THE POSSIBLE ASSOCIATION BETWEEN STAGE OF HIV DISEASE AND THE NUTRIENT COMPOSITION OF BREAST MILK

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DECLARATION OF INDEPENDANT WORK

I, GETA DE WET, identity number [redacted] and student number 20011318, do hereby declare that this research project submitted to the Central University of Technology Free State for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology Free State; and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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SIGNATURE OF STUDENT DATE

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SUMMARY

Breastfeeding is a major source of childhood nutrition and protection, but with South Africa having one of the highest HIV prevalence in the world the risk of HIV transmission from mother to infant through breastfeeding becomes a major issue. Infant mortality due to malnutrition and infections is also of great concern. Exclusive breastfeeding and giving antiretroviral drugs to the HIV-infected mother and the HIV-exposed infant is one of the most significant ways to improve infant survival rates and reduce transmission of HIV through breastfeeding. Whether HIV disease progression and its metabolic impact on the mother will affect the nutrient composition of breast milk is a question that arises.

The aim of this study was to determine the possible association between the stage of HIV disease, as measured by the immunological markers, and the nutrient composition of breast milk.

The study population consisted of 60 HIV infected female volunteers who were divided into two groups. Milk and blood samples were obtained from 30 HIV-positive women that was not on any ARV treatment and from 30 HIV-positive women that was on ARV treatment. Their HIV status and treatment regime were obtained from their files. Participants were also asked to complete a questionnaire.

Macro-nutrients that were measured included lactose, proteins, fat, total solids and the energy content of the breast milk. This was done on the MIRIS Human milk analyser. The micro-nutrients that were measured were calcium and phosphate on the DXC 800 chemistry analyser. Blood analysis was included to determine the stage of HIV disease progression in the HIV-positive mothers and comprised of a CD4/$CD8^+$ T cell count, viral load and a full blood count. CD4/$CD8^+$ T cells were determined using flowcytometry on the BD FACScalibur. The COBAS

AmpliPrep/COBAS TaqMan HIV-1 Test was used for the determination of the viral load and the full blood count was done using a Sysmex XT2000i haematology analyser.

When comparing the analysed haematological variables, the white blood cells and red blood cells indicated a significant difference between the two groups. Both of the groups were anaemic. The $CD3^+$ T cell count was higher and the $CD4^+$ T cell count was lower than the reference range in both groups. The median $CD4^+$ T cells and HIV-1 viral load for the HIV with treatment group was higher than the HIV-infected without treatment group.

The analyzed milk data yielded no p-value of great significance, suggesting that there was no statistically significant difference recorded of the measured nutrients between mothers receiving treatment and those who did not receive any treatment for HIV.

The Spearman Correlation Coefficient was used to determine if HIV disease progression would have an influence on the nutrients that were measured. For the HIV-infected without treatment group, a significant correlation was found between the HIV-1 viral load and percentage total solids in breast milk. For the HIV-infected with treatment group the only positive correlation was between the $CD4^+$ T cell count and the percentage total solids and energy content of the breast milk. No strong positive correlation could be established between the immunological markers of HIV disease progression and the analysed nutrients in the breast milk.

Taking this into consideration, HIV-positive mothers can breastfeed their babies even if their HIV status is at a more advance phase, but the emphasis should be placed on exclusive breastfeeding and getting the needed support to breastfeed.

Keywords: Breastfeeding, nutrients, HIV, breast milk, disease progression.

TABLE OF CONTENTS

CHAPTER 3

METHODOLOGY

LIST OF APPENDIXES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percentage
+	positive
=	equals
≤	less than or equal to
®	registered
µℓ	microlitre
3TC	2'3'-dideoxy-3'thiacytidine
ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ARA	arachidonic acid
ART	antiretroviral therapy
ARV	antiretroviral drugs
AZT	Azidovudine/Retrovir
BDNA	branched deribonucleic acid amplification
CCR-5	C-C chemokine receptor type 5
$CD3^+$	cluster of differentiation 3 positive
$CD4^+$	cluster of differentiation 4 positive
$CD8^+$	cluster of differentiation 8 positive
cDNA	complementary deoxyribonucleic acid
cm^2	cubic centimetre
cps	copies
CXCR4	CXC-chemokine receptor type 4
DHA	docosahexanoic acid
dℓ	decilitre
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EFV	Efavirenz

ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
fℓ	fentolitre
FTC	Emtricitabine
g	gram
HAART	highly active antiretroviral therapy
HB	haemoglobin
HCT	haematocrit
HIV	Human Immunodeficiency Virus
HLA	human leukocyte antigen
HPV	human papilloma virus
HSV-1	Herpes simplex virus type 1
IL-8	Interleucin-8
IR	Infrared
kcal	kilocalories
ℓ	liter
ℓ/ℓ	litre per litre
LPV	Lopinavir/ritonavir
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDG	millennium development goals
mℓ	millilitre
mm^3	cubic millimetre
mmol	millimol
mRNA	Messenger ribonucleic acid
MTCT	mother-to-child transmission
MUCPP	Mangaung University of the Free State Community Partnership Programme

NASBA	nucleic acid sequence-based amplification
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside analogue reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
PCR	polymerase chain reaction
pg	picogram
PI	protease inhibitor
PLT	platelets
PMTCT	preventing mother to child transmission
QS	quantitation standard
RANTES	regulating on activation normal T cell expressed and secreted
RBC	red blood cells
REE	resting energy expenditure
RNA	ribonucleic acid
RT	Reverse Transcriptase
SD	standard deviation
sIgA	secretory immunoglobulin A
SIV	Siminian immunodeficiency virus
SLPI	secretory leukocyte protease inhibitor
SLS	sulfolyser
SOP	standard operating procedures
TDF	Tenofovir
UNICEF	United Nations Children's Fund
US	United States
WBC	white blood cells
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

South Africa is one of the countries with the highest prevalence of HIV (Human immunodeficiency virus) in the world. To prevent mother-to-child transmission of HIV through breastfeeding, the South African government rolled out a policy providing free infant formula for all HIV-positive mothers over a decade ago (Bloemen, 2012). Although under-five mortality dropped by 35% worldwide between 1990 and 2010, nearly 21 000 children under the age of five die daily, mostly from preventable causes with 99% of these deaths occurring in developing countries (WHO, 2012). South Africa currently lags behind the Millennium Development Goal on reducing child mortality (Bloemen, 2012).

Although South Africa lagged behind in reaching its Millennium Development Goal there was a major policy shift in South Africa when the Tswane Declaration was accepted in 2011. This Declaration recommends that all babies should be exclusively breastfed for at least the first six months of their lives, and that HIV-positive mothers should receive antiretroviral therapy throughout the breastfeeding period. Their infants should also receive antiretroviral therapy (Motsoaledi, 2011). All of the abovementioned dramatically reduce the risk of transmission. However, with South Africa's breastfeeding rate at an extreme low of 8% it is extremely challenging to promote exclusive breastfeeding (Bloemen, 2012).

Globally 30% of children under five are estimated to be stunted and 18% have a low weight-for-height. This is mostly as a consequence of poor feeding and repeated infections. Under nutrition is associated with 35% of the disease burden for children under five, and infant and young child feeding is a key area to improve child survival and to promote development and healthy growth (WHO, 2010d). Also, the under-five mortality rate is higher in rural areas and among poorer, less educated communities and three-quarters of all child deaths are mainly due to preventable

causes (Bloemen, 2012). Pneumonia, responsible for nearly 1.4 million deaths every year, is the largest single cause of death in children under the age of five followed by diarrhoeal disease which accounts for 840 000 deaths in this age group (WHO, 2012).

Early and exclusively breastfeeding, is one of the most significant ways to improve infant survival rates. This is because breast milk is an important source of energy and nutrients for children (WHO, 2010d). It provides all the nutrients infants need to grow and strengthens their immune systems (Bloemen, 2012). The risk of mortality due to diarrhoea and other infections can increase in infants who are either partially breastfed or not breastfed at all (WHO, 2010d), since they will not be getting all the benefits of breast milk.

A woman, who is infected with HIV, can transmit the virus to her child during pregnancy, labour or delivery, and also through breast milk. Therefore, the risk of infants acquiring HIV through breastfeeding should be considered alongside the higher risk of death from causes such as malnutrition and illnesses such as diarrhoea and pneumonia (WHO, 2010d).

HIV-infected populations are burdened with malnutrition and numerous studies have reported that these deficiencies impair immune response and are associated with accelerated HIV disease progression (Dreyfuss and Fawzi, 2002). Energy requirements must increase by 10% to maintain body weight and physical activity in asymptomatic HIV-infected adults, and during symptomatic HIV energy requirements increase by approximately 20% to 30% to maintain body weight (WHO, 2003).

The $CD4^+$ T cell count serves as a major laboratory indicator of immune function in patients who have HIV infection and is one of the key factors in deciding whether to initiate antiretroviral therapy (HHS Panel on Antiretroviral Guidelines for Adults and Adolescents, 2011).

Giving antiretroviral drugs to the HIV-infected mother and the HIV-exposed infant can significantly reduce the risk of transmission of HIV through breastfeeding (1-2%).

HIV-infected mothers can therefore offer their infants the same protection against the most common causes of child mortality and the benefits associated with breastfeeding (WHO, 2010d).

It is not clear whether HIV disease progression and its metabolic impact on the mother will affect the nutrient composition of breast milk. No information that correlates the quality of breast milk with HIV disease progression could be found. It is therefore not known whether the stage of HIV disease will have a significant impact on the quality of the breast milk's nutritional value. This kind of information is important for Public Health purposes, as a decision making tool to establish at which point additional food, over and above breast milk, should be given to the infant and could provide additional nutrition guidelines to support the health of the infected mother. This would be to the benefit of both mother and feeding infant.

1.2 AIM

The main aim of this study was to investigate the possible association between the stage of HIV disease, as measured by the cluster of differentiation four positive ($CD4^+$) T cell counts, and the nutrient composition of breast milk in breastfeeding female subjects.

1.3 STRUCTURE OF THIS DISSERTATION

The structure of this dissertation is as follows:

Chapter 2 represents an extensive literature review in which the most critical information, that is needed to understand and interpret the aim of the study and the results of this study, is discussed.
Chapter 3 provides information about the blood and milk preparation and all the test procedures that were used in this study.
Chapter 4 reflects the results of this study.
In Chapter 5 the results are discussed and
Chapter 6 presents the limitations, conclusion and recommendations.

CHAPTER 2
LITERATURE REVIEW

2.1. HISTORICAL OVERVIEW

HIV has been defining our generation's medical and public health issues since the world first became aware of Acquired Immune Deficiency Syndrome (AIDS). According to Fauci (2003) this disease has spread around the globe and continues to have an enormous effect throughout the world, especially in sub-Saharan Africa. The progress that has been made since the 1950s in health, education, life expectancy and standards of living has been reversed in Africa by the spread of HIV/AIDS (Inungu and Karl, 2006).

The first description of HIV was in 1981 when young homosexual men in San Francisco and New York City presented with opportunistic infections that were typically associated with severe immune deficiency due to *Pneumocystis pneumonia* or *Karposi sarcoma.* At first, various factors such as lifestyle factors, chronic drug abuse and other infectious agents were considered as causing this disease (Bennett and Greenfield, 2011; Hooper, 2000). However, in 1983 HIV was identified as the etiological agent of AIDS (Bennett and Greenfield, 2011; Fauci, 2003).

The zoonotic nature of HIV was established in 1986 when the close phylogenetic relationship between HIV-2 and the siminian immunodeficiency virus (SIV) was established in sooty mangabeys in Western Africa. HIV-1 was considered to probably have originated from one or more cross-species transfers from the *Pan troglodytes troglodytes* specie of chimpanzees in Central Africa. One theory posits that the interspecies jump from chimpanzees to humans, probably occurred by accident because chimpanzees are killed for food in parts of sub-Saharan Africa (Bennett and Greenfield, 2011; Mayer, 2005; Fauci, 2003). Another theory holds that chimpanzee kidneys were used as a substrate for growing polio vaccines, thereby potentially providing a means for SIVs to infect humans (Martin, 2000).

The pathogenic mechanism of HIV disease is complex and multifactorial, meaning that the immune system is activated at the same time an individual might be experiencing immune deficiency. While this is taking place, regular cellular activity is interrupted and some cells become depleted. As a result, the depletion of $CD4^+$ T cells was recognized early on as a hallmark of the disease (Fauci, 2003; Grossman, Meier-Schellersheim, Sousa, Victorino and Paul, 2002).

