individuals at different levels of society. By implication, it is not the different levels of SES, but rather factors related to the different levels of SES that have an impact.

Significant differences could be seen in the haematological variables between the two respondent groups: Red blood cell count (RBC) ($p < 0.0001$), hemoglobin (Hb) levels ($p = 0.0119$), hematocrit (Hct) ($p = 0.0031$), mean corpuscular volume (MCV) ($p = 0.0005$), mean corpuscular hemoglobin (MCH) ($p = 0.0043$) and monocyte count ($p = 0.0275$). These differences, however, were not significant to this study.

Other differences that were significant were immunological parameters between the two respondent groups: CD4 cell count ($p < 0.0001$) and viral load, done only on the blood of the HIV-infected respondent group. The CD4 cell count is used as a guideline for the initiation of treatment for HIV-infected persons and is required to accurately assess the immune status of any patient at any given time. The viral load has long been established as a strong predictor of the rate of disease progression.

The only significant difference in the breast milk composition was reflected in the following variables between the two groups: percentage (%) proteins ($p < 0.0001$) and calcium levels ($p = 0.0081$). The median and mean values of the percentage proteins were elevated in the subject group of mothers living with HIV, while calcium levels in the same group showed a decrease in both median and mean values.

The lack of significant differences between the groups might be due to the small study population. If nothing else, this study highlights the need for further trials to evaluate the true effects of HIV on the nutrient composition of breast milk.
Key word: HIV, pregnant woman, breastfeeding

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

=	Equals
>	Larger / more than
≤	Less than or equal to
µℓ	Micro liter
%	Percent
+	Positive
®	Registered
-	Negative
/	Per

AIDS	Acquired immune deficiency syndrome
ARA	Arachidonic acid
ART	Antiretroviral treatment
ARV	Antiretroviral
AZT	Zidovudine
BD	Beckton Dickenson
CCR5	Chemokine receptor 5
cm^2	Square centimeter
CO_2	Carbon dioxide
cps	Copies
CXCR4	CXC chemokine receptor 4
DHA	Docosahexaenoic acid
dℓ	Deciliter
DNA	Deoxyribonucleic acid
DXC	Data Exchange Control
EDTA	Ethylenediamine tetra-acetic acid

env	Envelope
FBC	Full blood count
fℓ	Femtoliter
g	Gram
gp41	Glycoprotein 41
HAART	Highly active antiretroviral therapy
Hb	Hemoglobin
Hct	Hematocrit
HI	Human immunodeficiency
HIV	Human immunodeficiency virus
HTLV	Human T cell leukemia virus
IgA	Immunoglobin A
kcal	Kilocalorie
ℓ	Liter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
mℓ	Milliliter
mm^3	Cubic millimeter
mmol/ℓ	Millimole per liter
MTCT	Mother-to-child-transmission
MUCPP	Mangaung University of The Free State Community Partnership Programme
NNRTI	Nonnucleoside reverse transcriptase inhibitor
nr/n	Number
NRTI	Nucleoside analogue reverse transcriptase inhibitor
O_2	Oxygen
PCP	*Pneumocystis* pneumonia
PCR	Polymerase chain reaction

pg	Picogram
PI	Protease inhibitor
Plt	Platelets
QS	Quantification Standard
RBC	Red blood cells
RNA	Ribonucleic acid
SD	Standard deviation
SES	Socio-economic status
sIgA	Secretory immunoglobin A
SLS	Sulsolyser
SOP	Standard operating procedures
StatsSA	Statistics South Africa
STD	Sexually transmitted disease
STIs	Sexually transmitted infections
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
WBC	White blood cells
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

The first case of human immunodeficiency virus (HIV) was reported in South Africa in 1982. Since then the epidemic has spread at an alarming rate. South Africa is one of the countries with the highest HIV prevalence rate in the world. This is why HIV and AIDS (acquired immune deficiency syndrome) are rated amongst the most important factors that have been impacting social integration in general and family life as a whole. Regardless of race, class, gender and age the HIV epidemic has spread rapidly among black heterosexuals of both genders, and as such, is affecting this ethnic group's lives extensively (Smit, 2007; Crothers, 2001).

In sub-Saharan Africa, women are more likely to become HIV-infected than men. The most recent prevalence data shows that 13 women become HIV infected for every 10 men infected with HIV (UNAIDS, 2010). The most alarming factor in South Africa is the increase in child mortality that increased from 56.3 per 1 000 in 1990 to 65.5 per 1 000 in 2005 (Smit, 2007; UNAIDS, 2005), and it is further estimated that 50% of HIV-infected infants will die before the age of two. According to Gribble, McGrath, MacLaine & Lhotska (2011), guided decisions related to child nutrition and the improvement of quality of life are necessary to reduce morbidity and mortality among HIV-infected children.

Statistics South Africa (StatsSA) estimated South Africa's population at 50.59 million in 2011 with an overall HIV prevalence rate of approximately 10.6%, and people living with HIV at approximately 5.38 million. According to StatsSA, in 2011 approximately 16.6% adults between 15-49 years of age were infected with HIV and roughly 63 600 new HIV infections were among children aged 0 – 14 years (StatsSA, 2011).

HIV transmission from mother to child can happen during pregnancy, delivery or postnatal through breastfeeding. The latter type of transmission has emerged as an important mode of pediatric acquisition in the African breastfeeding population which is a major cause of child mortality in sub-Saharan Africa (Becquet, Bland, Leroy, Rollins, Ekouevi, Coutsoudis, Dabis, Coovadia, Salamon & Newell, 2009; Coovadia & Bland, 2007).

Since the 1980s breastfeeding has been well documented as a vehicle for HIV transmission. This poses a serious concern for the 15.7 million HIV-infected women of reproductive age (15-49) across the globe (Clasen, 2011), but more so in sub-Saharan Africa where MTCT of HIV is of particular concern. It is in this region that eight out of every ten women infected with HIV live and where 53% of infants under the age of four months are exclusively being breast fed (Clasen, 2011). Of the more than ten million children who die each year in the developing world, about 60% of these deaths are preventable. Labbok, Clark & Goldman (2004), posit that breastfeeding is the most effective means of reducing the death rate of children younger than five years.

The avoidance of breastfeeding in resource-limited countries puts infants at a much higher risk of infection and death. Thus to strike a balance between preventing HIV transmission and protecting infants from malnutrition and disease becomes difficult (Clasen, 2011). In South Africa limited research is available on the nutrient composition of HIV-infected mothers' breast milk.

1.2 AIM

The main aim of this study was to investigate the effect of HIV on the nutrient composition of breast milk.

1.3 STRUCTURE OF THIS DISSERTATION

The structure of this thesis is as follows:

- Chapter I is an introductory chapter that gives the background of the study.
- Chapter 2 is an extensive literature survey of the most critical information needed to understand and interpret the aim and results of this study.
- Chapter 3 provides detailed information about specimen preparation and all methological procedures used in this study.
- Chapter 4 provides the results of the study.
- Chapter 5 provides the discussion of this study.
- Chapter 6 summarizes the study by presenting a discussion and conclusion, with recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

The disease caused by HIV was first identified in 1981 among two groups - one in San Francisco and the other in New York, when young homosexual men presented with opportunistic infections associated with severe immune deficiency like *Pneumocystis* pneumonia (PCP) or aggressive Kaposi sarcoma. According to Bennett, it was only two years later that the human immunodeficiency (HI) virus itself was identified and various other causes including lifestyle factors, chronic drug abuse, and other infectious agents came to be associated with HIV Infection.

At that stage, HIV testing was not yet available and the HIV epidemic spread rapidly. Although society was unaware of the disease, clear clinical implications had arisen. Prior to the recognition of HIV, only one case of *Pneumocystis* pneumonia, not clearly associated with immune suppression, had been diagnosed in the United States between January 1976 and June 1980 (Bennett, 2011). A study that was published in December 1980 reported the isolation and characterization of the first human retrovirus, the human T cell leukemia virus (HTLV) (Blattner, 1991; Poiesz, Ruscetti, Gazdar, Bunn, Minna & Gallo, 1980). French scientists were the first people to isolate the virus in 1983 (Blattner, 1991; Barre-Sinoussi, Chermann, Rey, Nugeyre, Chamaret, Gruest, Dauget, Axler-Blin, Vézinet-Brun, Rouzioux, Rozenbaum & Montagnier, 1983). Owing to this breakthrough, a British seaman, who died of progressive immunodeficiency in 1959, has become the first person documented with HIV infection (Blattner, 1991; Crobitt, Bailey & William, 1990).

The HI virus is blood-borne and sexually transmissible. This virus is typically transmitted via sexual intercourse, shared intravenous drug paraphernalia, and through MTCT, which can occur during the birth process or during breastfeeding. What is also common is co-infection with other viruses that share similar routes of transmission such as hepatitis B, hepatitis C, and human herpes virus 8 (Bennett, 2011).

The incubation period of HIV from exposure to disease ranges from eight to ten years. That is characterized, according to Klatt (2011) and Blattner (1991), by a progressive depletion of CD4-positive T lymphocytes, as well as effects on other immune and central nervous system cell populations. Currently, two strains of the virus have been identified. HIV-1 originated from one or more cross-species transfers from chimpanzees in Central Africa, whereas HIV-2 is related to viruses that infect sooty mangabeys in Western Africa. Although HIV-1 and HIV-2 strains are superficially similar, each contains unique genes and has its own replication process (Bennett, 2011; Klatt, 2011).

Because of the sexual transmissibility of HIV-infection, the virus has been stigmatized and linked to sexual promiscuity. Stigmatization has led to discrimination against those infected, which in turn has resulted in a reluctance to be tested for the virus. However, since HIV causes relentless immune decline, eventual premature death in the vast majority of infected people and the fact that it is incurable has made testing crucial (Bennett, 2011; Skinner & Mfecane, 2004).

Despite many advantages being made globally in fighting the HIV epidemic, too many people are still getting sick and too many people are dying. The past decade has seen a historically unprecedented global response to the unique threat the HIV epidemic poses to human development (WHO, UNAIDS & UNICEF, 2011; Blattner, 1991). WHO (World Health Organization), UNAIDS

(Joint United Programme on HIV and AIDS) and UNICEF (United Nations Children's Fund) (2011) state that at the beginning of the 21st century, the international community faced formidable health and development challenges, none more so than countries in the poorest region of the world: sub-Saharan Africa. In this region, the rapidly expanding HIV epidemic dramatically reversed the decades of progress on the key developmental indicators, such as infant mortality and life expectancy (WHO, UNAIDS & UNICEF, 2011).

2.1.1 What is HIV?

HIV is a virus that infects cells of the human immune system and destroys or impairs their function (UNAIDS, 2008). It affects mostly the CD4 positive T cells and macrophages – a key component of the cellular immune system. Infection results in the progressive deterioration of the immune system, leading to immune deficiency. When the immune system can no longer fulfill its role of fighting off infections and diseases it is considered deficient. Once people are immunodeficient, they become susceptible to a wide range of infections known as opportunistic infections. They are thus called because they take advantage of a weakened immune response (UNAIDS, 2008).

2.1.2 What is AIDS?

AIDS is an acronym for **A**cquired **I**mmune **D**eficiency **S**yndrome, and is the result of HIV infection (UNAIDS, 2008).

Acquired means to get: the virus infects people, leading to the associated symptoms.

Immune means protected: it refers to the body's ability to fight off infections.

Deficiency means lack of: an HIV-infected person lacks protection and therefore cannot fight off common diseases.

Syndrome means a collection of signs and symptoms.

2.2 EPIDEMIOLOGY

Miller-Keane (2013) states that epidemiology is the science concerned with the study of the factors determining and influencing the frequency and distribution of disease, injury, and other health-related events and their causes in a defined human population for the purpose of establishing programs to prevent and control their development and spread.

2.2.1 Global

The global incidence of HIV infection peaked in the mid-1990s when more than three million people were being newly infected every year. AIDS then became one of the leading causes of adults dying in sub-Saharan Africa. Since 2006, more than 2.2 million people have died each year from AIDS-related causes (WHO, UNAIDS & UNICEF, 2011; UNAIDS 2009; WHO, 2004). According to the WHO and UNAIDS, an estimated 2.7 million people became newly infected with HIV in 2010. In Sub-Saharan Africa, where the majority of new HIV infections continue to occur, an estimated 1.8 million people became infected in 2009 (UNAIDS, Global Report, 2010). An estimated 34 million people have been living with HIV as from 2010, 16.8 million being women and 3.4 million children under the age of 15 (see Table 2.1 UNICEF, 2012).

TABLE 2.1: Global summary of the AIDS pandemic, 2011

Number of people living with HIV in 2011		
Total	34,2 million	[31,8 – 35,9]
Adults	30,7 million	[28,6 – 32,2]
Women	16,7 million	[15.7 – 17.8]
Children under 15 years	3.4 million	[3.1 – 3.9]
People newly infected with HIV in 2011		
Total	2.5 million	[2.2 – 2.8]
Adults	2.2 million	[2.0 – 2.4]
Children under 15 years	330,0000	[280,000 – 380,000]
AIDS deaths in 2011		
Total	1.7 million	[1.6 – 1.9]
Adults	1.5 million	[1.3 – 1.7]
Children under 15 years	230,000	[200,000 – 270,000]

Note: The numbers in brackets are ranges around the estimates that define the boundaries within which the actual numbers lie based on the best available information.
Source: UNAIDS. 2012.

2.2.2 Sub-Saharan Africa

Approximately 33.4 million people worldwide - one % (percent) of the global adult population aged 15-49 years - are currently infected with HIV. The vast majority of infections remain in sub-Saharan Africa, where 5.2% of the population is thought to be infected (UNICEF, 2012; Bennet, 2011; Klatt, 2011). WHO and UNAIDS estimated in 2007 that 15.4 million (13.9-16.6 million), almost half the

population of people living with HIV and AIDS, were women. In sub-Saharan Africa this number accounted for 68% of the world's disease burden (Sahasrabuddhe & Vermund 2009; McGowan, 2009).

Sub-Saharan Africa continues to bear an inordinate share of the global HIV burden. Though the number of new infections on the continent seems to have peaked in the mid-1990s, the epidemic continues to be a major challenge to the health and development of many African nations. The epidemic varies from country to country across the continent, with prevalence estimates ranging from 0.1 % in Madagascar to more than 15% in some of the countries in the southern cone. The epidemics in sub-Saharan Africa vary considerably, with South Africa still the most severely affected (USAID, 2011).

Sub-Saharan Africa is the region with the highest prevalence of HIV infection among women of reproductive age (WHO, UNAIDS & UNICEF, 2011). This region also accounted for 67 % of the world's AIDS-related deaths in 2010 (UNICEF, 2012). Table 2.2 summarizes the statistics of the AIDS pandemic for sub-Saharan Africa for the year 2009.

Table 2.2: AIDS statistics for sub-Saharan Africa, 2009 (UNAIDS)

	People living with HIV	**People newly infected with HIV**	**Children living with HIV**	**AIDS-related deaths**
SUB-SAHARAN AFRICA	22.5 million [20.9-24.2 million]	1.8 million [1.6-2.0 million]	2.3 million [1.4-3.1 million]	1.3 million [1.1-1.5 million]

2.2.3 South Africa

South Africa has a population of approximately 50.6 million people, accounting for 7% of the world's population (StatsSA, July 2011). Of these 50.6 million people, Statistics South Africa (StatsSA) estimated that 5.38 million people (17% of the global burden) were living with HIV in 2011. HIV prevalence for the adult population (15-29 years) has been estimated at 16.6%, and the overall population prevalence rate at 10.6%. This could be due to the South African Government's slowness in admitting to having an HIV epidemic and that AIDS was even a problem. Changes are now occurring, albeit slowly and at an unknown cost (Bennett, 2011).

In South Africa, both immediate and underlying factors are contributing to the transmission of HIV. Immediate factors of the HIV and AIDS epidemic include behavioral factors such as frequently unprotected sex, multiple sexual partners, and the high prevalence of sexually transmitted diseases (STDs). Underlying factors include poverty, the migrant labour system, commercial sex, illiteracy, stigmatization, discrimination, the low status of women and the lack of formal education. With more than five million people living with HIV and AIDS, South Africa still has the greatest disease burden of any single country in the world (Sahasrabuddhe & Vermund 2009) and remains the area most heavily affected by the HIV epidemic (UNICEF, 2012).