The next critical advancement in the identification of HIV was the development of a sensitive and specific test for antibodies. By 1985 the blood supplies in the United States and other developing countries were screened for HIV with the Enzyme-linked immunosorbent assay (ELISA) test and, according to Fauci (2003), millions of potential transfusion-related infections were prevented by this development.

The next development was a highly sensitive technique for the precise quantification of small amounts of nucleic acids, an essential component in the monitoring of individuals with HIV.

The course of the virus can be tracked by accurate measurement of the quantity of viral ribonucleic acid (RNA). This is done by assays using polymerase chain reactions (PCR) or nucleic acid sequence-based amplification (NASBA) of the viral source or branched deribonucleic acid amplification (BDNA) of the signal that can detect a virus down to a few hundred copies per millilitre in commonly used tests and down to a few copies using the latest ultrasensitive tests. Therapeutic decisions are guided by the PCR in conjunction with the $CD4^+$ T cell count. The relationship between the amounts of virus and the rate of disease progression, rate of viral turnover, the relationship between immune system activation and viral replication and the response to therapy are clarified by the abovementioned techniques (Sen, 2007; Fauci, 2003). A stable $CD4^+$ T cell count is associated with undetectable HIV RNA in peripheral blood and a decline in the $CD4^+$ T cell count is associated with increased HIV RNA (Sen, 2007; Osmond, 1998).

Second to the identification of HIV as the cause for AIDS, most advances have occurred in the development of effective antiretroviral drugs. HIV drug discovery

revolves around vulnerable targets in the replication cycle of the virus. Azidovudine (zidovudine) (AZT), a reverse transcriptase inhibitor, was the first effective drug against HIV. AZT, which was originally developed as an anticancer drug but was not effective in that capacity, was licensed in 1987 as the first antiretroviral drug (Fauci, 2003; WHO International Agency for Research on Cancer, 2000). It was identified through a screening process, using large numbers of compounds that had already been produced for other purposes.

Further drug development was made on the basis of targeting the vulnerable points in the virus's replication cycle. The HIV protease enzyme is expressed, purified, and crystallized to facilitate the tailored design of protease inhibitors – a class of antiretroviral drugs (ARV) that was first approved in 1995 by the United States Food and Drug Administration (FDA) (Fauci, 2003).

Fusion inhibitors are the newest class of drugs in development. They block the fusion of the viral envelope to the cell membrane and were approved in 2003 by the FDA. New and improved drugs, in all three classes, are actively pursued along with drugs against alternative targets such as the viral integrase. Since these drugs have been used in combinations of three or more drugs, the morbidity and mortality rate of individuals infected with HIV has sharply declined in developed nations (Hughes, Barber and Nelson, 2008; Fauci, 2003).

The future of a vaccine might be determined by the global distribution of clades. The B clade is rarely found in developing countries which are more severely affected by HIV (Bennett and Greenfield, 2011; Mayer, 2005; Fauci, 2003).

2.2. HIV PREVALENCE

Sixty million people have been infected with the virus since the beginning of the epidemic and nearly 30 million people have died of AIDS since then. An estimated 33.3 million people were living with HIV in 2009 and there were 2.6 million new infections 1.8 million of which were in the World Health Organization's (WHO) African Region (WHO, 2010a). Of the global total 1.8 million of AIDS related deaths,

72% (1.3 million) comprised of Africans and there were 11.3 million people living with HIV (WHO, 2010a; UNAIDS, 2010). It is estimated that 5.6 million people in Southern Africa were infected with HIV in 2009. Globally, 34% of people living with HIV were residing in the 10 countries in Southern Africa and, according to Figure 2.1. (UNAIDS, 2010), South Africa's epidemic was the largest in the world. An estimated 10.5% of the total population in South Africa was HIV-positive in 2010 (Statistics South Africa, 2010).

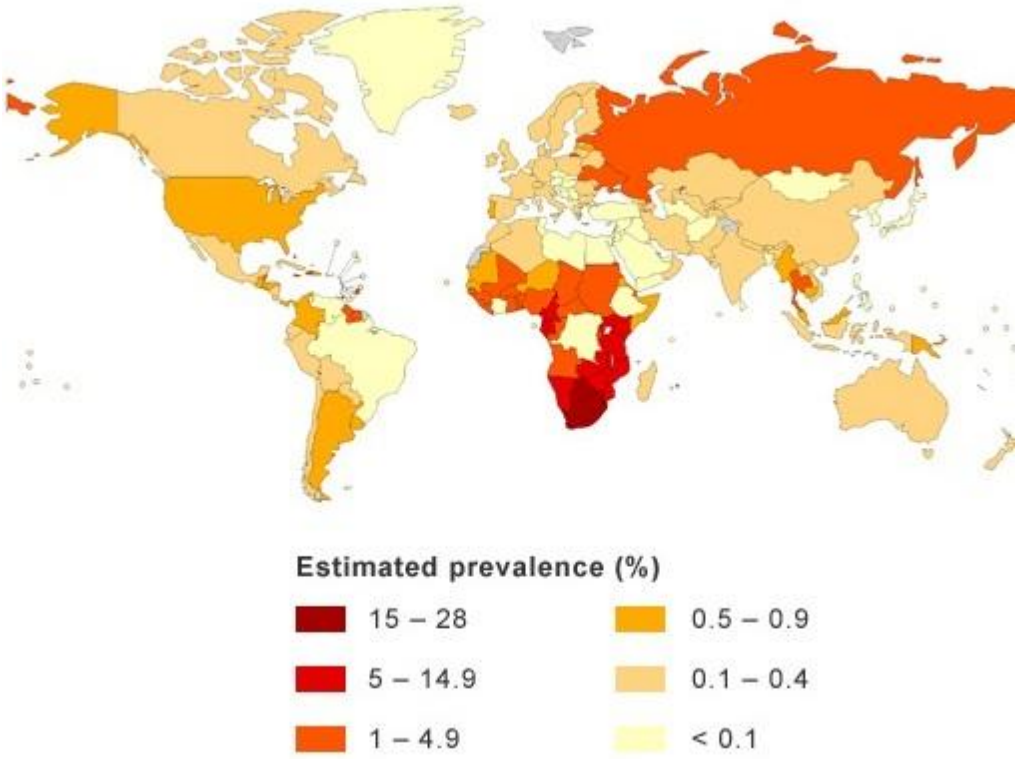


Figure 2.1 Estimated HIV prevalence among the population aged 15-49 years as seen in 2009 (Adapted from UNAIDS, 2010)

No matter where they live, girls and young women are especially vulnerable to HIV infection, and account for over 60% of all young people living with HIV. In sub-Saharan Africa, young women made up nearly 70% of all people living with HIV as seen in Figure 2.2 (UNICEF, 2010). Approximately 40% of all adult women with HIV were living in Southern Africa (UNAIDS, 2010) and approximately one-fifth of South African women who were in their reproductive years were HIV-positive (Statistics South Africa, 2010).

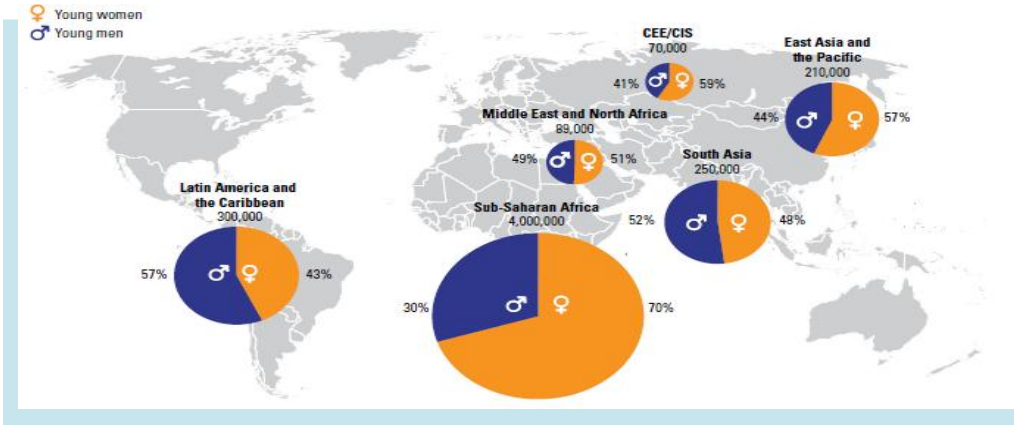


Figure 2.2 **Estimated numbers and percentage of young people 15–24 years old living with HIV, by region as seen in 2008** (Adapted from UNICEF, 2010)

Table 2.1 illustrates the HIV prevalence estimates and the number of people who were living with HIV for the years 2001 to 2010 in South Africa. As can be seen the numbers had increased since 2001, and in 2010, 19.7% of the population of women were HIV-positive.

Table 2.1 **HIV prevalence estimates and the number of people living with HIV in South Africa for the period 2001-2010** (Adapted from Statistics South Africa, 2010)

Population 15-49 years			Percentage of the total population	Total number of people living with HIV (in millions)
Year	Percentage of women	Percentage of the population		
2001	18.7	15.4	9.4	4.10
2002	19.2	15.8	9.6	4.38
2003	19.4	16.1	9.8	4.53
2004	19.6	16.3	9.9	4.64
2005	19.7	16.5	10.0	4.74
2006	19.7	16.6	10.1	4.85
2007	19.7	16.7	10.2	4.93
2008	19.7	16.9	10.3	5.02
2009	19.6	17.0	10.3	5.11
2010	19.7	17.3	10.5	5.24

In 2009, 2.5 million children had been infected with HIV, 2.3 million residing in sub-Saharan Africa as seen in Table 2.2. It also shows that, of the 15.9 million women who were infected with HIV, 12.1 million were residing in sub-Saharan Africa and 3.3 million in South Africa. In 2009 there were 16.6 million AIDS related orphans globally, 14.8 in Sub-Saharan Africa and 1.9 million in South Africa. This is shown in Table 2.3.

Table 2.2 Table comparing the Global, sub-Saharan Africa and South African estimates for HIV prevalence in adults and children; and women and children in 2001 and 2009; and adults in 2009
(Adapted from Statistics South Africa, 2010; UNAIDS, 2010)

YEAR	2001	2009	2009	2001	2009	2001	2009
	Adults & Children	Adults & Children	Adults 15+ years	Women 15+ years	Women 15+ years	Children 0-14 years	Children 0-14 years
GLOBAL	28 600 000	33 300 000	30 800 000	13 600 000	15 900 000	2 000 000	2 500 000
SUB-SAHARAN AFRICA	20 300 000	22 500 000	20 300 000	10 900 000	12 100 000	1 800 000	2 300 000
SOUTH AFRICA	5 300 000	5 600 000	5 300 000	2 600 000	3 300 000	170 000	330 000

Table 2.3 **Table comparing the Global, sub-Saharan Africa and South African estimates for HIV prevalence in young women and men; adults and children who became newly infected; AIDS-related deaths in adults and children and; living AIDS related orphans for 2009 and 2001** (Adapted from Statistics South Africa, 2010; UNAIDS, 2010)

YEAR	2009	2009	2009	2009	2009	2001	2009	2001
	Young women 15-24 years Prevalence (%)	Young men 15-24 years Prevalence (%)	Adults & children newly infected	Adults newly infected	Aids related deaths in adults & children	Aids related deaths in adults & children	Orphans 0-17 years currently living	Orphans 0-17 years currently living
GLOBAL	0.6	0.3	2 600 000	2 200 000	1 800 000	1 800 000	16 600 000	10 000 000
SUB-SAHARAN AFRICA	3.4	1.4	1 800 000	1 500 000	1 300 000	1 400 000	14 800 000	8 900 000
SOUTH AFRICA	13.6	4.5	390 000	340 000	310 000	220 000	1 900 000	580 000

2.3. PATHOPHYSIOLOGY

HIV-1 and HIV-2 are retroviruses in the Retroviridae family, *Lentivirus* genus. They are enveloped, diploid, single-stranded, positive-sense RNA viruses with a deoxyribonucleic acid (DNA) intermediate, which is an integrated viral genome that persists within the host-cell DNA (Bennett and Greenfield, 2011; Barré-Sinoussi, 1996).

2.3.1. HIV-types and diagnosis

There are various types and respective subtypes of HIV, according to Bennett and Greenfield (2011) and Klatt (2011), with HIV-1 and HIV-2 being the two major types. HIV-1 is responsible for the majority of infections globally while HIV-2 is rare outside West Africa. If the latter is reported outside West Africa there is usually some epidemiological link to that region (Grant and de Kock, 2001). Figure 2.3 shows the

difference in how the genes of HIV-1 and HIV-2 are laid out (Stowell, 2006). Neutralizing antibodies, rarely found in HIV-1, are present in HIV-2 and their presence is equivalent to a vaccine response. As a result, people with HIV-2 live longer and have a response associated with delayed disease progression. The different types and subtypes of HIV pose the problem that the detection methods for diagnosing HIV-1 and HIV-2 differ and not all methods designed to diagnose HIV-1 will detect HIV-2. Fortunately, perinatal transmission of HIV-2 occurs less often. The ARV treatment for HIV-2 also differs from that of HIV-1 because of a single amino acid, Leu-188 (Klatt, 2011).