Data from an antenatal survey suggests that HIV prevalence had plateaued, although at a high level of nearly 30%. This national figure masks provincial and district level differences with some districts in KwaZulu-Natal being above 40% to several districts in the Northern Cape and Western Cape below 10%. StatsSA (2011) identifies that high prevalence in access to ARVs represents longer life expectancy and not increased incidence of infection. StatsSA (2011) attributes this increase in life expectancy to the impact of antiretroviral (ARV) therapy.

However, StatsSA (2011) estimates that the disease still has a dramatic impact on life expectancy, which is currently 54.9 years for men and 59.1 years for women. Figures 2.1 and 2.2 show the HIV antenatal seroprevalence rates from 1990 to 2009 in South Africa as well as the districts in South Africa which have been influenced the most.

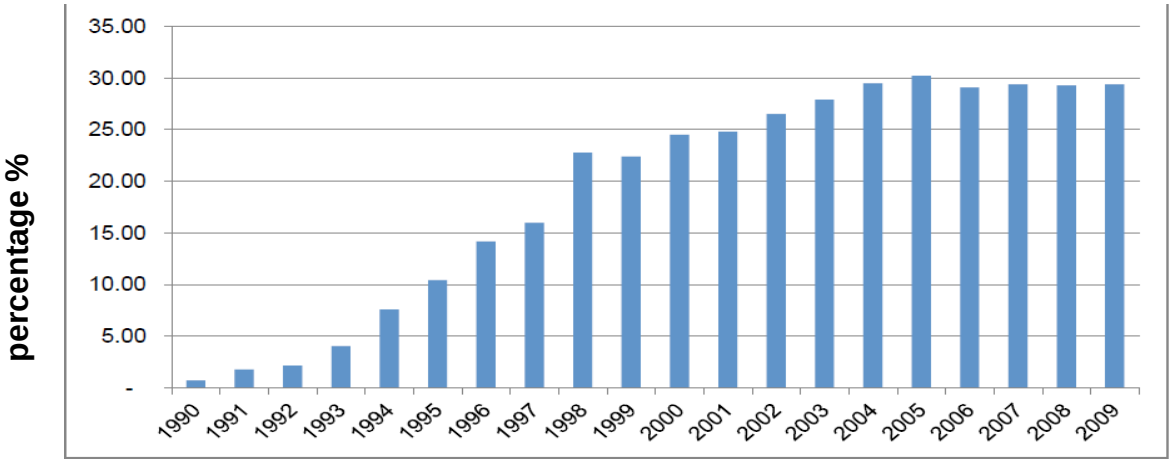


Figure 2.1: HIV Antenatal seroprevalence rates, 1990-2009

Source: Source: South African Department of Health (b), 2011)

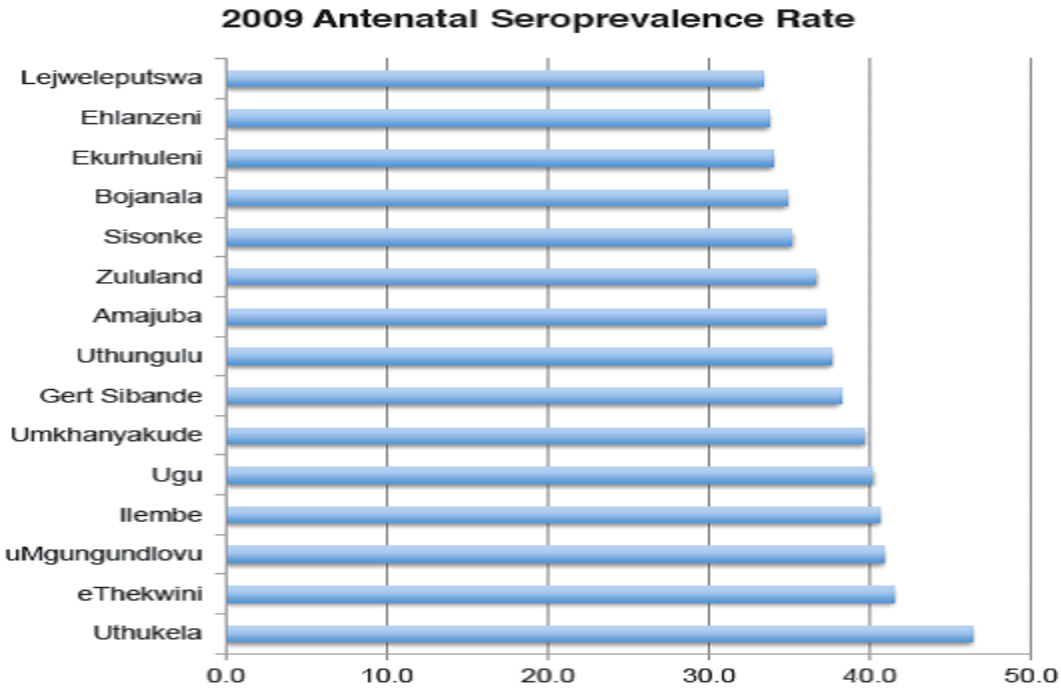


Figure 2.2: HIV Antenatal Seroprevalence Rates in 15 Districts with the Highest Rates in South Africa (**Source:** South African Department of Health (b), 2011)

2.2.3.1 Trends of HIV Transmission in South Africa

HIV infection is much higher in the South African black population than any other race groups. Adult women aged 15 years and above are more likely to be HIV positive than men of the same age and young women between 20 and 24 years are four times more likely than males of the same age to have the HI virus (South African Department of Health (a), 2011). The difference is even higher in teenage girls. It has been estimated that people living with HIV show considerable clustering in the eastern parts of the country, with the majority of adult people living with HIV (54%) located in Gauteng and KwaZulu-Natal (South African Department of Health (a), 2011). The levels of HIV in the informal settlements in urban areas are also high. Furthermore, a low SES is associated with HIV infection, since those who work in the informal sector have the overall highest HIV prevalence with almost a third of the African informal workers being HIV positive. Among women, those with less disposable income have a higher risk of being HIV positive (South Africa: Department of Health (a), 2011).

2.2.4 Children

More than 90% of children living with HIV and AIDS come from sub-Saharan Africa (Sahasrabuddhe & Vermund 2009). Diarrhea and pneumonia respectively are the third and fourth biggest causes of HIV-infected child-deaths under the age of five in South Africa (Doherty, Sanders, Goga & Jackson, 2010). De Cock and co-workers (2000) maintain that the global epidemiology of pediatric HIV infections reflect that of HIV infections in women. These estimates are equivalent to approximately one child being infected every second day in the United State, one every day in Europe, two a week in Asia, and over 1000 a day in Africa (McIntyre & Lallement, 2008).

The HIV pandemic has had a dramatic impact on child mortality, with 380 000 children having died of AIDS-related diseases. In 2006 an estimated 2.3 million

children under 15 years were living with the virus, mainly as a result of MTCT. Estimates, according to UNAIDS (2007), showed that around 420 000 children (350 000-540 000) were newly infected with HIV in 2007, again mainly through MTCT. More than 90% of these children are living in sub-Saharan Africa. The provision of a combination of antiretroviral treatment (ART) during pregnancy, ongoing for those with higher CD4 cell counts, together with the use of replacement feeding has become the standard for caring for HIV-infected pregnant women in well-resourced settings. As a result, there has been a drop in MTCT rates to below two percent (McIntyre & Gray, 2009; Fowler *et al*., 2007; Newell & Thorne, 2004).

Around 390 000 children were infected with HIV in 2010, bringing the total number of children infected with HIV to 3.4 million. All these children were under 15 years of age and more than 90% of them were living in sub-Saharan Africa (UNICEF, 2012).

2.3 HIV TRANSMISSION PHYSIOLOGY

HIV transmission primarily occurs through sexual contact. The virus breaches the epithelial barrier at sites of inflammation or micro-abrasions and via contact with Langerhans and dendritic cells. During this process HIV is transferred from the mucosal surface to the underlying target cells. The cells infected at the mucosal surface then present the HI virus to the CD4-positive lymphocytes and the virus is transported to deeper tissue (Figure 2.3). The HI virus can be detected in regional lymph nodes as early as two days after infection and then in the blood within 7 days. Following that, Viremia which has a dramatic impact on the immune system and leads to the destruction of CD4 memory T cells, occurs. This process mainly manifests in the gut-associated lymphoid tissue, where a large amount of these cells are located. It results in a crippling effect on the prime defenses against infection (Kuhn *et al*., 2007; Iliff *et al*., 2005; Brenchley *et al*., 2004; Coutsoudis, Pillay, Kuhn, Spooner, Tsia & Coovadia, 2001).

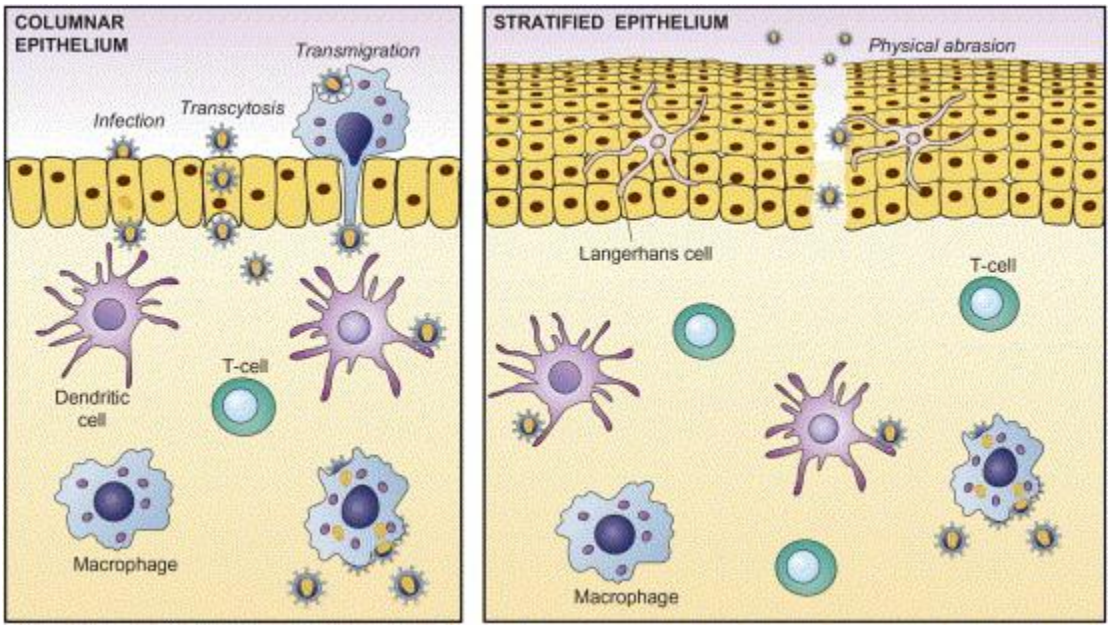


Figure 2.3: Mucosal transmission of HIV-1 probably occurs through multiple pathways. Target cells in the subepithelial area include CD4+ lymphocytes, macrophages and dendritic cells. Mucosal inflammation and epithelial disruption secondary to sexually transmitted infections (STIs) increase the risk of HIV-1 transmission trough recruitment of additional target cells. **Adapted from**: MgGowan, 2006

2.4 IMMUNOLOGY AND HOST DEFENSES

The adaptive immune response consists of two branches. The first branch, the humoral branch, controls infections through antibodies. Antibodies are proteins which are able to attach to antigens (such as a virus) because of the former's structures. When an antibody has bound to an antigen through a "lock and key" interaction, the invading pathogen can be destroyed through a number of intracellular and extracellular mechanisms (Klatt, 2011; Geise & Duerr, 2009; Goepfert, 2003). If the antibodies bind to the pathogen before they infect their target cells, the antibodies can provide a "sterilizing immunity"- that is, the immune response can prevent the establishment of any detectable infection. The cellular immune system is the second branch of the immune response and is much more complex (Klatt, 2011; Geise & Duerr, 2009; Goepfert, 2003). This system works best in clearing established infections such as when antigen-

presenting cells respond to selected pathogen peptides. When the antigen-presenting cells are detected by cytotoxic T cells, the pathogen peptides containing nine to 15 amino acids (epitopes) are displayed on the surface of the infected cells in conjunction with a “self” antigen (HLA). These infected cells can be destroyed either by phagocytosis, cytokine release or cell lysis (Klatt, 2011; Geise & Duerr, 2009; Goepfert, 2003).

The humoral branch of the immune system develops antibodies to the various protein constituents of HIV. Seeing that the structure of HIV is very complex and the HIV envelope protein limits antibody access to these surface proteins, those parts of the HIV envelope involved in T-cell binding are shielded by glycosolation and conformational masking, which shields the HIV from interaction with antibodies. Escaped isolates are easily developed and limit the antibody effects in neutralizing the circulating virus (Kwong *et al*., 1998). The proteins on the HIV envelope change due to rapid viral evolution, which makes it difficult for existing antibodies to recognize these new isolates. The CD8-positive lymphocytes dominate the cellular arm of the immune system and can control the infection temporarily through the recognition of infected cells, apoptosis, and cytokine secretion (Klatt, 2011; Geise & Duerr, 2009; Goepfert, 2003).

2.5 HIV LIFE CYCLE

The life cycle of HIV can be described in six steps (see Figure 2.4):

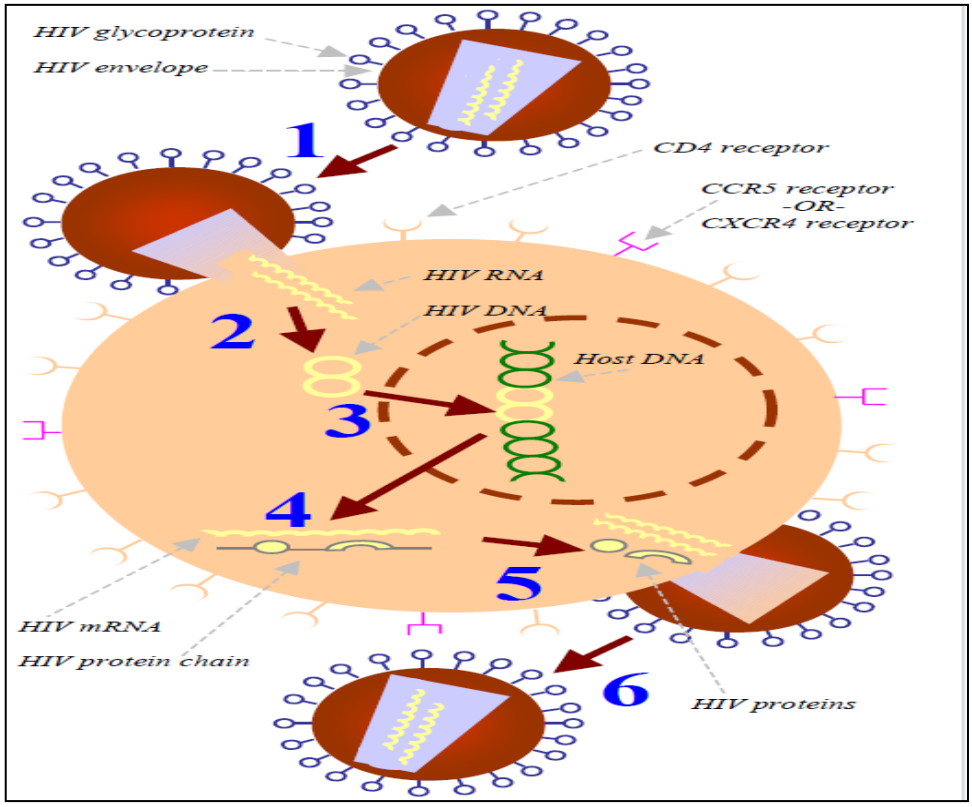


Figure 2.4: The aspects of the HIV life cycle (Adapted from: http://aidsinfo.nih.gov)

1. **Binding and Fusion**: HIV begins its life cycle when it binds to a CD4 receptor and one of two co-receptors on the surface of a CD4+ T-lymphocyte. The virus then fuses with the host cell. After fusion, the virus releases ribonucleic acid (RNA) into the host cell.