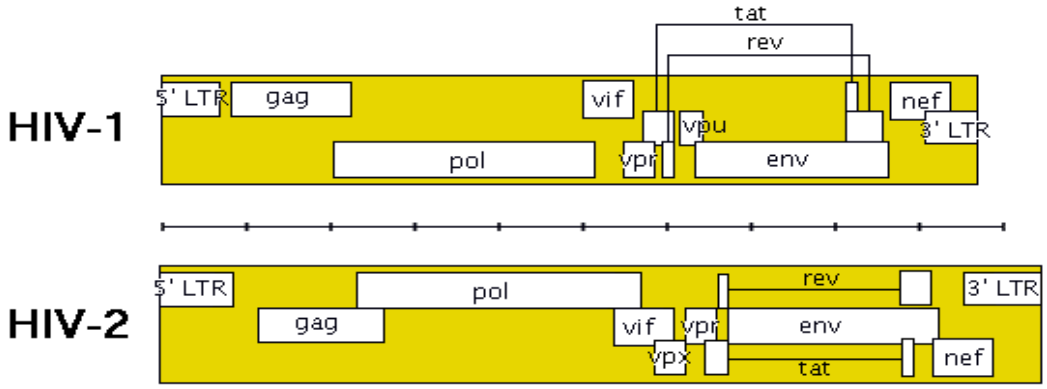


Figure 2.3 The different amino acids present in HIV-1 and HIV-2
(Adapted from Stowell, 2006)

Table 2.4 shows the different criteria for diagnosing HIV in adults and children as defined by the World Health Organization (WHO) in 2007.

Table 2.4 WHO case definition for HIV infection (Adapted from WHO, 2007)

HIV infection is diagnosed based on:	
Adults and children >18 months	**Children < 18 months**
Positive HIV antibody testing (rapid or laboratory-based enzyme immunoassay). This is confirmed by a second HIV antibody test (rapid or laboratory-based enzyme) relying on different antigens or of different operating characteristics; and/or; Positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination.	Positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination taken more than four weeks after birth. Positive HIV antibody testing is not recommended for definitive or confirmatory diagnoses of HIV infection in children until 18 months of age.

2.3.2. HIV life cycle; phases and staging

HIV is a blood-borne disease and there are numerous ways of transmission. These include sexual intercourse, shared intravenous drug paraphernalia, and mother-to-child transmission (MTCT), the latter which can occur during the birth process or during breastfeeding (Bennett and Greenfield, 2011).

Figure 2.4 shows the various steps in the life cycle of HIV starting with binding and fusion (step 1 as described below) and ending with budding (as described in step 6).

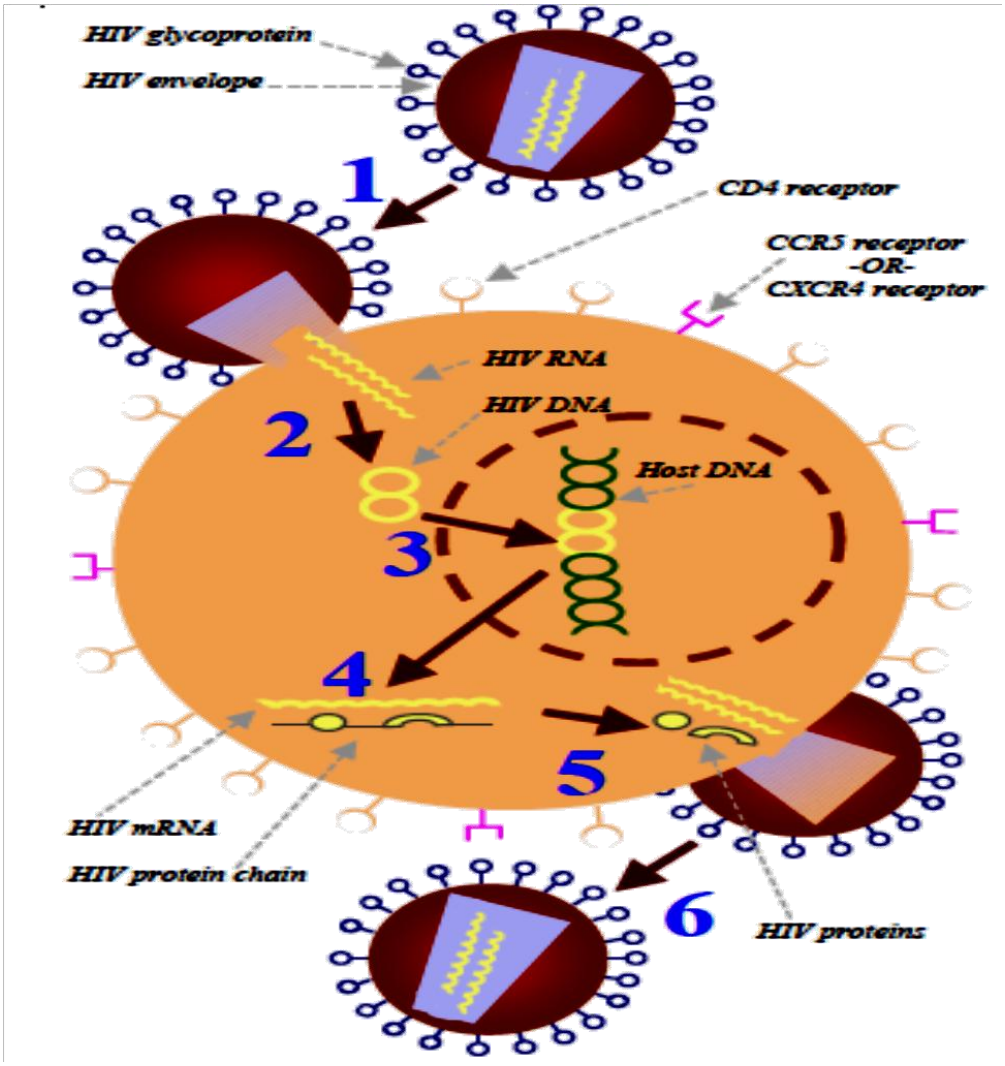


Figure 2.4 HIV life cycle (Adapted from AIDSinfo.com, 2005)

According to AIDSinfo (2005) the HIV life cycle is as follows:

1. Binding and Fusion: The HIV virus starts its life cycle when it binds to a CD4 receptor and one of two co-receptors on the surface of a $CD4^+$ T cell. Fusion with the host cell takes place and the virus releases RNA into the host cell.

2. Reverse Transcription: The single stranded HIV RNA is converted to double-stranded HIV DNA by reverse transcriptase (RT).

3. Integration: The HIV DNA enters the host cell's nucleus and the HIV DNA is hidden within the host cell's own DNA by the enzyme integrase. This is called a provirus and can remain inactive for several years.

4. Transcription: Copies of HIV genomic material are created by RNA polymerase when the host cell receives a signal to become active. Messenger RNA (mRNA) is also produced and is used to make long chains of HIV proteins.

5. Assembly: The long chain of HIV protein is cut into smaller individual proteins by protease and, together with copies of the HIV's RNA genetic material, a new virus particle is formed.

6. Budding: The new virus particle "buds" from the host cell. During this process the new virus steals part of the cell's outer envelope which is studded with protein/sugar combinations called HIV glycoproteins. These glycoproteins are needed for the binding of the virus to CD4 and co-receptors. New copies of HIV can move on to infect other cells.

The virus replication accelerates and massive viremia leads to the dissemination of the virus throughout the body's lymphoid tissue. Because there is usually only partial immunological control of virus replication, continual and accelerated production of viruses ensues. Lymphocyte depletion occurs as a result of a rapid turnover of $CD4^+$ T cells (Fauci, 2003).

The anti-HIV cluster of differentiation positive ($CD8^+$) T-cell response is inversely correlated to the size of the proviral reservoir which, in turn, correlates to the steady-state viral load. Aggressive treatment of acute infection may lower the proviral load. In this phase the viral load is high and the $CD4^+$ T cells drop. When anti-HIV antibodies appear and the $CD8^+$ T cells respond, the viral load drops to a steady state and the $CD4^+$ T cells return to levels within normal range although slightly lower than before infection. According to Bennett and Greenfield (2011) seroconversion may take anywhere from a few weeks up to several months.

Following seroconversion, plasma HIV-1 RNA levels are equilibrated to a value known as a "set point". The viral set point is most likely a measure of the dynamics between the virulence of the infecting virus strain and the host's immune system's

ability to contain the virus. A high viral set point correlates with faster disease progression. Viral replication during primary and early infection in infants is more variable than that seen in adults. Most infants experience a peak HIV-1 RNA viral load in the first few weeks after infection. This peak will decline in some infants to a set point, but in others will continue to gradually decrease for several months or years. High HIV-1 RNA viral load levels found in infants are highly predictive of disease progression, unlike in adults where the set point determines disease progression (Sen, 2007; Richardson *et al.,* 2003).

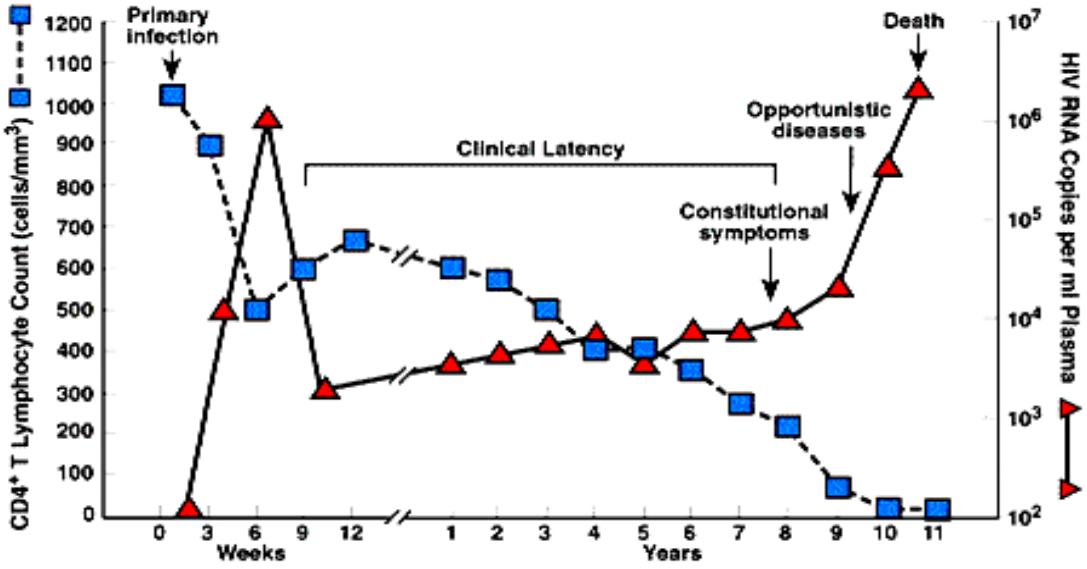


Figure 2.5 The typical course of HIV infection (Adapted from the Department for Work and Pensions, 2009)

Clinical HIV infection undergoes distinct phases: – acute seroconversion/primary infection, asymptomatic phase, symptomatic phase and advanced phase/AIDS (Bennett and Greenfield, 2011; Department for Work and Pensions, 2009). Figure 2.5 reflects these phases with the effect the infection has on the $CD4^+$ T cells and plasma HIV RNA viral load levels as described below.

2.3.2.1. <u>Primary infection:</u>

Between 2 and 6 weeks after exposure to the human immunodeficiency virus acute seroconversion takes place. The virus infects the exposed individual.

2.3.2.2. Asymptomatic phase ($CD4^+$ T cell count greater than 350/µℓ)

Persons in this stage show few or no signs of the infection for a few years to a decade or more. Although viral replication is ongoing during this period the immune response is still effective and vigorous. The viral load stays relatively steady but the $CD4^+$ T cells steadily decline (Bennett and Greenfield, 2011; Department for Work and Pensions, 2009).

2.3.2.3. Symptomatic phase ($CD4^+$ T cell count 200-350 /µℓ)

The person becomes increasingly susceptible to a number of infections like pulmonary tuberculosis, shingles, pneumococcal pneumonia, recurrent oral and vaginal candidiasis (thrush) and, rarely, oral hairy leukoplakia (white lesions on the side of the tongue caused by the Epstein-Barr virus) (Department for Work and Pensions, 2009).