2. **Reverse Transcription**: An HIV enzyme, called reverse transcriptase, converts the single-stranded HIV RNA to double-stranded HIV deoxyribonucleic acid (DNA).

3. **Integration**: The newly formed HIV DNA enters the host cell nucleus, where an HIV enzyme called integrase “hides” the HIV DNA within the host cell’s own DNA. The integrated HIV DNA, called provirus, may remain inactive for several years, producing new copies of HIV or none.

4. **Transcription:** When the host cell receives a signal to become active, the provirus uses a host enzyme called RNA polymerase to create copies of the HIV genomic material, as well as shorter strands of RNA called messenger RNA. These are used as a blueprint to make long chains of HIV proteins.

5. **Assembly**: An HIV enzyme called protease cuts the long chains of HIV proteins into smaller individual proteins. As the smaller HIV proteins come together with copies of HIV’s RNA genetic material, a new virus is assembled.

6. **Budding**: The newly assembled virus pushes out (“buds”) from the host cell. During budding, the new virus steals part of the original cell’s outer envelope. This envelope, which acts as a covering, is studded with protein/sugar combinations called HIV glycoproteins. These glycoproteins are necessary for the virus to bind CD4 and co-receptors. The new copies can now move to infect other cells (Klatt, 2011; Kwong *et al*., 1998)

2.6 HIV SUBTYPES

HIV can be classified into two distinct types since there are two distinct species. HIV-1 and HIV-2 have been identified and each is composed of clades or multiple subtypes. All clades of HIV-1 tend to cause similar disease, but the distribution of the clades differs (Bennett, 2011; Klatt, 2011). HIV-1 is responsible for the worldwide pandemic of AIDS whereas HIV-2 is clustered prominently in West-Africa. Although it is associated with AIDS in some cases, it is less virulent in its effects than HIV-1 (Klatt, 2011; Blattner, 1991).

There are several subtypes of HIV-1 which can be classified genotypically into several subtypes or clade groups on the basis of genotypic variation in the *env* region. Infection with HIV-2 progresses more slowly to AIDS, and has a slightly lower risk of transmission. This may be due to a less-aggressive infection rather than a specific property of the virus itself (Klatt, 2011). HIV-2 tends to have a lower viral load than HIV-1. The rapid progression to AIDS is associated with a greater viral load. Since HIV-2 is rare in the developed world most research has been focused on HIV-1 (Bennett, 2011; Klatt, 2011).

2.7 THE TYPICAL COURSE OF INFECTION

The clinical features of HIV can be determined by a CD4 lymphocyte count. When the number of virus particles (viral load) rises during the course of the illness, the number of CD4 lymphocytes fall. When the CD4 lymphocyte count falls below 350 cells per (/) cubic millimeter (mm^3), the person becomes susceptible to infection (see Figure 2.5). According to Klatt (2011) the majority of life threatening infections and tumors occur at CD4 counts below 200 cells/mm^3.

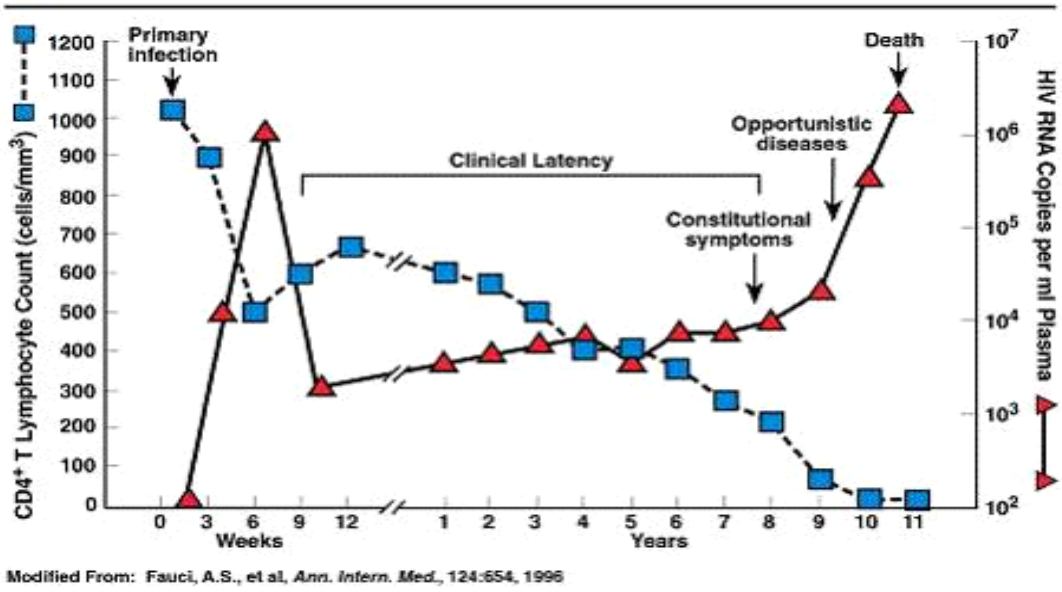


Figure 2.5: The Typical Course of HIV Infection (Bennett, 2011)

2.7.1 Primary Infection

Two to six weeks after exposure to HIV, the majority of people develop transient, often mild, non-specific illnesses (sero-conversion or acute HIV syndrome). This is caused by high circulating levels of HIV and a fall in the CD4 cell count. The most common symptoms are: malaise, joint pain, a rash, muscle pain, mouth ulcers and sore throats. Most of these symptoms resolve after seven to ten days. In a few people the illness is more severe and may be associated with opportunistic infection, such as pneumonia, when the CD4 count falls below 200 cells/mm^3.

2.7.2 Asymptomatic phase (CD4 count greater than 350cells/mm^3)

In this stage the CD4 cell count usually increases again, but still to a level below normal. Although people with a CD4 cell count greater than 350cells/$mm^{3,}$ have enlarged lymph nodes, they are usually asymptomatic. The length of the asymptomatic phase varies from person to person. In most people it lasts six to eight years, in about five to ten percent of people it can last for many years and in others for decades. However, in some people there is a rapid fall in the CD4 cell count and progression to the symptomatic phase happens within six to twelve months.

2.7.3 Symptomatic phase (CD4 count 200-350cells/mm^3)

With a CD4 cell count below 350cells/mm^3, the person becomes increasingly susceptible to a number of infections. These include: pulmonary tuberculosis, shingles, pneumoccocal pneumonia, recurrent oral and vaginal candidiasis and, although rarely, oral hairy leukoplakia. Individuals also become more susceptible to Karposi's sarcoma and lymphoma. People may develop intermittent or persistent non-specific constitutional symptoms, which include: lethargy, anorexia, weight loss, diarrhoea, fever and night sweats.

2.7.4 Advanced phase (CD4 count less than 200cells/mm^3 (AIDS)

When the CD4 cell count continues to fall, opportunistic infections and HIV related tumors may develop. It is said to be AIDS when a CD4 cell count is less than 200cells/mm^3, and/or an AIDS defining condition is detected. This is also referred to as Advanced HIV.

2.8 TREATMENT

2.8.1 Introduction

At the very beginning of the AIDS epidemic, people living with HIV were deemed not likely to live more than a few years. With the development of safe and effective drugs, HIV-infected people now have longer and healthier lives. There are no drugs to cure HIV infection but those that are available prevent the development of AIDS. Not only do they stop the virus from being made in the body, they also ultimately stop the virus from damaging the immune system. Nevertheless, the fact remains that even these drugs cannot eliminate HIV from the body (WHO, 2009).

The treatment for HIV, using anti-HIV drugs, is known as ART. The standard treatment involves a combination of at least three drugs to be taken at any one time in order to suppress HIV replication. These combination drugs are also known as “highly active antiretroviral therapy (HAART)”. They are used to reduce the likelihood of the virus developing resistance. ART has the potential to reduce mortality and morbidity rates among HIV-infected people, and to improve their quality of life (WHO, 2009).

With the introduction of combination antiretroviral therapy in 1996, the course of the disease in high-income countries has been altered for those already infected with HIV. However, in low and middle-income countries only a fraction of the people living with HIV have access to this therapy, keeping in mind that the people in the low and middle-income provide for 90% of the global HIV burden (WHO, UNAIDS & UNICEF, 2011; Flanigan, Campbell, Harwell & Kumarasamy, 2005).

Although the use of combination ART has improved the course of the disease, this therapy is not universally available. It should also be kept in mind that ART does not prevent the massive immune destruction that occurs soon after infection (Geise & Duerr, 2009; Brenchley *et al*., 2004) and it does not prevent the transmission of the virus (Geise & Duerr, 2009). The treatment of HIV-infected people, with antiretroviral drugs and drugs for the prevention and treatment of opportunistic infections, benefits individuals, communities, and nations. Support and effective care can enhance prevention by reducing stigma, increasing rates of HIV testing and possibly reducing transmission (Geise & Duerr, 2009). Access to HIV health services, in many low and middle-income countries, is constrained by under-resourced health systems and many ART programmes are not well-integrated with other health services (WHO, 2011). In many poor countries, access to HIV health services is limited by fragile health systems. Ten million people who are eligible do not have access to ART because of structural barriers such as discriminatory laws and outdated drug control policies. This exacerbates inequities in accessing treatment (Hirnschall & Schwartländer, 2011). According to Lockman (2011), breast milk will infect approximately 10%-15% of infants in the absence of ARV treatment. This is still the case, even though, significant advances have been made in antiretroviral therapy since the introduction of zidovudine (AZT) in 1987 (Rathbun, 2011).

The World Health Organization guidelines on ART were first published in 2002, with revisions in 2003, 2006, and 2010. The 2010 guidelines reflect the evidence that the earlier ART is started (≤ 350 CD4 cells/mm^3) the more cost effective it is, reducing HIV and tuberculosis transmission, and improving health outcomes (Hirnschall & Schwartländer, 2011; WHO, 2011 & 2009). In 2003, WHO laid out a strategic rationale for the rapid scale-up of ART in low-income and middle-income countries (Hirnschall & Schwartländer, 2011). When WHO and UNAIDS launched the "3 by 5" initiative on World AIDS Day in 2003, only 400 000 people in low and middle-income countries had access to antiretroviral therapy (WHO, UNAIDS & UNICEF, 2011 & 2009). HAART has nearly halved mortality among patients with AIDS and with it, the HIV-infection in patients who have access to medication and who achieve durable virologic suppression can now be managed like a chronic disease (Rathbun, 2011; Palella *et al.*, 1998).

High mortality rates remain five times higher in patients with AIDS than in HIV-infected patients without AIDS. Viral loads greater than 400 copies/mℓ (compared with < 400 copies/mℓ), and CD4 counts less than 200 cells/mm^3 (compared with > 200 cells/mm^3) and cytomegalovirus retinits are the biggest risk factors for excessive mortality (Rathbun, 2011; Puhan *et al.*, 2010). The UNAIDS Secretariat and WHO launched the Treatment 2.0 initiative in June 2010. It was designed to achieve and sustain universal access, to maximize the preventive benefits of antiretroviral therapy (ART), and also to dramatically improve the efficiency and impact of HIV care in resource-limited countries (WHO, UNAIDS & UNICEF, 2011; Hirnschall & Schwartländer, 2011; WHO, 2011).

By the end of 2010 the number of people receiving antiretroviral therapy had increased to 6.65 million. Effective antiretroviral regimens were given to almost 50% of pregnant women living with HIV, to prevent MTCT. That was an increase of over 1.4 million people, or 27% from December 2009. The greatest increase of people receiving ART was in sub-Saharan Africa, increasing from 3 911 000 in

December 2009 to about 5 064 000 a year later (WHO, UNAIDS & UNICEF, 2011). In 2010, WHO recommended that antiretroviral therapy should be initiated with a CD4 cell count less than 350 cells/mm^3, since clinical evidence shows that starting therapy that soon significantly reduced morbidity and mortality and also has significant benefits in preventing HIV infection (WHO, UNAIDS & UNICEF, 2011; WHO, 2010) (see Figure 2.6).

With the provision of antiretroviral prophylaxis to pregnant women living with HIV, more than 350 000 children have been prevented from acquiring HIV-infection since 1995 (WHO, UNAIDS & UNICEF, 2011). Regardless, for every person treated with ART four new people become infected (WHO, 2007) (see Figure 2.7).

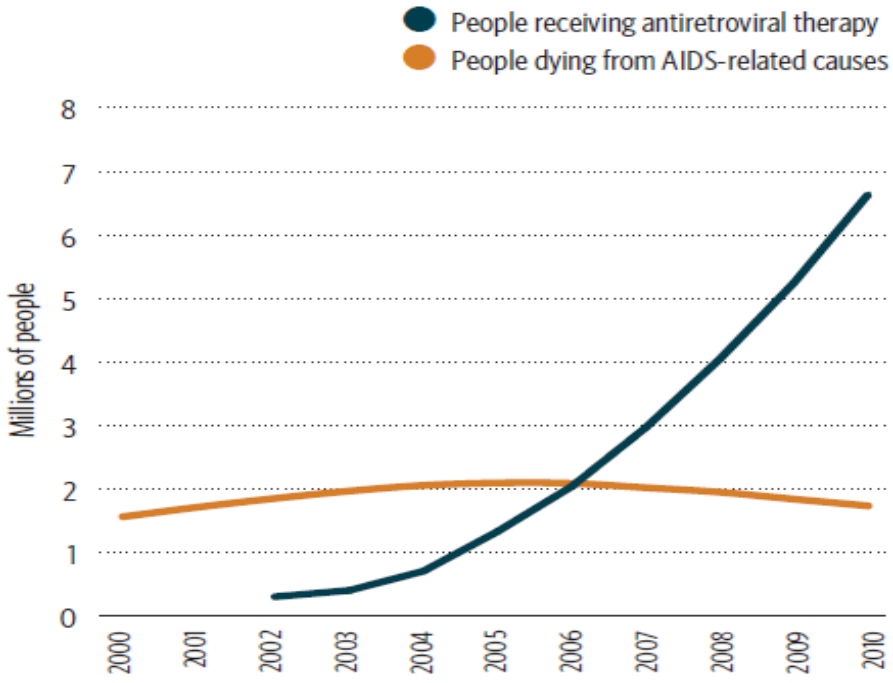


Figure 2.6: Number of people with access to antiretroviral therapy and the number of people dying from AIDS-related causes, low- and middle-income countries, 2000-2010 (WHO, UNAIDS & UNICEF, 2011)

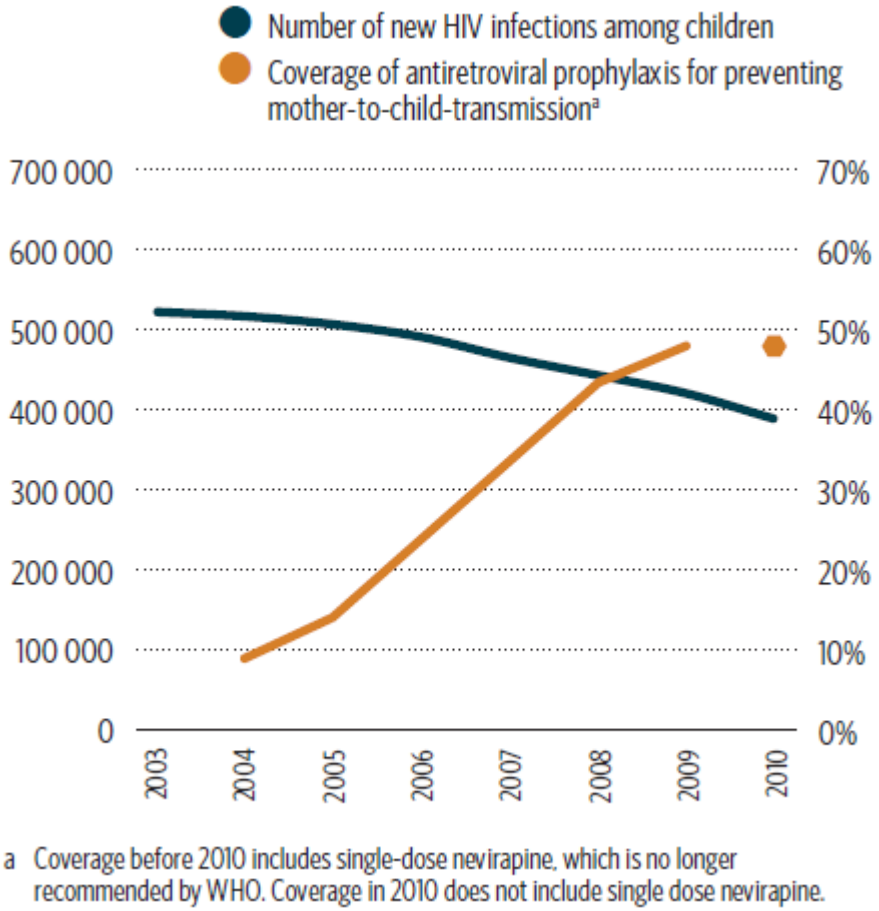


Figure 2.7: Coverage of antiretroviral prophylaxis for preventing the mother-to-child-transmission of HIV and the number of new HIV infections among children, low- and middle-income countries, 2003-2010 (WHO, UNAIDS & UNICEF, 2011).