2.3.2.4. Advanced phase ($CD4^+$ T cell count less than 200/µℓ) (AIDS)

The immune system responds to opportunistic and, normally harmless, commensal organisms and the $CD4^+$ T cells start to decline if the host fails to act on these infections (Bennett and Greenfield, 2011).

The time it takes for HIV infection to take place varies greatly. It ranges from 1 year or less in some persons to a still unknown upper limit in others. That upper limit has reached nearly 20 years in a few individuals. The epidemiology of HIV disease progression has tried to characterize the distribution of possible lengths of the incubation period and the AIDS survival period. They have also tried to determine what cofactors accelerate or retard the disease's progression rate. In the absence of a combination of treatments, it is likely that all HIV-infected persons will lose $CD4^+$ T cells and progress to AIDS. Even with a near normal $CD4^+$ T cell count lymphocytes show abnormalities that suggest their long-term immune functioning will be impaired. The median incubation period from HIV infection until AIDS development is approximately 10 years for young adults (Osmond, 1998). Although studies have

documented faster rates of disease progression and deaths in infants than in adults, children infected in utero and intrapartum have the fastest progression (Luzuriaga *et al*., 2006).

Advanced HIV infection is diagnosed on immunological $CD4^+$ T cell counts (as seen in Table 2.5) and/or clinical criteria (as seen in Table 2.6) in people with confirmed HIV infection. In cases where both the clinical as well as the immunological classification is available the immunological classification is usually used (WHO, 2007).

Table 2.5 WHO immunological classification for established HIV infection (Adopted from WHO, 2007)

AGE RELATED CD4 VALUES				
HIV-associated immunodeficiency	**<11 months (%$CD4^+$)**	**12-35 months (%$CD4^+$)**	**36-59 months (%$CD4^+$)**	**>5 years (absolute number per mm^3 or %$CD4^+$)**
None or not significant	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Advanced	25-29	20-24	15-19	200-349
Severe	<25	<20	<15	<200 or <15%

Although a four-stage clinical staging system for adults was developed in 1990 for clinical purposes, a three-stage system for children was only proposed in 2002 to support rolling out antiretroviral therapy (ART). The 2007 WHO case definitions of HIV, for surveillance and revised clinical staging and immunological classification, replaced the 2003 version. It stays in line with the 1990 classification of disease for adults and young people and proposes that the appearance of new or recurrent clinical staging events or immunodeficiency must be used to assess individuals once they receive ART. Clinical staging can be used once HIV infection has been confirmed (Weinberg and Kovarik, 2010; WHO, 2007).

Table 2.6 Criteria for HIV staging events in adults (15 years or older) (WHO, 2007)

Clinical Stage 1 (Asymptomatic)
○ Persistent generalized lymphadenopathy
Clinical Stage 2 (Mild symptoms)
○ Unexplained moderate weight loss (<10% of bodyweight) ○ Recurrent upper respiratory tract infections (current event plus one or more in last six-month period). ○ Herpes zoster ○ Angular cheilitis ○ Recurrent oral ulceration (two or more episodes in last six months) ○ Papular pruritic eruption ○ Seborrhoeic dermatitis ○ Fungal nail infection
Clinical Stage 3 (Advanced Symptoms)
○ Unexplained severe weight loss (more than 10% of body weight) ○ Unexplained chronic diarrhoea for longer than one month ○ Unexplained persistent fever (intermittent or constant and lasting for longer than one month) ○ Persistent oral candidiasis ○ Oral hairy leukoplakia ○ Pulmonary tuberculosis ○ Severe bacterial infection (such as pneumonia, meningitis, empyema, pyomyositis, bone or joint infection, bacteraemia and severe pelvic inflammatory disease) (current) ○ Acute necrotizing ulcerative gingivitis or necrotizing ulcerative periodontitis. ○ Unexplained anaemia (<8 g/dℓ), neutropaenia (<0.5 × 10^9 per litre) or chronic (more than one month) thrombocytopenia (<50 × 10^9 per litre)
Clinical Stage 4 (Severe Symptoms)
○ HIV wasting syndrome ○ *Pneumocystis* pneumonia ○ Recurrent bacterial pneumonia; (this episode plus one or more episodes in last six months) ○ Chronic herpes simplex virus infection (orolabial, genital or anorectal) of more than one month or visceral infection of any duration ○ Oesophageal candidiasis ○ Extra pulmonary tuberculosis

- Kaposi sarcoma
- Cytomegalovirus disease (other than liver, spleen or lymph node)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Extra pulmonary cryptococcosis (including meningitis)
- Disseminated nontuberculous mycobacterium infection
- Progressive multifocal leukoencephalopathy
- Chronic cryptosporidiosis (with diarrhoea lasting more than one month)
- Chronic isosporiasis
- Disseminated mycosis (coccidiomycosis or histoplasmosis)
- Recurrent non-typhoid *Salmonella* bacteraemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy
- Symptomatic HIV-associated cardiomyopathy

Table 2.7 indicates the criteria for the diagnosis of advanced HIV based on the clinical or immunological criteria.

Table 2.7 Criteria for diagnosis of advanced HIV (including AIDS) for reporting

(Adopted from WHO, 2007)

Clinical criteria for diagnosis of advanced HIV in adults and children with confirmed HIV infection:
Presumptive or definitive diagnosis of any stage 3 or stage 4 condition[a]. and/or;
Immunological criteria for diagnosing advanced HIV in adults and children, five years or older, with confirmed HIV infection:
CD4 count less than 350 per mm^3 of blood in an HIV-infected adult or child. and/or;
Immunological criteria for diagnosing advanced HIV in a child, younger than five years of age, with confirmed HIV infection:
%CD4+ < 30 among those younger than 12 months; %CD4+ < 25 among those aged 12-35 months; %CD4+ < 20 among those aged 36-59 months.

a) Criteria for HIV staging events provide criteria for presumptive or definitive diagnosis of all conditions.

AIDS in adults and children is defined as the clinical diagnosis (presumptive or definitive) of any stage 4 condition with confirmed HIV infection: OR immunological diagnosis in adults and children with confirmed HIV infection and >5 years of age; first-ever documented $CD4^+$ T cell count less than $200mm^3$ or % $CD4^+$ T cells <15: OR among children with confirmed HIV infection aged 12– 35 months first ever documented %$CD4^+$ T cells <20: OR among children of less than 12 months of age with confirmed HIV infection who documented % $CD4^+$ T cells <25 for the first time.

2.4. HIV DISEASE PROGRESSION

There are endogenous biologic or psychological factors that influence HIV disease progression. Other infections, behaviours or environmental factors that alter the natural history of HIV infection may be cofactors for disease progression, but to date the only cofactors, for which evidence is strong, are age and genetic differences in the chemotactic receptors that are required by HIV in order to infect cells. Some data suggests that genetic differences in human leukocyte antigen (HLA) molecules, smoking and nutrition may also be cofactors, but this data is less compelling (Osmond, 1998; Roger, 1998).

2.4.1. Genetic cofactors

When it was discovered that, in addition to the CD4 receptor, chemotactic receptors were needed for HIV to infect cells, the evidence suggested that these receptors affected the probability of infection and the subsequent progression rate of HIV disease. The heterozygous defect in the C-C chemokine receptor type-5 (CCR-5) gene offers partial protection, and there seems to be interaction between the HIV viral stain phenotype and the protective effect of CCR-5. Genetic differences in HLA also seem to be associated with the progression of HIV disease. In addition, the presence of HLA A1, Cw7, B8 and DR3 is also associated with $CD4^+$ T cell decline (Osmond, 1998; Roger, 1998).

2.4.2. Age as cofactor

Age is yet another cofactor in the progression of HIV disease. The effect age has is that the possibility of progression to AIDS is estimated as 1.5 per every 10 years of age (Osmond, 1998; Portela and Simpson 1997).

2.4.3. Other potential cofactors

Cause-and-effect relationships are difficult to separate. The use of drugs, smoking, poor nutrition, and depression may increase health problems. Cofactors that are not unique to HIV disease are difficult to evaluate and some researchers see them as having an association and others claim that there is no association (NAM Publications, 2013; Osmond, 1998).

2.4.4. Nutrition as cofactor

Micronutrient deficiency is prevalent in many HIV-infected populations. Studies have shown that these deficiencies impair immune response, weaken epithelial integrity and are associated with accelerated HIV disease progression (Friis, 2005; Dreyfuss and Fawzi, 2002).

HIV infection affects nutrient absorption and metabolism negatively and could lead to a vitamin A deficiency. Even when liver stores are adequate the serum, retinol, is suppressed during the acute phase's response to infection. Progression may also be explained by the increased risk of transmission (Friis, 2005; Dreyfuss and Fawzi, 2002; Kotler, 2000).

The potential benefits of micronutrients in showing clinical, immunological and virologic disease progression are important. Table 2.8 shows the evidence between micronutrients and HIV disease progression (Friis, 2005; Dreyfuss and Fawzi, 2002).

Table 2.8 Evidence of the relationship between micronutrients and HIV disease progression (Adapted from Dreyfuss and Fawzi, 2002)

Evidence of the relation between micronutrients and HIV disease progression		
Micronutrient	**Endpoint**	**Direction of association(type of exposure)**
Vitamin A	Clinical progression to AIDS	U-shaped (intake), - (serum)
	CD4+ cell count	↑(intake),↑(serum), - (supplements)
	HIV viral load	- (supplements)
	Death	U-shaped (intake), ↓(serum)
ß-Carotene	Clinical progression to AIDS	-(Intake)
	CD4+ cell count	-(Intake), - (supplements)
	HIV viral load	- (supplements)
	Death	↓(Intake)
Thiamine	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake), - (serum)
	Death	↓(Intake)
Riboflavin	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake), - (serum)
	Death	↓(Intake)
Niacin	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake)
	Death	↓(Intake), - (serum)
Vitamin B-6	Clinical progression to AIDS	↓(Intake), - (serum)
	CD4+ cell count	↑(Intake), - (serum)
	Death	↓(Intake), - (serum)
Vitamin B-12	Clinical progression to AIDS	-(Intake), ↓(serum)
	CD4+ cell count	↑(serum)
	Death	-(Intake), ↓(serum)
Folate	Clinical progression to AIDS	-(Intake), - (serum)
	CD4+ cell count	↑(Intake), - (serum)
	Death	-(Intake)
Vitamin C	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake)
	HIV viral load	↓(Supplements)
	Death	-(Intake)
Vitamin E	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake), - (serum)
	HIV viral load	↓(Supplements)
	Death	-(Intake), - (serum)
Iron	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake)
Zinc	Clinical progression to AIDS	↑(Intake), ↓(serum)
	CD4+ cell count	-(Intake), ↑(serum)
	Death	↑(Intake), ↓(serum)
Selenium	CD4+ cell count	- (supplements)
	Death	↓(serum)
Copper	Clinical progression to AIDS	-(Intake), ↑(serum)
Multivitamins and minerals	Clinical progression to AIDS	↓(Intake)
	CD4+ cell count	↑(Intake), ↑(Supplements)

↑, Increase in the endpoint with increasing intake of, serum concentration of, and supplementation with the nutrient;

↓, Decrease in the endpoint with increasing intake of, serum concentration of, and supplementation with the nutrient;

-, no association of the endpoint with the intake of, serum concentration of, or supplementation with the nutrient;

(intake), intake form diet and supplement use;

(serum), serum micronutrinient concentration;

(supplements); supplementation in randomized trials.

2.5. OPPORTUNISTIC INFECTIONS AND CONDITIONS

Oral lesions found in HIV-positive patients can be indicative of opportunistic infections. A decline in the number of $CD4^+$ T cells and an increase in viral load parallels the lesions found. These are independent indicators of the disease's progression (Coogan *et al*., 2005). Opportunistic diseases are caused by infectious agents when immunosuppression is increasing. *Pneumocystis carinii,* cytomegalovirus and varicella-zoster virus usually precede HIV infection in the host and are usually reactivated during HIV disease progression. Agents of bacterial pneumonia may be acquired during the course of the infection. Whether these opportunistic infections are markers of HIV-mediated immunosuppression, or act as a cofactor that accelerates HIV disease progression, has not been clearly determined. During periods of acute infection HIV replication, as well as cytokines, increases. HIV-mediated immunosuppression changes the host's control over the infectious agents. This inability to control the agents results in disease. The disease process, in turn, activates HIV, and this hastens the rate of immunosuppression (Osmond, 1998).