2.8.2 How does ARV therapy work?

The main aim of ARV therapy is to prevent the HI virus from multiplying inside a person. This virus is very active, multiplying itself before damaging the body's immune cells (CD4 cells). It also adapts quickly to whatever medicines are being taken as it tries to mutate so that these medicines no longer work (WHO, 2009). If the virus' growth stops, the body's immune CD4 cells are able to live longer and provide the body with protection from infections. At the close of 2008, more than four million people in low and middle-income countries were receiving

antiretroviral therapy (WHO, 2009). An additional 1.2 million received antiretroviral therapy in 2009, bringing the number of people receiving treatment to 5.2 million, a 30% increase (WHO, 2010).

2.8.3 How do antiretroviral drugs work?

As the HI virus multiplies inside an infected cell, it makes copies of itself that go on to infect other healthy cells within the body. The more cells are infected, the greater the impact on the immune system, and the more severe the deficiency in the immune system (WHO, 2009). Antiretroviral drugs interfere with the self copying of HIV and the way it spreads from cell to cell. There are several different classes of drugs that can be used (WHO, 2009).

2.9 DRUG CLASSIFICATION

There are currently six major classes of antiretroviral drugs: nucleoside drugs, nucleoside analogue reverse transcriptase inhibitors (NRTI's), non-nucleoside reverse transcriptase inhibitors (NNRTI's), protease inhibitors (PI's), fusion inhibitors, chemokine co-receptor antagonists (consisting of 2 subclasses: the chemokine receptor 5 (CCR5) antagonist and CXC chemokine receptor 4 (CXCR4) antagonist, and integrase inhibitors. The ways in which the drugs work on HIV are shown in Figure 2.8.

NRTIs: function by inhibiting the synthesis of DNA by reverse transcriptase, the viral enzyme that copies viral RNA into DNA in newly infected cells. Nucleoside analogues bear the structural resemblance of the natural building blocks of DNA, known as nucleosides adenosine, guanosine, thymidine and cytidine. They are triphosphorylated within the cell, and some undergo further modifications. Nucleoside analogues resemble monophosphorylated nucleosides, and therefore

require only two additional phosphorylations to become active inhibitors of DNA synthesis. Reverse transcriptase fails to distinguish the phosphorylated NRTIs from their natural counterparts, and attempts to use the drugs in the synthesis of viral DNA. When an NRTI is incorporated into a strand of DNA being synthesized, the addition of further nucleosides is prevented, and a full-length copy of the viral DNA is not produced. See Figure 2.8 below: The mechanisms of drugs on HIV (Spach & Gallant, 2012).

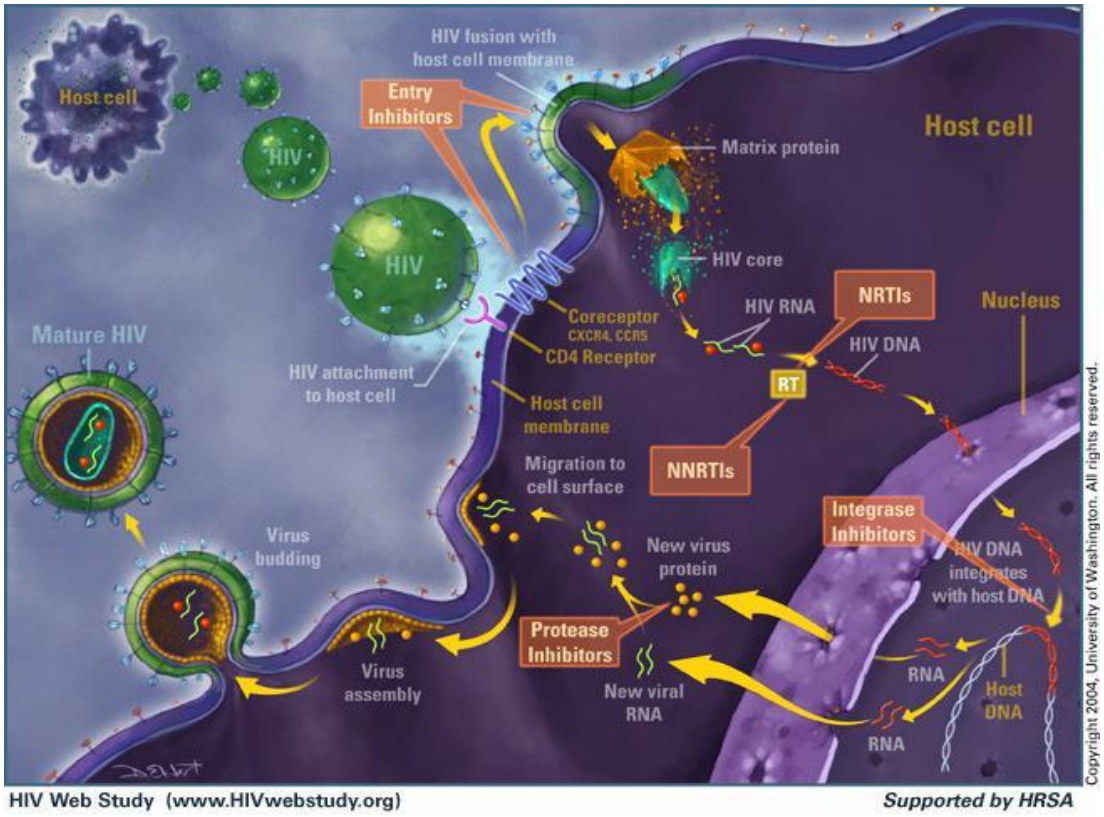


Figure 2.8: The mechanisms of drug inhibition on HIV (Spach & Gallant, 2012).

NNRTIs: also inhibit the synthesis of the viral DNA, but rather than act as a false nucleosides, the NNRTIs bind to reverse transcriptase in a way that inhibits the enzyme's activity (Klatt, 2011; Kwong *et al*., 1998).

PIs: bind to the active site of the viral protease enzyme, preventing the processing of viral proteins into functional conformations. Viral particles are still

produced when the protease is inhibited, but these particles are not infecting new cells (Klatt, 2011; Kwong *et al*., 1998).

Fusion inhibitors: prevent HIV from entering the target cells. Drugs of this class bind the HIV envelope glycoprotein 41 (gp41), which is involved in viral entry. By blocking the interactions between regions of the gp41 molecule, fusion inhibitors interfere with the conformational change (folding) of the envelope molecule required for fusion with the target cell membrane (Klatt, 2011; Kwong *et al*., 1998).

Chemokine co-receptor antagonists: prevent the entry of HIV into target cells. They bind to co-receptors (either CCR5 or CXCR4) on the surface of CD4 cells. By so doing, they block a required step in viral entry. Co-receptor antagonists bind human proteins (Klatt, 2011; Kwong *et al*., 1998).

Integrase inhibitors: bind a viral enzyme known as integrase and interfere with the incorporation of reverse-transcribed HIV DNA into the chromosomes of host cells (Klatt, 2011; Kwong *et al*., 1998).

2.10 BREASTFEEDING

2.10.1 Introduction

Lactation is the medical term for breastfeeding, a natural method of feeding an infant from birth to the time he or she can eat solid food. The saying that "breast is the best" has its origin in biologic merit and several studies have confirmed that even economically deprived and undernourished women have adequate breast milk, similar in quality to milk produced by well-nourished women (Leung & Sauve, 2005; Kramer, 2010). UNICEF declared breastfeeding the superior choice, both physically and economically and many experts see mother's milk as the ultimate health food. UNICEF (2012), not only views breast milk as the best food for newborn babies but also as the only food they need, hence the American Academy of Pediatrics recommends that babies be breastfed at least for six to twelve months.

Breastfeeding exclusively means that an infant receives only breast milk from his or her mother and no other liquids or solids , not even water, with the exception of oral rehydration solution, drops or syrups consisting of vitamins, mineral supplements or medicines (WHO, 2003). For optimal growth and development, infants should be exclusively breastfed for the first six months of life (Campbell, 2008), breastfeeding initiated as soon after delivery as possible (UNICEF, 2012; Leung & Sauve, 2005) since nutrition is crucial for newborn babies. The best source of that nutrition, according to just about every medical source, is breast milk (Champbell, 2008).

Adequate nutrition is the main aim of infant feeding. A baby's growth is more rapid during the first six months than at any other time in its life. Babies double their birth weight in and around four months and have tripled it by the age of one

year. All parts of the body develop quickly and gain tremendously in size. The baby's nutritional needs run parallel with its growth (Bartok, 2011). However, malnutrition has been responsible, directly or indirectly, for 60% of the 10.9 million deaths annually among children under the age of five (Champbell, 2008). The Bellagio Child Survival Group research evidence states that infants aged 0-5 months, not breastfed, had seven-fold and five-fold increased risks of death from diarrhoea and pneumonia respectively, compared with infants who were exclusively breastfed (Doherty *et al.,* 2010). At the same age, non-exclusive rather than exclusive breastfeeding resulted in a more than two-fold increased risk of dying from diarrhoea and pneumonia according to Doherty *et al.* (2010).

Exclusive breastfeeding is the most effective intervention to save the lives of millions of children in developing countries. If exclusive breastfeeding for infants younger than six months could universally be increased by 90%, approximately 1.3 million child deaths per year would be prevented (Doherty *et al.*, 2010; Sadoh, Sadoh, Adeniran & Abhulimhen-Iyoha, 2008). Studies from developing countries showed that infants who were not breastfed were six to ten times more likely to die in the first few months of life than infants who were breastfed. Pneumonia and diarrhoea were also more common and more severe in children who were not breastfed (WHO, 2003). Thus, adequate nutrition during infancy and early childhood is essential to ensure the growth, health and development of children to their full potential. It is said that one third of the estimated 9.5 million deaths that occurred in 2006 in children younger than five were because of poor nutrition (WHO, 2009; WHO, 2003).

Optimal infant and young child feeding practices are among the most effective interventions to improve child health. At least 35% of child deaths are associated with under-nutrition (WHO, 2003) with one in seven children in sub-Saharan Africa having died before their fifth birthday in 2008. This region accounted for half the child deaths worldwide for the same period and is the region with the

highest mortality rate in children under the age of five. Across all regions of the developing world, children from poorer households remain disproportionately vulnerable. In developing countries most children continue to die from preventable or treatable causes, pneumonia and diarrhoea the two main killers. Undernutrition contributes to more than a third of all deaths of under-fives (see Figure 2.9 UNICEF, 2012).

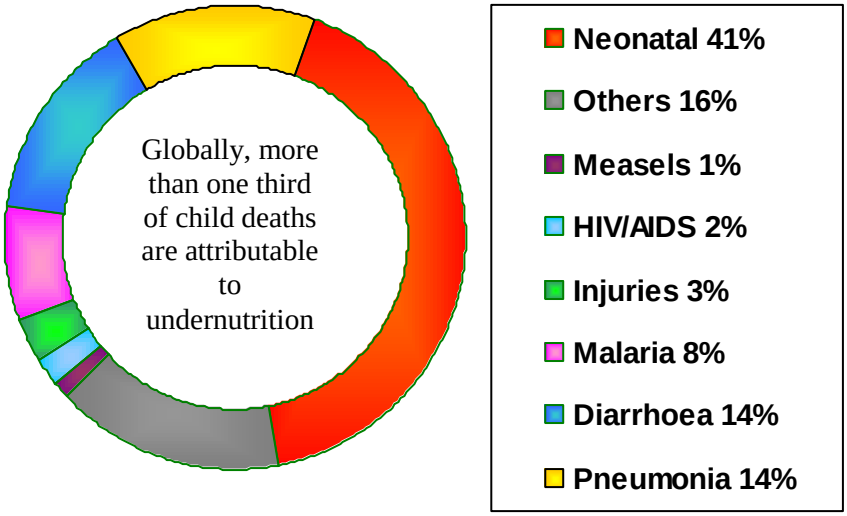


Figure 2.9: Causes of under-five deaths in 2008
Adapted from: UNICEF, 2012.

2.10.2 Breast milk composition

The first food most humans encounter is breast milk, which serves as the sole source of all nutrients required for biological functions and growth during the early stages of life. It is considered the optimal method of infant feeding (Leung & Sauve, 2005).

Colostrum is the special milk that is secreted in the first two to three days after delivery. It is produced in small amounts (40-50mℓ) on the first day. At that time it is all that a baby needs (WHO, 2003). Between the third and sixth day of lactation, the colostrums starts to change to milk, a process completed by the tenth day. This mature milk, thin and bluish in color like skim milk, has an ideal nutrient composition for the infant (Georgeson & Filteau, 2000). Colostrum is rich in antibodies and white blood cells, especially secretory immunoglobulin A (sIgA), and it contains a large amount of proteins, minerals and fat-soluble vitamins (vitamin A, E and K), more so than later milk. Colostrum provides immune protection against micro-organisms in the environment upon first exposure (WHO, 2003).

Breast milk is a species-specific liquid and contains unique substances such as living cells (e.g., macrophages), hormones, antibodies (e.g. immunoglobulins such as IgA), active enzymes (which promote gut maturation, facilitate digestion and stimulate passage of meconium) and other proteins that cannot be artificially supplied to the infant. It serves both a nutritive and immunological function essential for survival (Wagner, 2012). Nutrients that an infant needs in the first 6 months of life are found in breast milk, including fat, carbohydrates, proteins, vitamins, minerals and water. These nutrients are easily digested and efficiently used. Breast milk also contains factors that help with digestion and the absorption of nutrients as well as bioactive factors which augment the infant's immature immune system by providing protection against infection (WHO, 2003).

Humans do not need specific foods for survival; rather, they need the components of food, called nutrients. These nutrients are grouped into six general classes: carbohydrates, fats, proteins, vitamins, minerals, and water, which will be discussed in the following paragraph:

2.10.2.1 Fat

Fat provides about one half of the energy content of breast milk. It is secreted in small droplets and the amount increases as the feeding progresses. The hindmilk secreted towards the end of a feed is rich in fat, while the foremilk at the beginning of a feed contains less fat. The fat in breast milk contains long chain polyunsaturated fatty acids (docosahexaenoic acid or DHA, and arachidonic acid or ARA) which play an important role in the neurological development of the baby and are not available in other milk (WHO, 2003).

2.10.2.2 Carbohydrates

Milk sugar lactose, a disaccharide, is the main carbohydrate in breast milk. It is an important source of energy. Oligosaccharides, or sugar chains, are another kind of carbohydrate present in breast milk and play an important role in the protection against infection (WHO, 2003).

2.10.2.3 Protein

The protein in breast milk differs in quantity and quality from proteins in animal milk. Breast milk protein contains a balance of amino acids which makes it much more suitable for babies (WHO, 2003).