Opportunistic infections and conditions include the following:

- Aspergillosis
- Bacillary angiomatosis (cat scratch disease)
- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, oesophageal
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extra pulmonary
- Cryptococcosis, extra pulmonary
- Cryptosporidiosis, chronic intestinal (duration >1 month)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with vision loss)
- Encephalopathy, HIV-related
- Herpes simplex - Chronic ulcer or ulcers (duration >1 month) or bronchitis, pneumonitis, or esophagitis
- Hepatitis B and C

- Histoplasmosis, disseminated or extra pulmonary
- Human Papilloma Virus (HPV)
- Isosporiasis, chronic intestinal (duration >1 month)
- Kaposi sarcoma
- Lymphoma, Burkett (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primarily of the brain
- *Mycobacterium avium* complex or *Mycobacterium kansasii* infection, disseminated or extra pulmonary
- *Mycobacterium tuberculosis* infection, any site (pulmonary or extra pulmonary)
- *Mycobacterium* infection with other species or unidentified species, disseminated or extra pulmonary
- Microsporidiosis
- *Pneumocystis* pneumonia
- *Pneumocystis carinii*
- Pneumonia, recurrent
- Progressive multifocal leukoencephalopathy
- *Salmonella* septicaemia, recurrent
- Syphilis and Neurosyphilis
- Shingles
- Toxoplasmosis of the brain
- Wasting syndrome due to HIV infection

(Aegis, 2011; AIDS.org, 2011; Bennett and Greenfield, 2011)

The two strongest cofactors – age and genetically determined receptors for HIV - are not alterable. A reliable indicator for the current risk of acquiring opportunistic infections is the $CD4^+$ T cell count, and a count below 200 cells\µℓ is a good indicator (Bennett and Greenfield, 2011). Opportunistic infections may be averted or postponed with good medical care that includes prophylaxis (AIDS.org 2011; UCSF Medical Center, 2011; Osmond, 1998).

2.6. MOTHER TO CHILD TRANSMISSION

According to UNAIDS (2010), about 2.5 million children under the age of 15 years were living with HIV in 2009 and an estimated 370 000 had been newly infected with the virus. However, significant progress has been made in the prevention of MTCT of HIV, even in high burden and resource poor settings. The elimination of MTCT of HIV was important to achieving the millennium's development goals (WHO, 2010b) and the number has declined by 24% since 2004 (UNAIDS, 2010).

Transmission of HIV from mother to child can occur during pregnancy, at the time of delivery, or postnatal through breastfeeding. Although there is clear evidence that breast milk has protective factors, there is also evidence that breast milk can harbour potentially harmful bacteria and viruses (Dreyfuss and Fawzi, 2002; Georgeson and Filteau, 2000). The first reported case of breastfeeding-associated HIV transmission was in 1985 (Humphrey, 2010). It has been shown that if no intervention is provided, an estimated 20-25% of infants of HIV-infected women will acquire HIV up to and including during delivery. This percentage can go up to as high as 45% if these children are breastfed. Transmission is increased in women with more clinically advanced disease, low $CD4^+$ T cell counts and high HIV viral loads. In such cases, antiretroviral and optimal infant feeding practices are needed to reduce HIV transmission to the infant and to promote child survival (WHO, 2010e; Becquet *et al*., 2009; WHO, 2009).

If preventing a child from becoming HIV-infected were the only consideration, all HIV-infected mothers would be advised to give their baby infant formula milk. Although this is the recommendation given to mothers by medical experts in developed countries, it is very important for mothers living in resource poor countries to think about the risks of not breastfeeding. It may sound grim that an important health issue is based on whether you are poor or rich but, unfortunately, that is the stark reality. We are living in a world that is weighed down by inequity and the gap between rich and poor people and nations is widening (Shearer, 2008; Latham and Preple, 2000).

2.6.1. Transmission during pregnancy

The specific method for intrapartum MTCT is unknown, but transmission could most likely result from discrete exposure to virus in maternal blood or cervicovaginal secretions across infant mucosal surfaces (Luzuriaga *et al*., 2006).

2.6.2. Transmission during delivery

The aim of every obstetric unit, with regard to transmission during delivery, is to achieve the lowest possible transmission from mother to the foetus. The mode of delivery should be discussed with the patient and a multidisciplinary team be involved in her care. There should also be a counselling session, informing the patient of the facts and current statistics. According to Nikhil *et al* (2009) it would be prudent to have a delivery plan in place by weeks 34-36.

2.6.3. Transmission during breastfeeding

Many specific and nonspecific factors in human milk, including immunoglobins, lactoferrin, lysozyme, secretory leukocyte protease inhibitor (SLPI), oligosaccharides, glycoanubiglycans, RANTES (regulating on activation normal T cell expressed and secreted), and Interleucin-8 (IL-8) can protect against HIV infection in infants (Dreyfuss and Fawzi, 2002).

In many settings, breastfeeding cannot be avoided. Risk factors for transmission of HIV-1 during breastfeeding include the duration of breastfeeding, characteristics of the mother (her age, $CD4^+$ T cell count, viral load and breast abnormalities), the infant (oral candidiasis) kind of milk, e.g. human milk or type of breastfeeding (Read *et al*., 2003).

According to a study done in Malawi by Read and colleagues (2003), the risk of HIV-1 infection for infants of HIV-1 infected mothers who continued breastfeeding after 1 month of age was 3.5% at the end of 5 months, 7.0%, at the end of 11 months, 8.9% at the end of 17 months and 10.3% at the end of 23 months. Thus, the longer the

mothers infected with HIV-1 breastfed, the greater was the risk of HIV transmission to their infants. A risk assessment done in South Africa showed that a 15% increased risk of HIV-1 transmission was observed with breastfeeding than formula feeding. Early weaning from human milk - if feasible - would limit exposure to HIV-1 infected human milk while still allowing the child to experience the benefits of breastfeeding (Becquet *et al*., 2009; Read *et al*., 2003).

A lower $CD4^+$ T cell count indicates a more advanced maternal disease stage, which is associated with a higher viral load in breast milk. This increases the risk of HIV transmission to the infant. The presence of breast abnormalities like mastitis, breast abscesses and nipple lesions is also associated with an increased risk of MTCT of HIV-disease since a higher human milk viral load is seen in these abnormalities. Oral candidiasis, in infants under six months, is associated with late postnatal transmission of HIV-1 through breastfeeding (Becquet *et al*., 2009; Carter, 2003; Read *et al*., 2003; Rousseau *et al*., 2003).

Studies show that infants who are exclusively breastfed have a lower risk of being HIV-infected than those who are partially breastfed or receive mixed feeding. The first article on this was published in 1999 (Humphrey, 2010). Exposure to food and microbes in food or water may cause micro trauma to the infants' bowel which could provide an entry point for HIV transmission (Buskens *et al*., 2007; Rousseau *et al*., 2003).

2.7. NUTRITION AND HIV TRANSMISSION

Vertical transmission of HIV is influenced by both maternal and child factors, many of which relate to nutritional status. Although systemic cell-mediated immune function in pregnant women is an indicator of disease progression it may also protect against vertical transmission. Factors of maternal disease progression that increase the risk of vertical HIV transmission are of a clinical, immunologic and virological nature (Dreyfuss and Fawzi, 2002).

HIV infection affects nutrient absorption and metabolism. HIV or opportunistic infections may also lead to reduced dietary intake, decreased appetite and difficulty swallowing. The above, in combination with environmental factors such as the lack of a healthy balanced diet, creates a complex interaction between HIV and nutrition (WHO, 2010e).

Even when liver stores are adequate, serum retinol is depressed during the acute phase response to infection. Thus, low serum vitamin A concentrations may be the result of advanced HIV disease, and this disease progression may account for the increased risk of vertical transmission observed (Friis, 2005; Dreyfuss and Fawzi, 2002).

Various B vitamins influence both cellular and humeral immune function. Vitamin B_6 depletion in HIV-positive patients is associated with a reduced lymphocyte response to mitogens and natural killer cell cytotoxicity (Dreyfuss and Fawzi, 2002; Ehrenpreis, Carlson, Boorstein and Craig, 1994).

Neutrophil phagocytosis is impaired by low concentrations of folic acid and vitamin $B_{12.}$ Antioxidant vitamins are important enhancers of immune functions and vitamin C supplementation improves T and B lymphocyte proliferation. Zinc is needed for normal neutrophil, natural killer and macrophage function. Selenium is an important structural component of the antioxidant enzyme glutathione peroxidises, and is important in the maintenance of humoral and cell-mediated immunity (Kashou and Agarwal, 2011; Dreyfuss and Fawzi, 2002).

2.8. FACTORS FUELLING THE SPREAD OF HIV/AIDS IN AFRICA

People in Africa have their own unique culture, traditions and beliefs, some of which are fuelling the spread of HIV/AIDS in Africa. When seeking ways to stop the spread of HIV in Africa one cannot always use the same approach as in the rest of the world. The beliefs and cultures of the African people have to be taken into account.

2.8.1. HIV-associated stigma

Until recently, many African governments were hesitant to recognize the magnitude of the continent's HIV epidemic, dismissing critics as racists or being misguided. The silence surrounding HIV and AIDS that still perseveres has led to limited public discussion and continued stigmatization of those who are infected (Inungu and Karl, 2006). Buskens *et al*. (2007) argue that mothers often hide their HIV status from partners and relatives because of the fear of rejection, physical abuse and of losing financial and social support.

They further maintain that where mixed feeding is the norm, relatives and neighbours become suspicious when mothers breastfeed exclusively or only formula feed, often causing them to lie rather than disclose their status. However, the success of this strategy is often limited (Buskens *et al*., 2007).

When HIV-positive mothers attempt to wean at four to six months, particularly in rural areas where infants are traditionally weaned after one or two years, and when the mothers may need permission from the father or grandparents to do so, they may also attempt to lie (Buskens *et al*., 2007; Lunney, Jenkins, Tavenga, Majo, Chidhanguro, Iliff, Strickland, Piwoz, Iannotti and Humphrey, 2007).

2.8.2. Socio-economic status

The fact that the HIV epidemic has affected the impoverished regions of the world the most has led to closer examination of the relationship between HIV and poverty. This aforementioned relationship between poverty and HIV/AIDS is bidirectional in that poverty is a key factor in the transmission and HIV and that HIV/AIDS can impoverish people in such a way as to intensify the epidemic itself. Poor nutrition could result, weakening the immune system and making poverty-stricken people more susceptible to infectious diseases. The high cost of treatment and possible lack of (or inability to) work also make infected people more likely to fall into poverty (Inungu and Karl, 2006; Tladi, 2006).

Those who are poor tend to focus more on their daily survival than their health. Hopelessness leads to risky behaviours, such as prostitution, so many young women become sexually involved with numerous male friends or clients in exchange for financial support. In turn, this causes African prostitutes to have a higher prevalence of HIV when compared to the general population (Inungu and Karl, 2006).

2.8.3. Polygamy

Polygamy is a social practice in some parts of Africa, often used to ensure the continued status and survival of widows and orphans within an established family structure. In urban settings where polygamy is no longer the norm, men tend to have many sexual partners and use the services of prostitutes. There is also a false belief that men can rid themselves of HIV and AIDS by engaging in intercourse with a virgin. This perception puts young African girls at risk of contracting HIV (Mpungose, 2011; Inungu and Karl, 2006).

2.8.4. Widow inheritance

Many countries in sub-Saharan Africa practise widow inheritance, where a man's wife is viewed as property and is passed on to his adult sons or brothers after his death. In this traditional ritual, the widow agrees to marry her husband's younger brother to continue as part of the family. If she refuses she is expelled and left to care for herself and her children by herself. If the husband had died of AIDS, having infected his wife she will then in turn infect the new husband (Agot, Vander Stoep, Tracy, Obare, Bukusi, Ndinya-Achola, Moses and Weiss, 2010; Inungu and Karl, 2006).

2.8.5. Drug and alcohol abuse

It appears that the prevalence of HIV in intravenous drug users is more common than previously thought. Alcohol consumption and drug use reduce a person's ability to make informed choices concerning safe sex and protection from HIV infection (NAM Publications, 2013; Inungu and Karl, 2006).

2.8.6. Feeding preferences

Cultures have different feeding preferences. In those that believe in exclusively breastfeeding, if the mother feeds her baby with a breast milk substitute, the very fact that she chooses not to breastfeed may draw attention to her HIV status. According to Campbell (2008), not breastfeeding often leads to violence and abandonment by her family and the community. Most mothers and community members in Africa believe that breast milk is superior (God designed) to infant formula or animal milk, and that it creates a unique lifelong 'love link' between the mother and the infant (Buskens *et al*., 2007).

Although mothers know that nurses discourage mixed feeding, few of them practice exclusive breastfeeding. Buskens *et al*. (2007) found that the majority of HIV-positive women would rather formula feed (if they had the means) than breastfeed since nurses often present HIV transmission as a certainty instead of a probability. Some find this information impractical and generally counter-intuitive, conflicting with their own understanding or beliefs. Most mothers also give their infants water, from birth, believing that water is life (Buskens *et al*., 2007). They perceive water as vital in preventing constipation, dehydration and cleansing the infant's system (Field, Siziya, Katepa-Bwalya, Kankasa, Moland and Tylleskä, 2008; Buskens *et al*., 2007).

Most mothers in South Africa and Swaziland give their infants traditional medicines, herbal enemas or over-the-counter medicines, presumably to treat or protect them from diseases. Umfula is a solution given routinely, in rural South Africa, during the first month to clean the newborn infant (Buskens *et al*., 2007; Sikotoyi, 2004).
Porridge is sometimes introduced as early as ten days and, on occasion, from birth. Mothers who do this understand that milk is essential, but they consider it to be insufficient to make the child grow or to satisfy hunger (Field *et al*., 2008; Buskens *et al*., 2007).

2.8.7. Overriding authorities

Since grandmothers often help raise their grandchildren, the latter are seen as belonging to everyone and not exclusively to the mother. Both maternal and paternal grandmothers then often feel that they have a say in what the infants are fed, how they are cared for and what medical treatment they can receive. As the majority of mothers are unemployed they rely on the income of others to provide them with necessities. The member of the family who provides financially often also decides what the child is fed and how the child should be cared for (Buskens *et al*., 2007).

2.9. BREASTFEEDING VERSUS FORMULA FEEDING

When comparing breastfed infants to formula fed infants the comparative risk of death in the first two months of life, from infectious diseases, is six to ten times greater than in breastfed children in developing countries. Diarrhoea and pneumonia are more common and more severe in children who are artificially fed. Even in situations with adequate hygiene, diarrhoeal illness is more common in artificially fed infants than those breastfed (WHO, 2009; Latham and Preple, 2000).

According to Campbell (2008), there are difficulties in trying to prevent MTCT through breastfeeding, without increasing the risk of the child getting other illnesses since the absence of breastfeeding is associated with a significant increase in child mortality in uninfected infants. Other acute infections that are less common and less severe in breastfed infants are otitis media, *Haemophilus influenzae* meningitis and urinary tract infection (WHO, 2009; Kuhn *et al*., 2010).

Children who are artificially fed have an increased risk of long-term diseases with an immunological basis, like asthma, celiac disease, ulcerative colitis and Crohn's disease. According to WHO (2009), artificial feeding is also associated with childhood leukaemia. Even if infant formula is widely available it is often financially beyond the means of poor families (Campbell, 2008) and if infant formula is free there is often no mechanism to assure supply (Latham and Preple, 2000).

Formula milk can cause infections if it is used incorrectly. The formula is prepared by mixing milk powder with local water, which is most likely to be contaminated by sewage in poor communities (Campbell, 2008; Shearer, 2008,). In a study done by Shapiro (2007), the lack of a refrigerator and early weaning were the most predictive of infant illnesses. Weaning is often seen as a period of risk among infants in the developing world and supportive care during this period is very important. Shortening the normal duration of breastfeeding for uninfected children, born to HIV-infected mothers who live in resource-poor settings is associated with an increased child mortality, which extends into the second year of life. The lack of a refrigerator could be indicative of unsafe storage conditions of weaning foods or of a lower socioeconomic status (Kuhn *et al*., 2010; Shapiro *et al*., 2007).

Formula milk can cause malnutrition if over-diluted (Campbell, 2008). Globally, the prevalence of underweight in children under the age of 5 years has declined from 31% to 26% between 1990 and 2008. Countries where insufficient or no progress has been made are in sub-Saharan Africa and South Asia. In all regions of the world children living in rural areas are more likely to be underweight than children living in urban areas. Under-nutrition is caused by a combination of factors; such as lack of food in terms of quantity and quality, inadequate water and sanitation and health services, suboptimal care and feeding practices (UNICEF, 2010).

Stunting, however, is a problem of a larger magnitude than being underweight. Children under 2 years of age are most vulnerable of stunting and the effects are largely irreversible. Children of this age are put at risk of under-nutrition by suboptimal breastfeeding practices and inappropriate complementary feeding practices. It is, therefore, vital to focus on effective interventions for infant and young children feeding practices, especially those living in rural areas (UNICEF, 2010).

2.9.1. Water, sanitation and hygiene

Generations of women have been faced by the lack of water, sanitation and hygiene from as early as birthing. It is more than likely that a pregnant woman has had to collect and carry water for her baby's delivery from a hand pump outside her house,

herself. Globally, more than 40% of households do not have a water supply on their premises, 13% do not have a hand pump in their community and they have to rely on an unimproved water source. This water source is made riskier by the fact that many people in some communities lack even a basic toilet. This takes on greater meaning when a mother weans her child (Brocklehurst and Bartram, 2010; Swarts, Kruger and Dolman, 2010; Chopra, 2003).

Girls, around the age of six, should be going to school but much of their time will be spent doing chores around the house, water collection being one. These girls could be exposed to infestations, such as hookworm, which reduce physical growth and impair intellectual development, causing them to become anaemic. A lack of school toilets could contribute to sporadic attendance and sometimes in dropping out when a girl enters her menarche. If she does not overcome these constraints and drops out of school, she will likely face early marriage and early childbearing (Brocklehurst and Bartram, 2010).

The cycle is vicious and keeps women in poor health, out of education, and in poverty - doomed to bearing sickly children. Water, sanitation and hygiene provision will enable women to play bigger roles in their community (Brocklehurst and Bartram, 2010). The World Health Organization recommends that water for people living with HIV, should be in containers that allows minimal manual contact. A minimum of 20 litres per person, per day is recommended. To reduce diarrhoeal disease, disposal of faeces in a toilet, latrine or burial ground is recommended (WHO, 2010e). Hygiene interventions should include hygiene education and promotion of hand washing with soap, with soap being provided for people living with HIV, their caregivers and households (WHO, 2010e).

2.10. HIV TREATMENT

The introduction of ART has seen a great reduction in suffering and the death rate. Standard ART consists of the use of at least three ARV drugs in combination. WHO is providing countries with ongoing guidance, tools and support in ART treatment recommendations (WHO, 2011a). The up scaling of treatment has also affected

sub-Saharan Africa, so much so that by the end of 2009, 37% of adults and children who were eligible for ART were receiving it, compared to only 2% seven years earlier (UNAIDS, 2010). In 2005, approximately one million adults were in need of ART in South Africa. This total increased to nearly 1.5 million in 2009 (see Table 2.9).

Table 2.9 Number of persons in South Africa in need of ART for the period 2005-2010 (Adapted from Statistics South Africa, 2010)

Year	Adults (15+ years)	Children
2005	1,069,000	93,000
2006	1,153,000	99,000
2007	1,238,000	129,000
2008	1,332,000	132,000
2009	1,438,000	139,000
2010	1,555,000	183,000

In 2005, 133 000 adults received ART in South Africa, this had increased to 920 000 by 2009 as shown in Table 2.10.

Table 2.10 Estimated number of adults receiving ART and the percentage of children receiving ART and cotrimoxazole for the period 2005-2009 (Adapted from Statistics South Africa, 2010)

Year	Adults (15+ years)	Children	
	Estimated number receiving ART	Estimated percentage receiving ART	Estimated percentage receiving cotrimoxazole
2005	133,000	7	2
2006	239,000	8	4
2007	424,000	12	12
2008	679,000	29	21
2009	920,000	38	29

ART can be divided into various drug classes such as entry/fusion inhibitor, nucleoside analogue reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), chemokine co-receptor

antagonist (consisting of 2 subclasses: CCR-5 antagonist and CXC-chemokine receptor type 4 (CXCR4) antagonist), and integrase inhibitor (UCSF Medical Center, 2011; National Institute of Allergy and Infectious Diseases, 2009).

Figure 2.6 shows the various classes of ART at different points during the HIV life cycle as described elsewhere in this thesis.

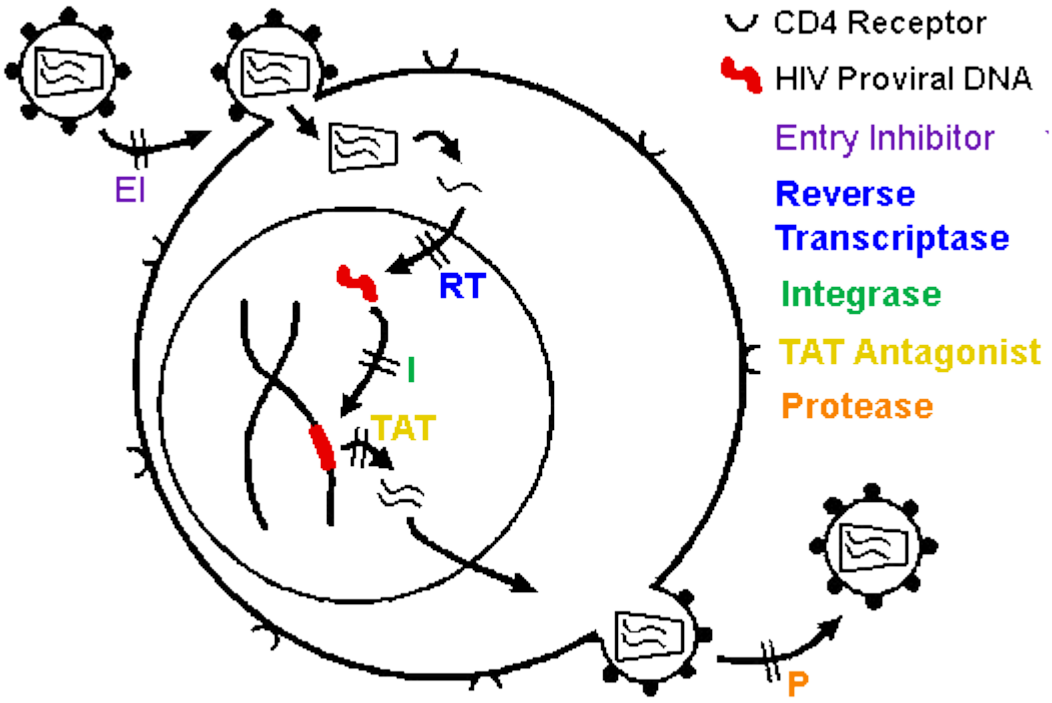


Figure 2.6 The various classes of ART work at different points during the HIV life cycle (Adapted from Klatt, 2011)

- **Reverse Transcriptase (RT) Inhibitors (blue)** interfere with the step during the HIV life cycle known as reverse transcription. There are two main types of RT inhibitors:
  - **Nucleoside/nucleotide RT inhibitors (NRTIs)** are faulty DNA building blocks. This drug acts by incorporating faulty pieces into the HIV DNA and the chain cannot be completed, thereby blocking HIV from replicating in the cell.
  - **Non-nucleoside RT inhibitors (NNRTIs)** bind to RT, interfering with the RT's ability to convert the HIV RNA into HIV DNA.
- **Protease Inhibitors (purple)** get in the way of the protease enzyme that HIV uses to generate infectious viral particles.

- **Fusion/Entry Inhibitors** prohibit the entry into the host cell by interfering with the virus' ability to fuse with the cellular membrane.
- **Integrase Inhibitors (green)** prevent occurrence of integrase, the enzyme HIV uses to integrate genetic material of the virus into its target host cell.
- **Chemokine co-receptor antagonists** bind to either CCR-5 or CXCR4 on the surface of $CD4^+$ T cells and, by so doing, obstruct a required step in viral entry. Whereas these drugs bind human proteins, drugs from other classes act on viral enzymes.

  (UCSF Medical Center, 2011; National Institute of Allergy and Infectious Diseases, 2009)

Hundreds of ARVs are available currently. The drugs that are recommended for the treatment and prevention of HIV in pregnant women, infants and young children are:

- Abacavir(ABC), AZT(Retrovir), Emtricitabine(FTC) and 2'3'-dideoxy-3'thiacytidine(3TC) – these belong to the NRTIs
- Efavirenz(EFV) and Nevirapine(NVP) – belongs to the NNRTIs
- Lopinavir/ritonavir(LPV) - is a protease inhibitor
- Tenofovir (TDF) - belongs to the nucleotide reverse transcriptase inhibitor (NtRTI) class of drugs.