2.10.2.4 Vitamins and Minerals

Unless a mother herself is deficient, breast milk normally contains sufficient vitamins for the infant. Vitamin D is the exception and the infant needs exposure to sunlight to generate endogenous vitamin D, or if this is not possible, a supplement. The minerals, iron and zinc, are present in breast milk in relatively

low concentrations and their bioavailability and absorption is high. If a mother's iron store is adequate, term infants are born with a store of iron to supply all their needs. Vitamin A plays an important role in the protection of the eyes and for the integrity of the epithelial surfaces (WHO, 2003)

2.10.2.5 Anti-infective factors

There are many factors in breast milk which help to protect an infant against infections:

- sIgA – prevents bacteria from entering the cells by coating the intestinal mucosa;
- White blood cells – can kill micro-organisms;
- Whey proteins (lysozyme and lactoferrin) – can kill bacteria, viruses and fungi;
- Oligosaccharides – prevents bacteria from attaching to mucosal surfaces (WHO, 2003).

The protection provided by these factors is uniquely valuable for the infant since it occurs without causing the effects of inflammation, such as fever, which can be dangerous for a young infant. The mother's body forms sIgA which contains antibodies against bacteria and other infections she may have encountered. These automatically protect the baby against bacteria that are particularly likely to be in the baby's environment (WHO, 2003; Georgeson & Filteau, 2000).

2.10.2.6 Other bioactive factors:

Breast milk also contains the following:

- Bile-salt stimulated lipase – digests the fat completely once the milk has reached the small intestine. In artificial milk the fats are digested less;
- Epidermal growth factor – stimulates the maturation of the lining of the infant's intestine. This enhances the digestion and absorption of nutrients, and is less easily infected or sensitized to foreign proteins;
- Other growth factors – the developing and maturation of nerves and the retina (WHO, 2003).

2.10.3 Breastfeeding versus Formula feeding

In resource-poor regions of the world where unhygienic settings and very little financial income prevail, breastfeeding is of particular importance. According to Leung & Sauve (2005), economically breastfeeding is less expensive than formula feeding. Studies have also shown health benefits in that mothers who are poor and HIV-positive, but exclusively breastfeed their infants have a lower risk of infecting the infants with HIV than those who are partially breastfed or who receive mixed feeding (Buskens, Jaffe & Mkhatshwa, 2007; Iliff *et al*., 2005).

For the vast majority of women in these regions the avoidance of breastfeeding is not a realistic option. Studies have shown that even after counselling on feeding choices, HIV-infected women chose to breastfeed their babies, causing one to wonder whether this choice was made through freedom or necessity (Coutsoudis *et al*., 2001).

2.10.4 Factors affecting successful formula feeding:

- Access to a steady, sustainable supply of the feed
- Availability of clean water to prepare the feed
- Access to basic sanitation facilities
- Availability of fuel to prepare the feed
- The mother's health
- Local community infant feeding norms and levels of stigma surrounding replacement feeding
- The mother's ability and willingness to disclose her HIV status to her partner and family (Campbell, 2008; Sadoh *et al*., 2008)

2.10.5 Advantages of breastfeeding for mothers

Advantages for mothers who breastfeed, include the following: early involution of the uterus, enhanced bonding between mother and child, lactation amenorrhea, and the reduction incidence of ovarian and breast cancer (Leung & Sauve, 2005). It is also labour saving since the milk is at the right temperature, and there is no risk of contamination with pathogens. It develops the mother-baby relationship and gives the baby a feeling of security and is the safest way to feed an infant (Leung & Sauve, 2005).

2.10.6 Advantages of breastfeeding for the infant

Breastfeeding can reduce the incidence and severity of infections since the mother's milk contains and provides natural defenses, prevents allergies, obesity and hypertension and insulin-dependent diabetes mellitus and enhances

cognitive development (Leung & Sauve, 2005). The incidence of infection, especially enteral infection, has been found to be higher in artificially fed than in breast fed babies, even in highly developed communities. Breastfeeding, therefore, is generally associated with significantly decreased infant morbidity and mortality rates (Leung & Sauve, 2005).

2.11 HIV AND BREASTFEEDING

2.11.1 Background

The greatest biomedical challenge of this century is HIV-1 infection leading to AIDS. This disease has been devastating because of its concentration in young adults, thus affecting social stability from the level of individual families to national economies since an estimated 33 million people worldwide are infected with HIV (Kourtis, Bulterys, Hu & Jamieson, 2012). HIV, unfortunately, among other diseases may be transferred to the infant through breast milk. There is a continuing debate among developed and developing populations in the world about the breastfeeding of infants born to HIV-infected mothers (Shearer, 2008) with studies having shown that the transmission risk (in percentage) of HIV through breastfeeding may vary between four and nineteen percent. Breastfeeding is responsible for 40% of all MTCT of HIV (Lockman, 2011) and HIV transmission specifically occurs through the cell-rich colostrum fraction of breast milk (Shearer, 2008).

Risk of transmission of HIV from mother to the child is considered to be at its highest during breastfeeding, the breast milk itself serving as a vector of HIV transmission. The first case of MTCT of HIV, with breast milk as the expected carrier, was reported in 1985 (Humphrey & Iliff, 2001). Only then was it shown that the virus was present in breast milk and could reach concentrations

adequate for transmission (Rousseau *et al.*, 2003). Of the estimated 3.6 million children then infected with HIV, 1.2 to 1.8 million were infected through breastfeeding.

Women in developing countries have the difficult choice of balancing the risk of transmitting HIV through breast milk against the substantial benefits of breastfeeding. However, it is questionable whether the benefits of breastfeeding when the mother is HIV-infected can be compared to the benefits when the mother is not HIV-infected, especially in terms of nutrient content. A dangerous situation therefore arises, especially in poor developing environments, with HIV infected mothers being considered as a risk for transmitting HIV to their infant via breast milk, and on the other hand, the risk of infection with deadly microbial contaminants in bottle feeding preparations. HIV-infected populations are burdened with malnutrition and numerous studies have reported that these deficiencies impair immune responses and are associated with accelerated HIV disease progression (Dreyfuss & Fawzi, 2002). As a result, WHO, UNICEF and UNAIDS have adapted infant feeding options for HIV-infected women and now support the possibility that an HIV-infected woman informed of her HIV-positive status will choose the safest feeding option. They recommend that HIV-infected women who decide not to breastfeed their children must be ensured access to sufficient quantities of nutritionally adequate breast-milk substitutes they can prepare safely (Dabis *et al*., 2000).

According to Lockman (2011), WHO released revised guidelines on infant feeding by HIV-infected mothers in low and middle-income settings in July 2010. These guidelines take into account that factors such as cost, lack of access to clean water, refrigeration, materials for boiling water and stigma, do not make formula feeding in developing countries feasible. Also, in low and middle-income countries replacement feeding is being associated with high rates of infant morbidity and mortality related to pneumonia, diarrhea, and other illnesses,

making the avoidance of breastfeeding very risky (Lockman, 2011). The risks and benefits must be weighed when deciding on a feeding option, in fact, the first principle must be a feeding option to optimize the health of the HIV-exposed baby (Lockman, 2011).

Exclusive breastfeeding for the first six months of life, and continued breastfeeding after that for some undetermined duration, will provide the greatest chance of infection-free survival (Shearer, 2008; Humphrey, 2010) since exclusive breastfeeding during the first few months of life carries a lower risk of HIV transmission than mixed feeding. Seeing that breast milk is the natural, not a lifestyle choice, for the poor it becomes the difference between life and death for mothers and babies in developing countries (Coovadia, Rollins, Bland, Little, Bennish & Newell, 2007; WHO, 2003).

2.12 MOTHER-TO-CHILD TRANSMISSION OF HIV

The first description of AIDS in children and the possible transmission from mother to child were first published in the early 1980s (McIntyre & Gray, 2009; Oleske *et al*., 1983; Cowan *et al*., 1984). Breast-milk transmission accounts for up to half of the global HIV infections in children (John-Stewart, 2007). Prolonged breastfeeding in developing countries is common and MTCT of HIV remains considerable, accounting for forty two percent of HIV infections in infants and young children in Africa (Buskens *et al*., 2007).

Although the exact mechanism of transmission from breast milk is not fully understood, it is thought that exposure to microbes in water and food may cause micro-trauma to infants' bowels, which provides an entry point for HIV transmission (Buskens *et al*., 2007). Infection can occur through the entry of cell-free HIV virions or of cell-associated HIV. Both forms of HIV have been detected

in colostrums and breast milk although the viral load in breast milk proved lower than in plasma (Walter, Kuhn & Aldrovandi, 2008; Lehman & Farquhar, 2007; Rousseau *et al*., 2003). The cell-associated virus correlates with the risk of early transmission and both, cell-associated and cell-free, with later transmission (Koulinska *et al*., 2006). The cell-free virus could penetrate the mucosal lining of the gastro-intestinal tract of infants. The virus then infects the inter-epithelial dendritic cells and can be sampled by M cells of the Peyer's patches. It can also enter the submucosa directly through the mechanisms which allow intact proteins to transverse the immature mucosal barrier. Another entry point could be through damaged mucosal foci. These mucosal factors may explain the protective effect of exclusive breastfeeding over mixed feeding, which has been documented in several studies (Kuhn *et al*., 2007; Iliff *et al*., 2005; Coutsoudis *et al*., 2001).

Although antiretrovirals suppress the cell-free they do not do so for the cell-associated virus (Shapiro *et al*., 2005). Nevertheless, the risk of MTCT can still be reduced by antiretroviral strategies. This will necessitate balancing the risk of morbidity and mortality caused by replacement feeding in low resource settings (McIntyre & Gray, 2009) since the comparative risk of death in the first two months of life from other infectious diseases, especially diarrhoea, is six-fold greater in formula-fed children than breastfed children in developing countries (Buskens *et al*., 2007; Coutsoudis *et al*., 2001).

The South African Department of Health and the National AIDS Council released revised guidelines for the prevention of MTCT of HIV in April 2010, stating that all HIV-infected pregnant women with CD4 cell counts of ≤ 350 cells/mm^3 should receive HAART. HIV-exposed infants whose mothers are not on HAART should receive six weeks of ARV prophylaxis and nevirapine with continued prophylaxis until one week after complete cessation of breastfeeding and all confirmed HIV-positive infants should receive HAART from as early as six weeks of age. These

guidelines also state that the approach to infant feeding should not only be to avoid HIV transmission, but also to maximize child survival (Doherty *et al.,* 2010). If ART is administered to mothers during pregnancy and intrapartum and to the infant in the neonatal period it could reduce the overall risk of vertical transmission of HIV-1 to approximately eight percent. ART regimens in resource-poor settings have also resulted in the reductions of MTCT of HIV-1 by 33% to 50% (Newell, 2005; Dabis *et al*., 2000).

Breastfeeding adds to the risk of vertical transmission of HIV-1, especially in infants who are fed a combination of breast milk and other liquids and solids. An estimated 1.5 million children infected with HIV were living in the world at the end of the twentieth century. An additional 500 000 to 600 000 infants were acquiring infections each year from their mothers, the majority living in sub-Saharan Arica. Women now account for nearly half of the five million or so incident infections in adults and with most of them are in their childbearing years, they transmit the infection to their children at a rate of approximately 1500 cases per day (Newell, 2005; Dabis *et al*., 2000).

The most overwhelming source of HIV-1 infection in young children is caused by MTCT. Despite effective antiretroviral therapy, there were approximately 700 000 new infections in children worldwide in the year 2003, according to WHO. The majority of these infections were reported from resource-limited countries (Newell, 2005; Scarlatti., 2004). In 2005, an estimated 630 000 – 820 000 infants were newly infected, 280 000 – 360 000 would have been infected through breastfeeding (Coovadia *et al*., 2007; Newell, 2005). An estimated 1.49 million pregnant women in low and middle-income countries were living with HIV in 2010. Seventy five percent of these pregnant women were concentrated in ten countries, amongst them Kenya, Mozambique, Nigeria and South Africa (UNICEF, 2012).

An HIV infected mother can pass HIV to her unborn baby at any of the following 3 stages:

- During pregnancy: accounts for 15-20% of MTCT cases.
- During delivery: is responsible for the bulk of infections in children as 60-70% of infections happen at this stage.
- Breastfeeding is one of the ways HIV gets passed from mother to child, accounting for 15-20% of infections (UNICEF, 2012).

2.12.1 Potential factors affecting mother-to-child HIV transmission include

The following potential factors affecting MTCT include:

- The pattern of breastfeeding (babies who are breastfed may have a lower risk of becoming infected than those who also consume other liquids, milks, or solid foods in the first months of life) (Coutsoudis *et al.*, 2001)
- Breast health (mastitis, cracked and bloody nipples, and or other indications of breast inflammation are associated with higher risks of transmission)
- Breastfeeding duration
- Maternal viral load (which is higher with recent infection or advanced disease in the mother)
- Maternal immune status, CD4 cell counts
- Maternal nutritional status (Campbell, 2008; Newell, 2005)
- Mode of delivery
- Time of rupture of membranes to delivery
- Prematurity
- Infant birth weight (Newell, 2005)

2.13 WHO GUIDELINES ON HIV AND INFANT FEEDING 2010

World Health Organization guidelines of 2010 on HIV and infant feeding state that the risk of postnatal transmission of HIV through breastfeeding can be reduced with ARV interventions, to either the HIV-infected mother or HIV-exposed infant. This has implications for how women living with HIV might feed their infants. The breastfeeding and ARV intervention can significantly improve infants' chances of surviving.

CHAPTER 3

METHODS

3.1 STUDY DESIGN AND WORKPLAN

This study used a descriptive design. Breast milk of HIV seronegative and HIV seropositive women was measured for nutrient composition. Respondents were all volunteer lodging or day visitors at the Pediatric/Neonatal wards of hospitals and healthcare clinics in the Bloemfontein area: National, Pelonomi and Universitas Hospitals as well as Heidedal, Botshabelo and the Mangaung University of The Free State Community Partnership Programme (MUCPP) clinics.

The breast milk and blood samples were obtained from the volunteers themselves and their clinical data was obtained from their patient files, if available, after permission was granted by the appropriate authorities. Laboratory procedures and measurements were performed by the laboratory of Mowbray Maternity Hospital Milk Depot in Cape Town and the Pathcare Laboratory in Bloemfontein. Below is a summarized layout of the data collection (Figure 3.1).

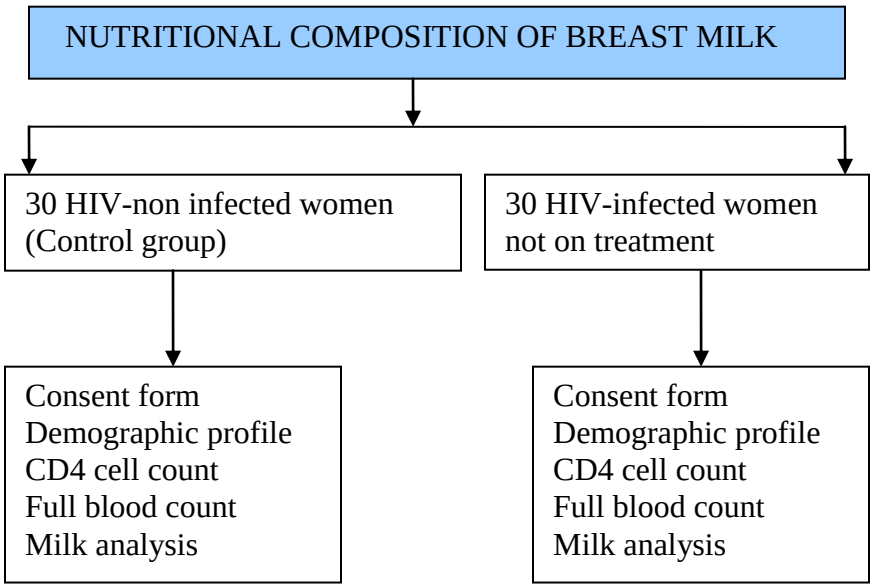


Figure 3.1 Summary lay out of the data collection

3.2 ETHICAL APPROVAL

Approval for the study was obtained from the Ethics Committee of the University of the Free State (ETOVS 107/08). The patients understood that they could withdraw from the study without having to explain their reasons for doing so. After their roles in the study were explained to each participant and they signed an informed consent form.

3.3 POPULATION

Two groups of female volunteers, from the same socio-economic background, were chosen to participate in this study. Participation was voluntary, from lodging or day visiting mothers at the Pediatric/Neonatal wards of the following hospitals in the Bloemfontein area: National, Pelonomi, Universitas as well as clinics in Heidedal, Botshabelo and MUCPP. Volunteers, who met the inclusion criteria and had given written consent, were selected after a screening visit.

3.4 SAMPLE SIZE

The first group consisted of 30 healthy, lactating HIV-non infected women, who represented the control group. The second group consisted of 30 lactating HIV-infected women not on treatment.

3.5 INCLUSION / EXCLUSION CRITERIA

The criteria were set to ensure a homogeneous subject population with accompanying diseases. The 60 respondents who were selected had to meet all inclusion criteria and none of the exclusion criteria and had to have given written consent.

3.5.1 Inclusion Criteria

- All lactating women who exclusively breastfed.
- Respondents had to at least be five days postpartum to avoid the analysis of colostrums as the analysis had to be performed on mature milk. Colostrum is the deep yellow-colored milk secreted the first days after childbirth and its secretion lasts for about five days after birth. It changes to mature milk gradually.
- Respondents must not be breastfeeding for longer than 45 days.
- Respondents had to be between 18-40 years of age.
- Mothers should not been breastfeeding a previous child

3.5.2 Exclusion Criteria

- Evidence of psychiatric disorder, antagonistic personality, poor motivation to participate in this study or non-compliance with protocol requirements.
- History of or current compulsive alcohol abuse (more than 10 drinks weekly) and/or regular exposure to other substance abuse.
- Participation in another study with an experimental drug within eight weeks of the first administration of the study medication.
- Heavy smoking (i.e. more than twenty cigarettes per day).
- Diabetic HIV-positive individuals.

3.6 WITHDRAWAL CRITERIA

Although none of the volunteers withdrew, they had the right to withdraw from the study at any time, no matter what the reason.

3.7 RESPONDENT IDENTIFICATION

Each respondent received a number (01-60) and retained this number throughout the study. The participants' initials and date of birth were obtained from a copy of their identification book.

3.8 RESPONDENT INFORMED CONSENT

After the respondents met the inclusion criteria, they were informed verbally and in writing about the nature of the study. The purpose and the possible risks in taking part in this study were also explained verbally and in writing. In order to facilitate understanding and provide respondents with adequate information, both the written information regarding the respondents and informed consent discussions were included. After verbal consent to participate in the study was given by the volunteer respondents and they had accepted the terms of the study, they signed and dated the informed consent form. The person who explained and conducted the informed consent discussion also signed with the respondents that they had accepted the terms and understood everything.

The respondent information sheets and the informed consent forms were made available in English, Afrikaans and Sesotho (the indigenous language of the region). Respondents were given a copy of both the information sheet and informed consent form in their choice of language.

3.9 SAMPLE COLLECTION

3.9.1 Questionnaire

After the respondents consented to participate in the study, they were interviewed and asked to complete a questionnaire (Appendix A).

3.9.2 Breast milk samples

Breast milk samples were collected just before the mothers started breastfeeding. Participants were asked to abstain from breastfeeding their babies for three hours while the procedure was explained to each participating mother and they were asked to take the sample themselves. Five mℓ of breast milk was collected from each participant in sterile (50mℓ) plastic containers.

3.9.3 Blood samples

Two 5mℓ EDTA tubes of blood were collected from each respondent by a registered medical nurse.

3.9.4 Data from patient files

The patients' medical history, current treatment regimes, as well as their CD4 count were obtained from their medical files, if and where available.

3.10 LABORATORY PROCEDURES

3.10.1 Sample Preparation

3.10.1.1 Breast milk samples

After the breast milk samples were collected in 50mℓ sterile plastic containers, the milk was divided into two separate sterile containers. This was done to ensure a homogenous sample. The milk was frozen for analysis and then sent to two different laboratories (Pathcare in Bloemfontein and Mowbray Maternity

Hospital Milk Depot in Cape Town). The samples were transported, within two weeks of sampling, to Cape Town on dry ice to ensure that they stayed frozen until analyses could be done. The refrigerated milk was defrosted under a stream of warm tap water for the analysis.

3.10.1.2 Blood samples

Blood samples (2 x 5mℓ EDTA tubes) were collected from each respondent by a registered medical nurse. The samples were sent to Pathcare pathology laboratory for analysis. All blood samples were processed according to the SOP of Pathcare. Upon arrival at the laboratory, a full blood count (FBC) and CD4 cell count was done. The EDTA tubes for the viral load count analysis were sent to Pathcare, Cape Town.

3.11 BLOOD ANALYSIS

The following procedures were performed on the blood samples: FBC and CD4 cell counts.

3.11.1 Full blood count

Methodology of the test

The EDTA tubes containing the patients' blood were shaken for a few seconds to allow for proper mixing before being checked for clots with applicator sticks.

A Sysmex® XT-2000*i* analyzer was used for the analysis of the FBC. The Sysmex® XT-2000*i* is an automated heamatology analyzer for *in vitro* diagnostic use in clinical laboratories. The Sysmex® XT-2000*i* performs analysis of the white blood cells (WBC) and reticulocytes with an optical detector block based on

the flow cytometry method, using a semiconductor laser. RBC and platelet (Plt) counts were analyzed by the red blood cell detector using the Hydro Dynamic Focusing method. Hemoglobin was analyzed by the hemoglobin detector based on the sulsolyser (SLS) hemoglobin detection method. The Hct saw to the direct measurement of the red cell transducer. The MCV, MCH and the mean corpuscular hemoglobin concentration (MCHC) are parameters calculated from the red cell parameters.

Quality assurance

Three levels of commercial controls were used for quality assurance: e-Checks 1, 2 and 3.

3.11.2 CD4 cell count

Methodology of the test

All reagents and controls were put on benches to reach room temperature. The EDTA tubes containing the patients' blood samples were vortexed for a few seconds to allow for the proper mixing of the samples. The samples were then checked for clots with applicator sticks. Twenty µℓ of MultiTEST CD3/CD8/CD45/CD4 (catalog nr 342417) reagent was pipetted from the bottom of a TruCOUNT Tube (catalog nr 342447). Fifty µℓ of the patients' blood was added to the bottom of the TruCOUNT tube. The tube was vortexed, gently but thoroughly to allow for the proper mixing of the reagent and the EDTA blood. Afterwards, samples were incubated for 15 minutes in the dark, at room temperature. Four hundred and fifty µℓ FACS Lysing solution (catalog nr 349202) was added to the tube after incubation, before it was vortexed again. The tubes were incubated in the dark for 15 minutes and samples analyzed on the BD FACSCalibur analyzer (manufactured by Beckton Dickenson). Flow cytometry was used to determine the CD4-, CD3- and CD8 positive cells. The CD4 cell count was done to determine the immunological status of the patient. It is an

essential measure for the risk of contracting opportunistic infections and is thus used as an indicator for instituting disease prophylaxis.

Quality assurance

The same procedure was repeated with the Trucount controls, in which low, MultiTEST 4-colour reagents use a time-saving lyse/no wash method for direct immunoflourenscence staining of human peripheral blood specimens. When whole blood is added to TruCOUNT tubes containg MultiTEST reagents, the fluorochrome-labelled antibodies bind specifically to antigens on the surface of the lymphocytes. The FACSCalibur flow cytometer detects four fluorescent colours as well as forward scatter and side scatter.

3.11.3 Viral load

The viral load measures and determines the quantification of HIV. This is the test for staging HIV disease, and monitoring the efficiency of the treatment. The viral load count was analyzed using the COBAS AmpliPrep and the Cobas TaqMan HIV-1 Test, both manufactured by COBAS. This is a nucleic acid amplification test for the quantification of HIV Type 1 RNA in human plasma. It uses the COBAS AmpliPrep instrument for automated specimen processing and the COBAS Taqman analyzer for automated amplification and detection.

Methodology of the test

The COBAS AmpliPrep/COBAS Taqman HIV-1 test is based on three major processes:

- specimen preparation to isolate HIV-1 RNA;
- reverse transcription of the target RNA to generate complementary DNA;

- simultaneous polymerase chain reaction (PCR) amplification of target complementary DNA and detection of cleaved dual-labelled oligonucleotide detection probe specific for the test.

Automated specimen preparations were done by the COBAS AmpliPrep/Cobra TaqMan HIV-1. This step was followed by automated reverse transcription and the PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantification Standard (QS) Armoured RNA. The Master Mix was developed to ensure comparable quantification of group M subtype of HIV-1 and the Master Mix reagent contained a primer pair, specific for both HIV-1 RNA and HIV-1 QS RNA. A target-specific and QS-specific dual-labelled oligonucleotide probe was used for the detection of amplified DNA. This probe permitted independent identification of HIV-1 and HIV-1 QS amplicons.

The HIV-1 QS is used for the quantitation of HIV-1 viral RNA. It is a non-infectious Armored RNA construct and contains HIV sequences with identical primer binding sites to the HIV-1 RNA target and a unique probe binding region. The HIV-1 QS was added to each specimen, at a known copy number, and was carried through specimen preparation, reverse transcription, PCR amplification and detection of cleaved dual-labelled oligonucleotide detection probes. The HIV-1 RNA concentrations were calculated by the COBAS TaqMan analyzer by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control. Accurate quantifications of HIV-1 RNA in each specimen were allowed by the HIV-1 QS, which compensated for effects of inhibition and controlled the preparation and amplification processes. Selection of the target RNA sequence for HIV-1 depended on identification of regions within the HIV-1 genome that showed maximum sequence conservation among the various HIV-1 groups M subtypes and HIV-1 group O specimens. Two regions of the HIV genome were simultaneously targeted for amplification and detection by the COBRAS AmpliPrep/Cobas TaqMan HIV-1 Test in order to address the high genetic variability of the virus. Two target-specific and one QS-specific dual-labelled

oligonucleotide probes allowed for independent identification of the HIV-1 amplicon and of the HIV-1 QS amplicon. The selection of the primers and the dual-labelled oligonucleotide probes was critical for the ability of the test to amplify and detect the HIV-1 group M subtypes and HIV-1 group O. The COBAS AmpliPrep/Cobas TaqMan HIV-1 test used reverse transcription and PCR amplification primers that defined sequences within the highly conserved regions of the HIV-1 *gag* gene and of the HIV-1 LTR region. This test could quantitate HIV-1 RNA over the range of 40 – 10 000 000 copies/mℓ. Qualified medical technologists/technicians performed the viral load testing as per Pathcare Laboratory's SOP for viral load testing.

Quality assurance

HIV-1 L (+) C, HIV-1 H (+) C and CTM (-) C

3.12 BREAST MILK ANALYSIS

Each laboratory's standard operating procedures were followed for the analysis of the biochemical variables in the breast milk. The following variables were included in the analysis of the breast milk.

Macro-nutrients:	Protein (%);
	Lactose (%);
Micro-nutrients:	Calcium (mmol/ℓ);
	Phosphate (mmol/ℓ).
Other:	Fat (%);
	Energy (kcal/100mℓ)

Proteins, lactose and fat were analyzed at the Mowbray Maternity Hospital Milk Depot in Cape Town, while calcium and phosphate were analyzed at both Bloemfontein and Cape Town Pathcare laboratories.

3.12.1 Measurement of Macro-nutrients:

Methodology of the test

The Miris Human Milk Analyzer (manufactured by Miris) was evaluated for breast milk analysis at the University Hospital in Lund, Sweden. All components were analyzed in a single run and the accuracy of the tests was extremely high. The Miris Human Milk Analyzer uses infrared transmission spectroscopy, which is based on approved IR-technology in combination with a new, unique patented technique.

Quality Assurance

On board quality assurance

3.12.2 Measurement of Micro-nutrients:

For the analysis of the micro-nutrients the DXC 800 analyzer (manufactured by Beckman Coulter) was used.

Methodology of the tests:

Phosphate

A timed-rate method using a PHOSm reagent measured the phosphorus concentration in the samples. The inorganic phosphorus reacted with

nammonium molybdate in an acidic solution to form a colored phosphomolybdate complex (107 Beckman Coulter October 2006).

Calcium

The total calcium concentration in the samples was measured by indirect potentiometry. Calcium ion selective electrodes were used in conjunction with a sodium reference electrode. A calcium ion selective electrode measured the unbound free calcium ions in the solution (108, Beckman Coulter, October 2005).

Quality assurance

Synchron 1, 2 and 3.

3.13 STATISTICAL ANALYSIS

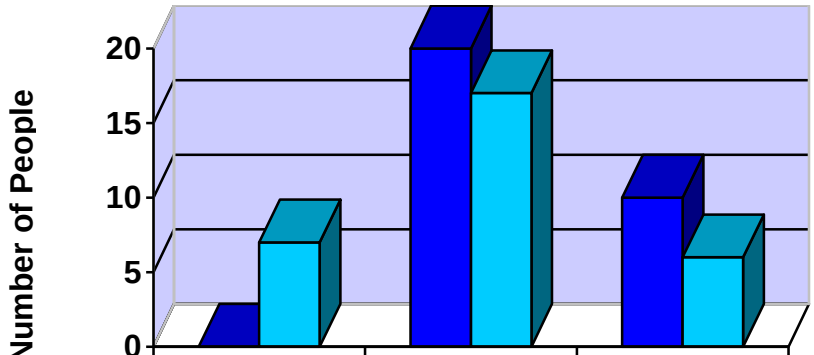
The data for this study was analyzed by the STATISTICA®' 98 Edition, Statsoft Inc. Software. Linear and logistic regression analyses were used to determine the effect of HIV on the nutrient composition of breast milk. Descriptive statistics were used to present data in both tabled and graphic format. Categorical data was compared using the chi-square non-parametric analysis. A p-value of less than 0.5 was considered to be significant.

CHAPTER 4
RESULTS

The results in this chapter will focus on socio demographic status, haematological data and milk composition. The results are given in table and figure form with emphasis on the significant differences. The study population consisted of 60 mothers who were divided into two groups of 30 mothers each. Group one consisted of HIV non-infected mothers who were also the control group. Group two consisted of HIV-infected mothers who did not receive any treatment.

4.1 Socio-economic status

Firstly, the number of people contributing to the household income of the respondents will be discussed.



	None	One Contributin	Two Contributins
HIV non-infected	0	20	10
HIV-infected	7	17	6

p-value 0.0051

Figure 4.1: Number of people contributing to household income (n=60).