(NAM Aidsmap, 2011; WHO, 2010b; WHO, 2010e)

2.10.1. Treatment and prevention of HIV in pregnant women, infants and young children

Significant progress is being made globally in the prevention of MTCT of HIV, even in high burden and resource-limited settings (European Collaborative Study collaborators, 2010). The incidence and impact of HIV among children younger than 15 years in Southern Africa has been reduced. In 2009, there was a 32% decrease in newly infected children and there were 26% fewer AIDS-related deaths among children, compared with 2004 statistics (UNAIDS, 2010). Elimination of MTCT is considered a realistic public health goal and is an important part to the campaign to achieve the millennium development goals (MDG). WHO recommends a broad

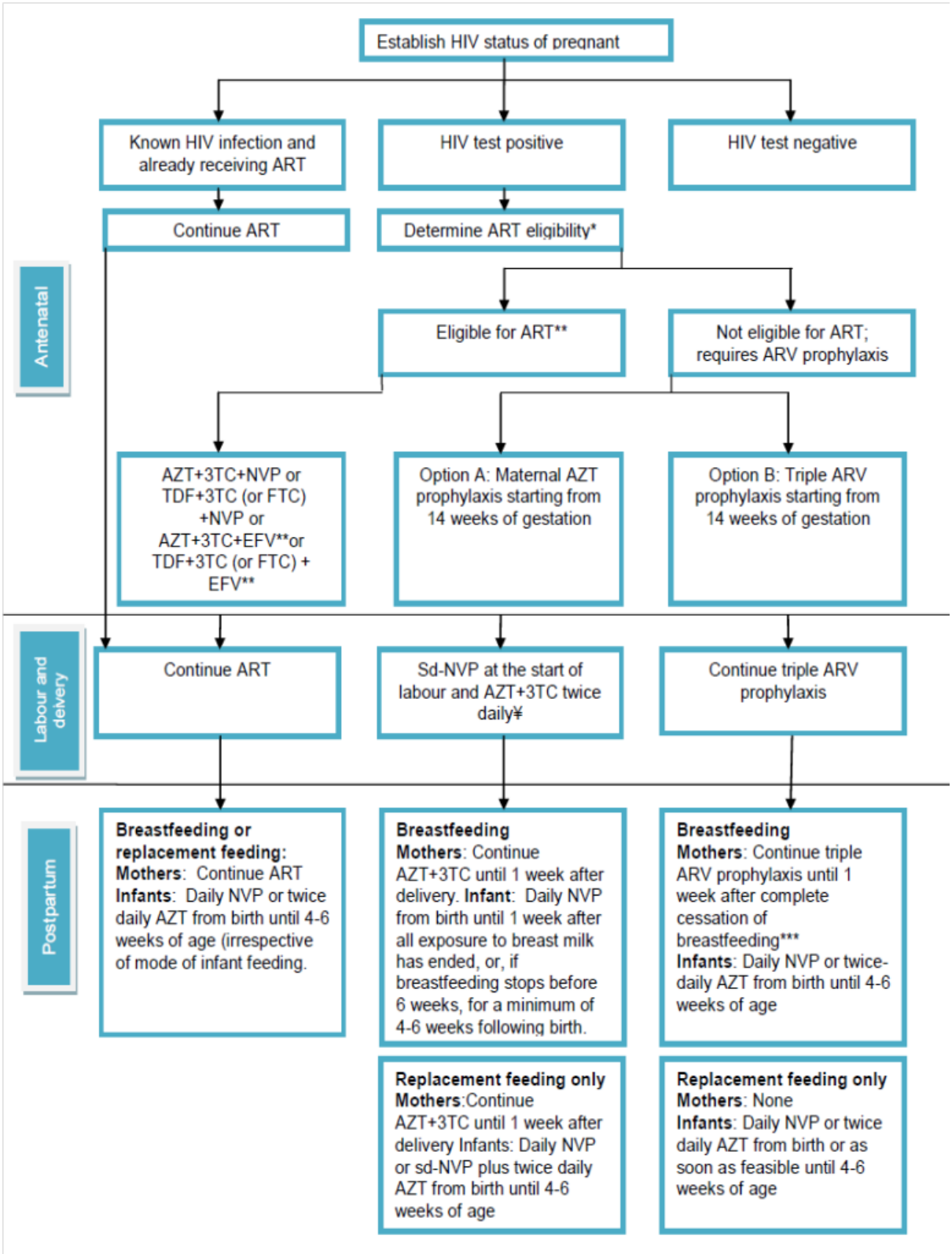
approach in the prevention of HIV in infants and young children and so the 2010 guidelines were developed to provide international standards, primarily for low- and middle-income settings. These guidelines focus on four areas:

- the primary prevention of HIV transmission
- the prevention of unintended pregnancies in women who are HIV-positive
- the prevention of HIV transmission from women who are HIV-positive to their children
- treatment, care and support for women who are HIV-positive, their children and families.

According to WHO (2010b), once implemented these recommendations have the ability to reduce the risk of MTCT in resource-poor settings. The revised 2010 Preventing Mother to Child Transmission (PMTCT) recommendations as seen in Table 2.11 were based on two key approaches:

1. Lifelong ART, which is also safe and effective in the reduction of MTCT, for HIV-positive women in need of treatment for their own health.
2. ARV prophylaxis that prevents MTCT during pregnancy, delivery and breastfeeding for HIV-positive women not in need of treatment for their own health.

Table 2.11 Algorithm for the 2010 PMTCT recommendations (Adapted from WHO, 2010b)



For the first time, evidence allowed for new recommendations on ARV prophylaxis to either mother or infant, during breastfeeding, in areas where breastfeeding was judged to be the most suitable choice of infant feeding for women who were HIV-infected. This option was conceivable mostly because it also offered a culturally appropriate alternative to HIV-infected mothers (Peltier *et al*., 2009).

PMTCT is not an intervention that stops at delivery and, ideally, postpartum and breastfeeding follow-ups should also be provided for the mother and the infant. It should be made clear whether treatment is provided for the mother's HIV disease or for MTCT prophylaxis when deciding on treatment (WHO, 2010b; WHO, 2010e). The reduction of HIV RNA levels and the use of ARVs both appear to have an independent effect on the reduction of perinatal transmission of HIV. Maximal and sustained suppression of HIV RNA should be achieved during pregnancy. The most critical suppression would be during late pregnancy and the time of delivery when MTCT occurs most frequently (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2011).

A $CD4^+$ T cell count is essential to determine whether a woman should initiate lifelong ART. Prophylaxis interventions are restricted to women who are not eligible for treatment according recommendations (WHO, 2010b; WHO, 2010e). The single dose NVP regimen is the most feasible and inexpensive strategy for low-income countries that are only able to deliver a minimal range of ARVs (Orne-Gliemann *et al*., 2008).

The Kesho Bora study (2011) found that giving a combination of three ARVs to pregnant mothers who were HIV-infected from the last trimester, through delivery and six months during breastfeeding reduced the risk of transmitting HIV to the baby and improved survival. The study compared the triple-ARV regimen against a control regimen of zidovudine and single-dose nevirapine. The triple-ARV regimen cut HIV infections in infants by 43% compared to the control regimen and reduced the risk of HIV transmission during breastfeeding by more than half. There was no apparent risk to the health of mothers or their babies associated with the triple-ARV regimen when compared with the control regimen. Findings from the Kesho Bora

study strongly influenced the WHO guidelines on ARVs which were issued in July 2010, and were aimed at preventing MTCT of HIV and infant feeding (WHO, 2011b).

2.10.2. Pregnant women eligible for ART

It is recommended that women with $CD4^+$ T cells ≤350 cells/mm^3 start ART for their own health, irrespective of WHO clinical staging, and women in WHO clinical stages 3 or 4, do the same irrespective of the $CD4^+$ T cell count. Once on ART, treatment should be maintained throughout the pregnancy, during delivery and breastfeeding and thereafter for optimum health, irrespective of the gestational age (WHO, 2010b; WHO, 2010e). Table 2.12 indicates the recommended regimens for HIV-infected pregnant women who are eligible for treatment, and their infants.

Table 2.12 Antiretroviral treatment options recommended for HIV-infected pregnant women who are eligible for treatment (Adapted from WHO, 2010b; WHO, 2010e)

Maternal ART and infant ARV prophylaxis
Mother
Maternal antepartum daily ART, starting as soon as possible irrespective of gestational age, and continued during pregnancy, delivery and thereafter. Recommended regimens include: AZT + 3TC + NVP or AZT + 3TC + EFV* or TDF + 3TC (or FTC) + NVP or TDF + 3TC (or FTC) + EFV*
Infant
Daily NVP or twice-daily AZT from birth until 4-6 weeks of age (irrespective of the mode of infant feeding).

*The use of EFV should be avoided in the first trimester and NVP should be used instead.

All HIV-infected pregnant women who are not eligible for ART for health reasons, should be given an effective ARV prophylaxis to prevent HIV transmission during

pregnancy, labour and delivery, postpartum and during the breastfeeding phase. ARV therapy should start in the second trimester or as soon as possible afterwards (WHO, 2010b; WHO, 2010e). Table 2.13 shows the ARV-prophylaxis options recommended for HIV-infected pregnant women who do not need treatment for their own health benefit and the options recommended for their infants.

Table 2.13 ARV-prophylaxis options recommended for HIV-infected pregnant women who do not need treatment for their own health (Adapted from WHO, 2010b; WHO, 2010e)

Maternal AZT + infant ARV prophylaxis (Option A)	**Maternal triple ARV prophylaxis (Option B)**
Mother	**Mother**
Antepartum twice-daily AZT starting from as early as 14 weeks of gestation and continued during pregnancy. At onset of labour, sd-NVP and initiation of AZT + 3TC twice daily for 7 days postpartum. (Note: If maternal AZT was provided for more than 4 weeks antenatal, omission of sd-NVP and AZT + 3TC can be considered; in this case, continue maternal AZT during labour and stop at delivery.	Triple ARV prophylaxis starting from as early as 14 weeks of gestation and continued until delivery, or, if breastfeeding, continued until 1 week after all infant exposure to breast milk has ended. Recommended regimens include: AZT + 3TC + LPV/r or AZT + 3TC + ABC or AZT + 3TC + EFV or TDF + 3TC (or FTC) + EFV
Infant	**Infant**
For breastfeeding infants Daily NVP from birth for a minimum of 4-6 weeks and until 1 week after all exposure to breast milk has ended. ***Infants receiving replacement feeding only*** Daily NVP or sd-NVP + twice daily AZT from birth until 4-6 weeks of age.	***Irrespective of mode of infant feeding*** Daily NVP or twice daily AZT from birth until 4-6 weeks of age

2.10.3. Implications for the new WHO guidelines on HIV and infant feeding for child survival in South Africa

In April 2010 the South African Department of Health and the National AIDS Council released revised clinical guidelines for the prevention of MTCT of HIV. These guidelines contain many promising changes, including highly active antiretroviral therapy (HAART) for all HIV-infected pregnant women with $CD4^+$ T cell counts ≤350 cells/μℓ; 6 weeks of ARV prophylaxis with nevirapine for all HIV-exposed infants; continued infant nevirapine until 1 week after complete cessation of breastfeeding for HIV-exposed infants whose mothers are not on HAART; and HAART for all confirmed HIV-positive infants from as early as 6 weeks of age (Doherty *et al*., 2011).

The new guidelines (Table 2.14) recommend that public health facilities continue providing free formula milk to women opting not to breastfeed. This guideline was made after WHO guidelines which suggested that each country's authorities should base their decision on whether health services would mainly be to support and counsel HIV-infected mothers on ARVs to breastfeed, or advocate all avoidance of breastfeeding. Doherty *et al*. (2011) and Cousoudis *et al*. (2002) see the provision of free formula milk as an incentive that could cloud feeding decisions.

Table 2.14 Infant feeding recommendations in the 2010 South African guideline on the prevention of MTCT of HIV (Adapted from Doherty *et al*., 2011)

For all mothers:
• Counselling on infant feeding must commence after the first post-test counselling session in pregnancy. • Infant feeding should be discussed with women at every antenatal visit. • Mixed feeding during the first 6 months of life should be strongly discouraged as it increases the risk of childhood infections • Provide nutritional support for all breastfeeding, HIV-positive mothers and for formula-feeding mothers with food insecurity.
Breastfeeding HIV-positive women:

- All mothers who are known to be HIV-infected, either on lifelong ART or not, who exclusively breastfeed their infants should do so for 6 months, introduce appropriate complementary foods thereafter and continue breastfeeding for the first 12 months of life.
- Trained health-care personnel should provide high quality, unambiguous and unbiased information about risks of HIV transmission through breastfeeding, ART prophylaxis to reduce the risk, and risks of replacement feeding.
- Mothers who are known to be HIV-infected, and not on lifelong ART, who decide to stop breastfeeding at any time should do so gradually, over one month, while the baby continues to receive daily NVP and should continue for one week after all breastfeeding has stopped.