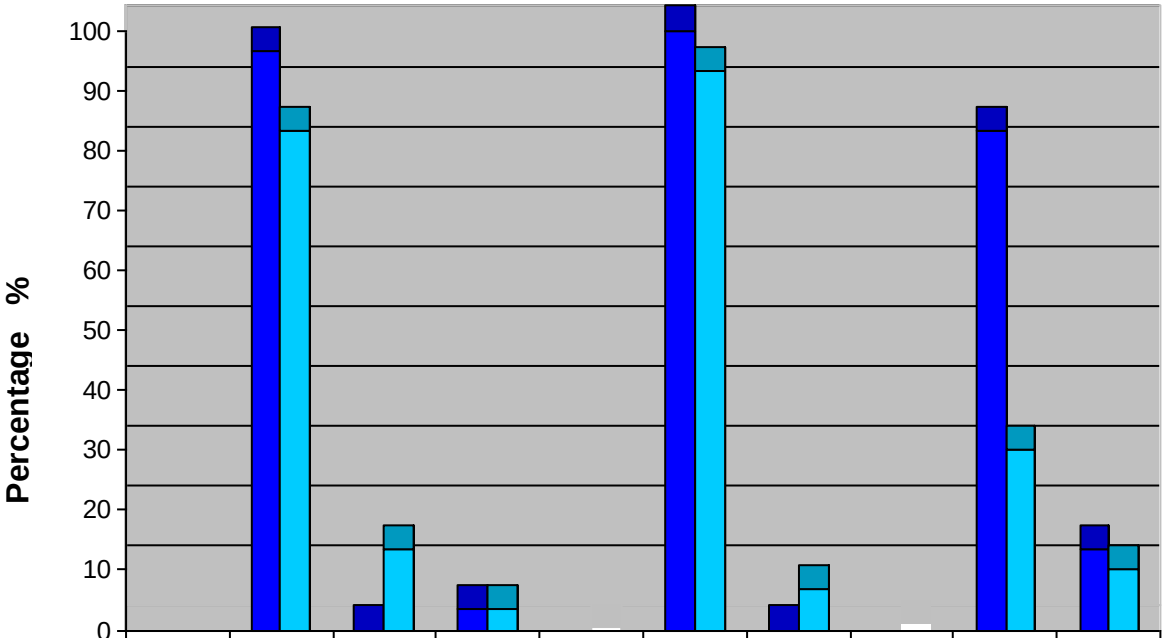
There was a significant difference between the number of people contributing to the household income (p=0.0051). Seven (23.33%) of the mothers living with HIV had no financial support or financial income to support them in the upbringing of their children, seventeen (56.67%) had one person contributing to the household income and six (20.99%) had two people contributing to their household income. In the HIV non-infected group 20 (66.67%) mothers had one person contributing to household income and ten (33.33%) mothers had two people contributing to their household income. The employment status of both the respondent groups showed a significant difference ($p < 0.0001$), see Table 4.1.

Table 4.1: Employment status of the study population (n=60)

Parameters	HIV non-infected (n=30)	HIV-infected (n=30)	Fisher's Exact Test
Employment status			**p < 0.0001***
Housewife by choice	24 (80%)	9 (30%)	
Unemployed	2 (6.67%)	16 (53.33%)	
Self employed	1 (3.33%)	0 (0%)	
Salary earner	3 (10%)	4 (13.33%)	
Other	0 (0%)	1 (3.33%)	

p-value* - shows a significant difference with a value < 0.05

In the HIV non-infected group 24 (80%) most mothers were housewives by choice, two (6.67%) were unemployed, one (3.33%) was self employed and three (10%) were salary earners. In the group of mothers living with HIV, nine (30%) mothers were housewives by choice, 16 (53.33%) unemployed, four (13.33%) salary earners and one (3.33%) had other means of income. In light of this we also looked at the availability of water, electricity and if the respondents had access to refrigeration. Figure 4.2 shows the percentages for the basic household necessities of the two different respondent groups.



	Water	Inside Home	Outside Home	Other Source	Electricity	Yes	No	Fridges	Yes	No
HIV non-infected	p-value	96.67	0	3.33	p-value	100	0	p-value	83.33	13.33
HIV-infected	0.1124*	83.33	13.33	3.33	0.4915	93.33	6.67	0.7755	30	10

p-value* - shows a significant difference with a value < 0.05

Figure 4.2: Basic household necessities of the study population (n=60)

There was no significant difference between the two respondent groups with reference to the availability of water sources (p=0.1124), electricity (p=0.4915) or refrigeration (p=0.7755). In the HIV non-infected respondent group, 29 (96.67%) mothers had access to running tap water in their homes and one (3.33%) had another source of tap water. In the group of mothers living with HIV, 25 (83.33%) mothers had access to running tap water in their homes, four (13.33%) had access to running tap water outside their homes and one (3.33%) had another source of tap water.

With reference to the availability of electricity in the homes of both groups, 30 (100%) mothers in the HIV non-infected respondent group had electricity in their homes and in the group of mothers living with HIV, 28 (93.33%) had electricity in their homes and only two (6.67%) did not. Twenty five (83.33%) mothers in the HIV non-infected group and 24 (80%) mothers living with HIV had fridges in their homes. In the HIV non-infected respondent group four (13.33%) mothers did not have fridges in their homes and one (3.33%) mother used a neighbor's fridge. In

the group of mothers living with HIV, three (10%) mothers did not have fridges in their homes and three (10%) used a neighbor's fridge (Figure 4.2).

Table 4.2 shows the percentage of exclusive breastfeeding between the two respondent groups. There were no significant differences between the 2 respondent groups with reference to exclusive breastfeeding (p=0.3533). In the HIV non-infected group 26 (86.67%) mothers breastfed exclusively and four (13.33%) did not. The frequency of breastfeeding in this group was as follows: 24 (92.31%) mothers breastfed eight times a day, one (3.85%) mother 20 times a day and one (3.85%) 24 times a day. In the group of mothers living with HIV, 29 (96.67%) breastfed exclusively and one (3.33%) did not. The frequency of breastfeeding in this group was as follows: one (3.45%) mother breastfed three times a day, one (3.45%) four times a day and 27 (93.10%) seven times a day. Without a doubt, the demographic questionnaire indicated that both respondent groups thought that their breast milk was the ultimate baby food.

Table 4.2 : Exclusive breastfeeding comparison between the respondent groups

Parameters	HIV non-infected (n=30)	HIV-infected (n=30)	p-value
Exclusive breastfeeding			0.3533
Yes	26 (86.67%)	29 (96.67%)	
No	4 (13.33%)	1 (3.33%)	

p-value* - shows a significant difference with a value < 0.05

4.2 Haematological and immunological data

Table 4.3 shows the median, mean, standard deviation, minimum value, maximum value, normal ranges and p-value of each of the haematological and immunological parameters for the two respondent groups. The WBC, MCHC, Plt,

neutrophil, eosinophil and basophil median values were within the normal reference ranges for both respondent groups. The significant differences can be seen in the following variables between the two respondent groups: RBC ($p < 0.0001$), Hb levels ($p = 0.0119$), Hct ($p = 0.0031$), MCV ($p = 0.0005$), MCH ($p = 0.0043$) and monocyte count ($p = 0.0275$) (see Table 4.3).

In the respondent group of women living with HIV, the median as well as the mean values of the following variables showed a decrease in value: RBC, Hb level and the Hct. With the exception of the Hb, the RBC and Hct counts were all within normal range. The following variables showed elevated results: MCV, MCHC and the monocyte differential count. Although elevated, these variables were still within the normal ranges.

Table 4.3: Haematological parameters for the study population

Haematological Variables	Group (n=30)	Median	Mean	SD	Minimum	Maximum	Normal ranges	p-value
WBC ($10^3/\mu\ell$)	Neg	6.64	6.91	2.13	3.46	12.30	4.0-11.0	0.7618
	Pos	6.59	6.78	2.31	3.48	13.5		
RBC ($10^6/\mu\ell$)	Neg	4.72	4.69	0.38	3.93	5.38	3.7-5.3	**<0.0001***
	Pos	4.00	3.88	0.54	2.83	4.97		
Hb (g/dℓ)	Neg	12.70	12.56	1.57	7.90	15.10	12.0-16.0	**0.0119***
	Pos	11.60	11.42	1.82	6.80	14.70		
Hct (ℓ/ℓ)	Neg	0.40	0.39	0.04	0.28	0.45	0.35-0.45	**0.0031***
	Pos	0.36	0.35	0.05	0.21	0.44		
MCV (fℓ)	Neg	84.00	83.43	6.98	61.00	94.00	81-100	**0.0005***
	Pos	90.00	91.43	10.88	67.00	123.00		
MCH (pg)	Neg	27.5	26.93	2.88	17.00	31.00	28.0-35.0	**0.0043***
	Pos	29.00	29.63	3.93	21.00	38.00		
MCHC (g/dℓ)	Neg	32.00	32.27	1.39	28.00	34.00	32.0-37.0	0.6692
	Pos	32.00	32.47	1.66	29.00	37.00		
Plt ($10^3/\mu\ell$)	Neg	337.50	334.93	73.31	205.00	468.00	140-420	0.5161
	Pos	346.00	350.83	111.32	150.00	656.00		
Neutrophils ($10^3/\mu\ell$)	Neg	3.62	3.95	1.94	1.16	9.26	2.0-7.5	0.8245
	Pos	3.29	3.89	1.96	1.34	9.46		
Lymphocytes ($10^3/\mu\ell$)	Neg	2.27	2.33	0.51	1.55	3.89	1.0-4.0	0.1808
	Pos	2.04	2.14	0.80	0.92	4.27		
Monocytes ($10^3/\mu\ell$)	Neg	0.39	0.42	0.15	0.19	0.81	0.0-0.8	**0.0275***
	Pos	0.49	0.51	0.18	0.24	0.98		
Eosinophils ($10^3/\mu\ell$)	Neg	0.12	0.19	0.19	0.01	0.76	0.0-0.4	0.4197
	Pos	0.16	0.21	0.23	0.02	1.07		
Basophils ($10^3/\mu\ell$)	Neg	0.02	0.03	0.02	0.01	0.1	0.0-0.1	0.9256
	Pos	0.02	0.03	0.01	0.01	0.07		

p-value* shows a significant difference with a p-value <0.05
SD=standard deviation, WBC = white blood cells, RBC = red blood cells, Hb = hemoglobin, Hct = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, Plt = platelets

A significant difference in both subject groups with reference to the immunological status were detected ($p < 0.0001$) (see Table 4.4).

Table 4.4: Immunological parameters for the study population

Haematological Variables	Group (n=30)	Median	Mean	SD	Minimum	Maximum	Normal ranges	p-value
Total T Cell Count (cm^2)	**Neg**	1709.50	1715.90	437.19	1051.00	2991.00	1100-1700	0.9832
	Pos	1756.00	1770.93	735.25	712.00	3742.00		
CD 4 Count (cm^2)	**Neg**	988.00	992.30	290.44	549.00	1958.00	700-1100	**<0.0001***
	Pos	430.50	577.97	377.72	138.00	1447.00		
CD 8 Count (cm^2)	**Neg**	620.50	658.93	211.37	339.00	1050.00	500-900	**<0.0001***
	Pos	1081.50	1138.57	467.13	498.00	2452.00		
CD4/CD8 Ratio	**Neg**	1.64	1.61	0.51	0.81	2.78	1.0-1.5	**<0.0001***
	Pos	0.42	0.53	0.31	0.10	1.27		
HIV viral load (cps/mℓ)	**Neg**	-	-	-	-	-	-	-
	Pos	676	37812	95462	40	478135	-	-

p-value* shows a significant difference with a p=value <0.05
SD=standard deviation

4.3 Composition of milk nutrients

Table 4.5 shows the median, mean, standard deviation, minimum value, maximum value, normal ranges and p-value of each of the chemical variables for the two subject groups. The only significant differences that could be seen in the following variables between the two groups were the percentage proteins (p = <0.0001) and calcium levels (p = 0.0081). The median as well as the mean values of the proteins were elevated in the subject group of mothers living with HIV. The calcium levels in the same group showed a decrease in both median and mean values. The reference ranges used in Table 4.5 were obtained from previous studies.

Table 4.5: Composition of milk nutrients

Variables	Group (n=30)	Median	Mean	SD	Minimum	Maximum	Normal ranges	p-value
Fat (%)	**Neg**	2.85	4.14	4.10	0.80	18.00	3 – 5 % [a] 4.5 % [b]	0.3146
	Pos	3.10	4.32	4.54	1.30	26.90		
Proteins (%)	**Neg**	0.90	1.05	0.79	0.10	4.00	0.8 – 0.9 % [a] 1.1 % [b]	**<0.0001***
	Pos	1.80	2.04	1.50	0.50	7.50		
Lactose (%)	**Neg**	5.75	5.79	1.20	1.80	10.50	6.9 – 7.2% [a] 6.8 % [b]	0.1427
	Pos	5.45	5.46	1.52	1.90	7.80		
Energy (kcal/100mℓ)	**Neg**	55.00	66.53	41.91	32.00	211.00	60 – 75 [a]	0.1450
	Pos	61.50	70.20	39.19	33.00	263.00		
Calcium (mmol/ℓ)	**Neg**	5.43	5.33	0.90	3.75	7.10	2.5 – 3.0 mmol/ℓ [a]	**0.0081***
	Pos	4.43	4.49	1.57	0.65	8.15		
Phosphate (mmol/ℓ)	**Neg**	1.43	1.52	0.47	0.50	2.85	1.3 – 1.6 mmol/ℓ [a]	0.1448
	Pos	1.18	1.34	0.72	0.50	2.95		

p-value * shows a significant difference with a p-value < 0.05
SD=standard deviation
[a] Values obtained from the Perintol, 1979.
[b] Values obtained from Mustafa, 2001

CHAPTER 5
DISCUSSION

5.1 INTRODUCTION

Since the first case of HIV and AIDS was reported 25 years ago, it has become one of the most highly studied diseases in history. The complexity, place-specific, social, economic, behavioral and psychological drivers of the spread of HIV and AIDS remain less well-delineated. And still unfolding today is the consequences of increased illness and death in poor communities and countries (Gillespie, Greener, Whiteside & Whitworth, 2007).

5.2 SOCIO-ECONOMIC STATUS

There is an increasing interest among researchers with regard to the relationship between SES and health. The physical and social environment in which individuals work and live is associated with SES. Individuals with fewer socio-economic resources usually encounter less social support and have decreased access to means for restoring and maintaining health (Adler & Snibbe, 2003). The inverse relationship between SES and health is one of the most consistent social epidemiological findings. The SES-health relationship is not only found in more developed and industrial countries, but also in developing countries such as South Africa (Mulatu & Schooler, 2002).

Two variables that differed significantly between the two respondent groups were the number of people contributing to the household income ($p=0.0051$) as well as the respondents' employment status ($p<0.0001$). The question that arises is whether income and employment levels have independent effects on an individual's health status (See Figure 4.1 and Table 4.1).

South Africa is a country struggling with a legacy of poverty and economic inequality in the midst of an HIV epidemic and is one of the countries which is affected the worst (Gillespie *et al*., 2007; Booysen & Summerton, 2002). In 2005 a United Nations publication stated that poverty increases vulnerability to HIV and AIDS. This statement is complex in the fact that the HIV burden is concentrated mainly in the poorest regions of the world but not always among the poorest populations in the areas. In South Africa, for example, the prevalence rates of HIV are highest among the youth living in poor urban informal settlements as compared to other areas (Gillespie, Kadiyala & Greener, 2007; Dinkelman, Lam & Leibbrandt, 2007; Booysen & Summerton, 2002).

In the 1990's, *The New England Journal of Medicine*, published various articles which documented marked differences in death rates among individuals at different income and educational levels. These showed that there was a graded association between income and mortality and that the effect was greatest at the lowest income levels, especially for infant mortality (Adler & Snibbe, 2003). Consequently, higher incidence and prevalence of health problems, disease and death are associated with a lower SES. This negative relationship can be found whether social inequality is measured at individual level or at the level of neighborhood community or even society (Mulatu & Schooler, 2002). So it is often argued that poverty is the root cause of the spread of HIV (Gillespie *et al*., 2007).

The results that were obtained in reference to household necessities, showed no significant difference between the two respondent groups' water sources ($p=0.1124$), electricity ($p=0.4915$) and refrigeration ($p=0.7755$). Based on this, it could be argued that should the mother choose not to breastfeed her child, she would have the means available to her for alternative feeding. If a mother whished to bottle feed her child, she would need access to clean water, refrigeration and materials for boiling water. This study showed that these

mothers, HIV-infected or not, did indeed have access to water and sanitation infrastructure (See Figure 4.2).

Since the outbreak of HIV more than 25 years ago, there have been numerous debates about whether an HIV-infected mother should breastfeed or not even though the benefits of breast feeding have been proven and established throughout the years (Anatolitou, 2012; Symon, 2012). Although the HI-virus can be transmitted through breastfeeding and this should be taken into account, there are many more factors to consider before making a decision about infant feeding. No significant differences were found in this study with reference to exclusive breastfeeding (p=0.3533). Although all the respondents regarded their breast milk as the best food for their babies, the choice to breastfeed whether or not there is HIV infection, still remains a personal one.

The South African Government stated in August 2011 that South Africa is one of only 12 countries in the world where infant mortality has been on the increase. The Tswane declaration in support of excusive breastfeeding was therefore brought to light and the 2010 WHO guidelines on HIV and infant feeding which recommend that all HIV infected mothers should breastfeed, were adopted. As such, it has been recommended that South Africa moves to an exclusive breastfeeding strategy, except when an authorized health practitioner recommends milk formula (Department of Health, 2012).

5.3 HAEMATOLOGICAL AND IMMUNOLOGICAL VARIABLES

5.3.1 Haematological Variables

Haematological variables are sometimes difficult and intriguing to interpret, because when the results are put into perspective, there are always other variables excluded from the study, which may have been worth analyzing for interpretation purposes.

In this study there were a few statistical differences in the haematological variables of the different respondent groups. Significant differences were seen in the decreased values of the RBC ($p<0.0001$), Hb ($p=0.0119$) and Hct ($p=0.0031$) (see Table 4.3). RBC, also known as erythrocytes, contains Hb which transports oxygen (O_2) and carbon dioxide (CO_2) acts as acid-base buffer and supply energy and ions to the body. The RBC count usually rises or falls with the Hb and Hct parameters, but decreases in conditions such as anemias, hemolysis, chronic renal failure, hemorrhage and failure of marrow production (Curry, 2012), since it shows the number of red blood cells per unit volume of blood.

An important clinical problem in HIV-infected patients and those with AIDS is anemia. Since blood loss is the most obvious cause of anemia, the pathophysiology of HIV-associated anemia may include three basic meganisms, a decrease in red blood cell production, an increase in red blood cell destruction or ineffective red blood cell production (Masaisa, Gahutu, Mukiibi, Delanhe & Philippé, 2011; Behler, Shade, Gregory, Abrams, Volberding, 2005).

Decreased RBC production - may be a consequence of infiltration of the bone marrow by neoplasm, infection, use of myelosuppressive medication, HIV infection itself, a decreased production of endogenous erythropoietin, blunted response to erythropoietin or hypogonadism (Masaisa, Gahutu, Mukiibi, Delanhe & Philippé, 2011; Behler *et al*., 2005; Claster, 2002).

Increased RBC destruction – premature or increased RBC destruction in the spleen or the circulator system may occur in patients with HIV infection. Hemolytic anemia may occur as a result of RBC autoantibodies, hemophagocytic syndrome, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura or glucose-6-phosphate dehydrogenase deficiency (Masaisa, Gahutu, Mukiibi, Delanhe & Philippé, 2011; Behler *et al*., 2005; Claster, 2002).

Ineffective RBC production – may result from nutritional deficiencies, most commonly, deficiencies in iron, folic acid or vitamin $B_{12.}$ In HIV-infected persons, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology (Masaisa, Gahutu, Mukiibi, Delanhe & Philippé, 2011; Behler *et al*., 2005; Claster, 2002).

Hemoglobin is the main component of red blood cells and serves as the transporter of O_2 and CO_2 in the blood. The Hb can be used to determine the presence of anemia or polycythemia (Merritt, 2012) and the Hct is the fraction of whole blood composed of RBC. Causes of a decrease in Hct include, but are not limited to anemia, bleeding, red blood cell destruction, bone marrow suppression or underproduction, malnutrition and nutritional deficiencies, infection, overhydration and pregnancy (O'Leary, 2012). Studies have shown that hemoglobin is an independent prognostic factor in both ART-naïve individuals and those commencing therapy (British HIV Association guidelines, 2012). The Hb levels of the HIV-infected group were lower than the normal reference levels and were also lower than the Hb levels of the control group (see Table 4.3). The risk factors currently associated with anemia in HIV infection could include: a history of clinical AIDS, a CD4 cell count less than 200 cells/μℓ, plasma viral load, women, black race, Zidovudine use, increasing age, lower body mass index, history of bacterial pneumonia, oral candidiasis and a history of fever.

The variables that showed an increase were the MCV (p=0.0005), MCH (p=0.0043) and the monocyte count (p=0.0275). The increases in the above mentioned parameters were evident in the values of the HIV-infected respondent group, but were still within normal reference ranges (see Table 4.3). MCV is the average volume of red cells in a specimen and is elevated or decreased in accordance with the average red cell size. A high MCV indicates macrocytic (large average RBC size), a common cause of macrocytic anemia (increased MCV) as well as folate deficiency anemia, Vitamin B deficiency anemia, liver

disease, hemolytic anemias, hypothyroidism, excessive alcohol intake, aplastic anemia and myelodysplastic syndrome (Curry, 2012).

MCH is the content (weight) of Hb of the average red cell. MCH is an indicator of red blood cell, used in the diagnosis of anemia to determine if an anemia is hypo, normo or hyperchromic. MCH on its own does not add significant, clinically relevant information (Merritt, 2012). The WBC count, on the other hand, is a component of a complete blood cell count and is the enumeration of white blood cells in a small volume of whole blood. The only variable that showed a significant difference was the monocyte count (p=0.0275) (see Table 4.3). Monocytes are phagocytes or myeloids that surround, engulf and digest bacteria or other particles. Common causes of an elevated monocyte count or monocytosis include chronic infections such as tuberculosis, bacterial endocarditis, rickettsiosis, malaria, collagen vascular disease and inflammatory bowel disease (Naushad, 2012).

5.3.2 Immunological Variables

The only two immunological variables tested in this study were the CD4 cell count and viral load. These two immunological variables are the most common markers used for HIV confirmation and are also used for the initiation of therapy. The respondents' HIV status was obtained from their medical records and CD4 cell counts as well as a viral load were done on their blood and used as a confirmatory test.

The CD4 cell count is used as a guideline for the initiation of treatment for HIV-infected persons and is required to accurately assess the immune status of any given patient at any given time. HIV has a particular tropism for cells with the CD4 protein on their surface. Macrophages, glial cells in the brain, T-helper cells and T-regulator cells all have CD4 on their surfaces. $CD4^+$ T cells can be lost through a number of mechanisms and cell death can occur as part of the natural

immune response and cell activation that occurs with any chronic infection. HIV itself is also directly cytotoxic to T cells. Long before these mechanisms were fully understood, AIDS was characterized by a specific loss of $CD4^+$ T cells (Bennett, 2012).

Several areas of the immune system are affected by the loss of $CD4^+$ T cells. Both cellular CD8 responses and humoral antibody responses become less effective due to the lack of T-cell help. In adults, the $CD4^+$ T cell count has the most influence. CD4 is a protein that lives on the surface of infection fighting WBC called T-helper cells. HIV targets these immune cells. The CD4 count declines over time in untreated individuals and may vary from time to time (see Table 4.4). In people infected with HIV who are not getting treated, CD4 cell counts generally decreases as HIV progresses (Carpenter, 2012). The positive subject group's mothers are known to be living with HIV, without receiving any treatment.

The mean value of the CD4 cell count of the HIV-infected respondents was 577 cells/mm^3; this is lower than the normal reference ranges as given in Table 4.4. Persons with a CD4 cell count above 350 cells/mm^3 are usually asymptomatic or early stage infection, although they have enlarged lymph nodes. The immunological results obtained in Table 4.4 showed a comparison between the early stages of HIV infection and the haematological findings in Table 4.3. The length of the asymptomatic phase varies from person to person, lasting anything form six to eight years in most people. In about five to ten percent of people it can last for many years and in some for decades. In others there is a rapid fall in the CD4 cell count and progression to the symptomatic phase happens within six to 12 months (Carpenter, 2012).

Primary HIV infections are associated with high plasma viral loads (see Table 4.4). These viral loads decline about four to six months after infection to a nearly steady level, with a small but asymptomatic phase of infection. In the advanced

stages of HIV, the viral load increases sharply, coinciding with the onset of AIDS. The viral load has long been established as a strong predictor of the rate of disease progression and may influence the choice of antiretroviral agents (British HIV Association guidelines, 2012).

5.4 MACRO- AND MICRO NUTRIENTS

No significant differences were seen between the two respondent groups with reference to the fat (p=0.3146), lactose (p=0.1427), energy (p=0.1450) and phosphate levels (p=0.1448). The significant differences were in the percentage protein (p<0.0001) and calcium levels (p<0.0081) (see Table 4.4). Human milk contains a wide variety of proteins that contribute to its unique qualities, so due to financial constraints, this study could only analyze the total protein levels of said milk. The fact that only the proteins were analyzed made interpretation difficult. Human milk proteins provide an important source of amino acids to rapidly growing infants and also play a role in facilitating the digestion and uptake of other nutrients in breast milk. Breast milk proteins exert numerous physiologic activities such as the enhancement of immune function, defense against phatogenic bacteria, viruses and yeast, and the development of the gut and its functions (Lönnerdal, 2003). In this study, the protein levels among the HIV-infected respondent group showed an elevation (see Table 4.4). Comparison to other studies was particularly difficult since a wide variety of analytical methods had been used over years in these studies. Several different methods were used for protein analysis in different studies and yielded different results 9g/ℓ (WHO, 2009) for mature milk and 14-16g/ℓ during early lactation (Lönnerdal, 2003). It was shown that protein concentration changes as lactation progresses. Although the protein content of human milk decreases rapidly during the first month of lactation and the decline is much slower after, it is relatively invariable between women at any given stage of lactation (WHO, 2002). In general it appears that neither protein malnutrition nor protein supplementation has little if any effect on the protein concentration in human milk (Lönnerdal, 2003).

Calcium is essential for tissue formation, bone structure and function and its metabolism is modulated by other nutrients, hormones and trace elements (Loui, Raab, Obladen & Brätter, 2002). In this study, the calcium levels of the HIV-infected group were lower than those of the HIV non-infected group and both respondent groups' calcium levels were higher than the reference ranges given (see Table 4.4). Comparison to other studies was particularly difficult since a wide variety of analytical methods were used over years in these studies. As said by the WHO, human milk contains 250-300mg/ℓ calcium with no pronounced changes during lactation. Maternal diet does not appear to influence the concentration of calcium in milk, but recent studies from Gambia (2002) indicated that poorly nourished women on low calcium diets produced milk with lower than normal calcium levels, which did not increase with calcium supplementation (WHO, 2002).

CHAPTER 6
CONCLUSION

The HIV pandemic has caused an ongoing debate about the best way for HIV-infected mothers to feed their children. This has led to confusion around infant feeding messages and an erosion of breastfeeding practices since HIV can be transmitted from mother-to-child trough breastfeeding. In developing countries the key issue in HIV-affected populations is reduced to decisions on how to balance the hazards of MTCT of HIV, against the risks of mortality and heightened morbidity among infants.

6.1 CONCLUSION

Since there is very limited research data available in the 20th century concerning the effect of HIV on breast milk, the results of this study could prove useful for future investigations dealing with the effect of HIV on the nutrient composition of breast milk. This study originated from the question whether HIV had an effect on the nutrient composition of breast milk.

Previous studies (Adler & Snibbe, 2003; Malatu & Schooler, 2002) implied that SES had a direct influence on the prevalence of HIV and that health problems of individuals associated with different levels of SES were not necessarily associated with socio-economic class itself, but rather with the associated factors related to the different levels of socio-economic status such as HIV.

In conclusion, the results from this study indicate that there are differences in the nutrient composition of breast milk of mothers that are HIV-infected. The differences can be seen in the percentage proteins and calcium levels of breast milk. Although limited research data was available, the normal reference ranges

on the composition of breast milk showed a correlation between the results obtained from this study to those from other studies. However, it also seems that if the individual is relieved from the burden associated with a specific socio-economic background, everyone seems to have the same health status.

6.2 RECOMMENDATIONS

The results from this study clearly underline the need for further investigation. The same study design can be used but a larger study population should be included in a subsequent study. The true effect of SES on the prevalence of HIV can also be evaluated. The problem associated with the interpretation of the results is hidden within the fact that the word "socio-economic status" could be very controversial, as no quantitative value could be ascribed directly to it.

Only a limited panel of tests could be performed on the composition of the breast milk. Further investigations need to be done on the composition of breast milk with a wider range of tests being conducted.

Major factors influencing the public's point of view on HIV and breastfeeding that need urgent attention include the role of peer pressure, advertising, government health regulations and general breastfeeding education. However, it will not be easy to evaluate the specific role of these factors on the mothers' feeding choice for her infant. From this study we can indicate that breast is still regarded as the best.

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APPENDIX A

UNIVERSITEIT VAN DIE VRYSTAAT
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2008-07-25

MS M HATTINGH
C/O PROF FJ VELDMAN,
SCHOOL OF HEALTH TECHNOLOGY
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BLOEMFONTEIN
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Dear Ms Hattingh

ETOVS NR 107/08
MS M HATTINGH DEPT OF BIOMEDICAL TECHNOLOGY, CUT
PROJECT TITLE: NUTRIENT COMPOSITION OF BREAST MILK IN HIV-SEROPOSITIVE WOMEN

- You are hereby informed that The Ethics Committee approved the above study at the meeting on 22 July 2008.
- Committee guidance documents: Declaration of Helsinki, ICH, GCP and MRC Guidelines on Bio Medical Research. Clinical Trial Guidelines 2000 Department of Health RSA; Ethics in Health Research: Principles Structure and Processes Department of Health RSA 2004; Dept of Health: Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition 2006; the Constitution of the Ethics Committee of the Faculty of Health Sciences and the Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines.
- Any amendment, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.
- The Committee must be informed of any serious adverse event and/or termination of the study.
- A progress report should be submitted within one year of approval of long term studies and a final report at completion of both short term and long term studies.
- Kindly refer to the ETOVS reference number in correspondence to the Ethics Committee secretariat.

Yours faithfully

for **PROF WH KRUGER**
CHAIR: ETHICS COMMITTEE
Cc Prof FJ Veldman, CUT, Bloemfontein

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APPENDIX B

QUESTIONNAIRE

NUTRIENT COMPOCITION OF BREAST MILK IN HIV SEROPOSITIVE WOMEN

SOCIO ECONOMIC STATUS AND PERSONAL INFORMATION

Name: _____

Questionnaire number: ☐☐☐ 1 -3

Birth date: ☐☐☐☐☐☐☐☐ 4 - 11

Interview date: _____

Address: _____

_____

1. Name of the baby: _____

2. Birth date of the baby: ☐☐☐☐☐☐☐☐ 12 -

3. Sex of the baby:

Boy		Girl		☐ 20

4. How many children are you breastfeeding: _____ ☐ 21

5. How many people contribute to the income of the family: _____ ☐ 22

6. What is the highest education level of the caregiver: _____ ☐☐ 23 -

7. Do you have access to the following:

Refrigerator in the house	1	2	☐	25
Refrigerator of a neighbour	1	2	☐	26
Running tap water in the house	1	2	☐	27
Running tap water outside the house	1	2	☐	28
Other source of water Specify _____			☐☐	29 -
Electricity	1	2	☐	31

8. Are the baby being breast-fed exclusive?

Yes	1	No	2	☐ 32

9. If the above answer is yes, how many times a day do you breastfeed the baby:

	Times	☐ 33

10. What is the best drink for your baby:

Tea	1	
Cow's Milk	2	
Formula	3	
Mothers Milk	4	
Other, specify _____	5	☐ 34

11. Employment status:

Housewife by choice	1
Unemployed	2
Self employed	3
Full time wage/salary earner	4
Other, specify _____	5
Don't know	6

☐ 35

12. How many days per week do you work:

_____ ☐ 36

13. Do you follow any special diet?

Yes	1	No	2

☐ 37

14. If yes, please specify:

Diabetic	1
Slimming	2
Allergies	3
Other, specify _____	4

☐ 38

15. Do you take any vitamin supplements?

Yes	1	No	2

☐ 39

16. Indicate which of the following best describe the eating patterns you usually follow:

More than 3 meals per day with eating between meals?	1
3 Meals per day with eating between meals?	2
3 Meals per day with no eating between meals?	3
2 Meals per day with eating between meals?	4
2 Meals per day with no eating between meals?	5
1 Meal per day with eating between meals?	6
1 Meal per day with no eating between meals?	7
Nibble the whole day, no specific meals?	8

☐ 40