Formula feeding HIV-positive women:

- Free commercial infant formula will be provided to infants for at least 6 months.
- Women should receive practical support, including demonstrations on how to safely prepare formula and feed the infant.
- At 6 months of age, infants with - or at risk of - poor growth should be referred for continued nutritional monitoring and dietary assistance.
- An appropriate formula milk product for the infant's age and circumstances should be chosen.
- In cases in which commercial formula is provided free of charge at health facilities, managers, supervisors and health care personnel should ensure an uninterrupted supply at clinic level.

Recent estimates of proportional causes of mortality of children under the age of five in South Africa have put diarrhoea and pneumonia third and fourth, respectively, behind HIV/AIDS and neonatal causes. But, according to Doherty *et al*. (2011) most deaths of HIV-infected children are due to supervening infections, most commonly diarrhoea and pneumonia. A South African Demographic and Health Survey found that access to piped water in a dwelling was 58% for urban residents and 11% for rural residents, 87% of urban residents and 56% of rural residents used electricity for cooking and 74% of urban residents and 5% of rural residents had a flush toilet (Doherty *et al*., 2011; Swarts, Kruger and Dolman, 2010).

The revised South African clinical PMTCT guidelines provide an opportunity to reduce postnatal HIV transmission by making ARVs available to women who needed them and for infants during breastfeeding (Doherty *et al*., 2011).

2.11. NUTRIENT REQUIREMENTS FOR PEOPLE LIVING WITH HIV/AIDS

People living with HIV more so pregnant and lactating women, have different nutrient requirements than non-infected persons.

2.11.1. Adults

Studies that were done on HIV-infected adults showed increased resting energy expenditure (REE). This explains the weight-loss and wasting seen in these individuals. It is recommended that thus infected individuals increase their energy needs by 10% of a healthy adult's needs. During periods of symptomatic disease or opportunistic infections the recommended increase is 20% - 30% to maintain body weight, taking the increase in REE into account (WHO, 2003).

The World Health Organization recommends that whenever feasible, people with HIV who lack the means to meet their basic dietary needs, and their families should be assisted in achieving food security (WHO, 2010e).

2.11.2. Pregnant and lactating women

The recommended energy intake for adults also applies to HIV-infected pregnant and lactating women. Iron-folate supplementation is a standard component of antenatal care for preventing anaemia and improving foetal iron stores. WHO recommends daily iron-folate supplementation during the first six months of pregnancy to prevent anaemia, and supplements twice daily to treat severe anaemia. Like other chronic infections, HIV causes disturbances of iron metabolism and anaemia. Adequate micronutrient intake is best achieved through an adequate diet, but in resource-poor settings a multiple micronutrient supplement may be needed in pregnancy and lactation (WHO, 2003).

Optimal infant and young feeding practices rank among the most effective interventions to improve child health. An estimated 9.5 million children died before their fifth birthday in 2006 and two thirds of those deaths occurred in the first year of life. In 2008, half of the 8.8 million deaths of children under five years old occurred in sub-Saharan Africa where under-nutrition is associated with at least 35% of child deaths. Children who survive, often also do not reach full developmental potential. Around 32% of children under the age of five are stunted and 10% are wasted in developing countries. It is also there that mothers and families need support to initiate and sustain appropriate feeding practices in infants and young children (WHO, 2009).

2.12. BREAST MILK

All female mammals are uniquely equipped to provide species-specific nourishment and immunity through the provision of milk to their newborns (Jahn, 2009; Katz, 2006). The properties of human milk facilitate the transition of life from in utero to ex utero, and provide bioactive substances to the developing infant during critical periods of brain, immune and gut development (Wagner, 2010). The process of producing milk and its removal by an infant is called lactation, and women produce breast milk as a response to the baby's suckling. Prolactin and oxytocin are two hormones which play a role in the supply and demand system. Prolactin is essential for the initiation and maintenance of milk production, while oxytocin stimulates milk ejection (Katz, 2006).

The synthesis of milk remains constant, at approximately 800mℓ per day, but the actual volume of milk secreted may be adjusted to the infant's requirements by a feedback inhibitor of lactation. Thus the rate of milk synthesis is related to the degree of breast emptiness or fullness. Stress and fatigue affect a woman's milk supply (Wagner, 2010).

Colostrum, the first milk that is produced after giving birth, meets all the nutritional requirements of the newborn during growth and maturation. It is thicker, richer in minerals and has a higher protein, sodium, chlorine and fat-soluble (A, E and K)

vitamin concentration. The high vitamin A concentration is often what gives colostrum its yellowish colour. Colostrum is rich in white blood cells and antibodies, especially sIgA, which strengthens the newborn's immune system. It acts as a laxative to remove meconium (first faeces) from the digestive tract. Colostrum becomes transitional milk within one or two days, and then it changes into mature milk. This rate varies from woman to woman (Katz, 2006; Georgeson and Filteau, 2000).

Human milk contains the right mixture of proteins, carbohydrates, fats, vitamins, water and minerals to meet all the nutritional needs of infants for the first six months of life. It contains bioactive factors that augment the infant's immature immune system providing protection against infection, and other factors such as epidermal growth factor that helps digestion and absorption of nutrients (WHO, 2009). No baby is allergic to breast milk although he/she may have a reaction to something the mother had eaten. Breast milk contains at least 100 ingredients not found in formula milk (Jahn, 2009).

Wagner (2010) lauds Olive Wendell Holmes for having been spot on when he said that a pair of substantial mammary glands had the advantage over two hemispheres of the most learned professor's brain in the art of compounding a nutritious fluid for infants.

Breast milk composition is remarkably stable around the world. It changes only slightly with maternal diet and under different environmental conditions (Katz, 2006).

2.12.1. Fats

Breast milk contains about 3.5g of fat per 100mℓ of milk, which provides about half the energy content of the milk. The amount of fat increases as the feed progresses, thus as a result, hind milk (milk secreted towards to end of a feed) is rich in fat. The fore milk (milk at the beginning of the feed) contains less fat. Cholesterol, triglycerides, short-chain fatty acids and long chain fatty acids are present in human milk. Long-chain polyunsaturated fatty acids called docosahexanoic acid (DHA) and

arachidonic acid (ARA) are fatty acids that are not available in other milk. Since they are important for the neurological development of a child, they may be considered essential fatty acids. The amount of ARA and DHA varies with the maternal diet. The antiprotozoan activity against *Giardia lamblia* has been shown to be directly related to the release of free fatty acids from milk triglycerides by bile salt dependant lipase and lipoprotein lipase (Wagner, 2010; WHO, 2009; Hamosh, 2001; Jenness, 1979).

2.12.2. Carbohydrates

Lactose is the main carbohydrate in milk and another important source of energy. Breast milk contains about 7g lactose per 100mℓ. Oligosaccharide is a carbohydrate which provides important protection against infection (WHO, 2009).

2.12.3. Protein

Breast milk contains 0.9g of protein per 100mℓ of milk. This concentration is much lower than that found in animal milks. High concentrations of protein can overload the infant's immature kidneys. The protein casein found in breast milk, has a different molecular structure than that found in other milks, making it more easily digestible. Whereas human milk contains whey which is alpha-lactalbumin, cow's milk has betalactoglobulin to which infants can become intolerant (WHO, 2009).

2.12.4. Vitamins and minerals

Although breast milk normally contains sufficient vitamins for an infant unless the mother herself is deficient, the infant needs exposure to sunlight to generate vitamin D. Iron and zinc are present, albeit in low concentrations, but their bioavailability and absorption are high. If maternal iron stores are adequate, term infants are born with a store of iron that is sufficient for their needs (WHO, 2009; Katz, 2006).

2.12.5. Water

The mother can still exclusively breastfed even in hot climates because breast milk contains enough water for the baby preventing dehydration (Katz, 2006).

2.12.6. Other bioactive factors

- Once the milk has reached the small intestine, complete digestion of fat is facilitated by bile salt-stimulated lipase. Fat in artificial milk, however, is not completely digested.
- The lining of the infant's intestine is stimulated by epidermal growth factor so that it is better able to digest and absorb nutrients and is less easily infected by foreign proteins.
- Other growth factors target the development and maturation of the nerves and retina (WHO, 2009).

2.12.7. Anti-infective factors

- Immunoglobulin, principally secretory immunoglobulin A (sIgA), which coats the intestinal mucosa prevents bacteria from entering the cells. The sIgA contains antibodies that are formed in the mother's body to guard against bacteria in her gut and against infections she has encountered. This is of incredible value considering that infants' immune system does not develop fully until they are two years old.
- Immunoglobulin M, D, G, E.
- White blood cells which can kill micro-organisms.
- Whey proteins (lysozyme and lactoferrin) which can kill bacteria, viruses and fungi. Lysozyme lyses mostly gram-positive and a few gram-negative bacteria. Lactoferrin activates natural killer cells and plays a role in complement activation, affects coagulation and inhibits *E.coli* and *S. flexneri* from adhering to cell walls.
- Oligosaccharides which prevent bacteria from attaching to mucosal surfaces.

- *Lactobacillus bifidus* which are beneficial bacteria that prevent the growth of harmful organisms.
- Interferon and fibronectin have antiviral activities and enhance lytic properties of milk leucocytes.

(Wagner, 2010; Jahn, 2009; WHO, 2009; Georgeson and Filteau, 2000; Hamosh, 2001)

2.13. OTHER BENEFITS OF BREASTFEEDING

Breastfeeding holds benefits for both the baby and the mother.

2.13.1. For the baby:

- Breastfed infants are at lower risk for sudden infant death syndrome.
- The risk of Type 1 diabetes decreases for children with a family history of diabetes if the infant is exclusively breastfed for a minimum of four months. Incidences of Type 2 diabetes may also be reduced later in life.
- Obesity, high blood pressure and high cholesterol are reduced in breastfed children.
- The instances of eczema and asthma are lower for infants who are exclusively breastfed for four months.
- Studies show that children who are breastfed score on average 3.2 points higher on cognitive functions than children who were artificially fed and this may affect an individual's ability to contribute to society.
- Breastfeeding is very convenient. The stress of making sure that there is sufficient formula, that bottles are sterile, and the chore of needing to heat up bottles are eliminated.
- Breast milk is always fresh and healthy.
- Oxytocin raises the pain threshold and creates a sense of calm in both mother and baby.
- Bonding between mother and baby.

(WHO, 2009; WHO and UNICEF, 2009; Jahn, 2009; Katz, 2006)

2.13.2. For the mother:

- Reduced risk of ovarian and premenopausal breast cancer.
- Decreased risk of heart disease.
- Decreased risk of osteoporosis.
- Lower chance of developing Type 2 diabetes.
- Breast milk suppresses ovulation and delays the return of a woman's fertility. This is very important especially in HIV-infected mothers, since another pregnancy in a short period of time can cause severe stresses on their body. This may place the next infant at risk of HIV and of becoming an orphan.

(Jahn, 2009; WHO and UNICEF, 2009; Campbell, 2008; Latham and Preple, 2000)

2.14. ANIMAL MILK AND INFANT FORMULA

In the 19th century, improved production of cow's milk produced large surpluses. Preservation technologies developed and this led to the possibility of using cow's milk as a breast milk substitute in Europe and North America. Before this, a baby was breastfed by its mother or a wet nurse. In the early 20th century, advertising in Britain led to greater use of artificial feeding products. Powdered milk for infants was marketed in the 1950s and 1960s. Nestlé saleswomen in nurses' uniforms gave out free samples and promoted artificial feeding in maternity wards and clinics (Campbell, 2008).

The use of milk substitutes offered greater independence to woman and many people believed that these substitutes were beneficial for the children, encouraging faster weight gain. As a result, bottle feeding was associated with scientific motherhood (Campbell, 2008). As well, in the 1920s the breast was sexualized in such a way that it made public feeding more sensitive than it had ever been previously (Campbell, 2008).

The quality and quantity of nutrients found in animal milks vary greatly from those found in human milk. Although animal milks can be home-modified as a short term replacement for breast milk, in exceptionally difficult situations, they can never be

equivalent to or have the same anti-infective properties as breast milk. This modification can be done by the addition of water, sugar and micronutrients (OVCSupport.net., 2013; WHO, 2009).

Infant formula is usually made from industrially modified cow milk or soy products. The quantities are adjusted during the manufacturing process to make them compare better with breast milk. The qualitative differences in the fat and protein cannot be altered and anti-infective and bio-active factors cannot be added (WHO, 2009).

Infections in newborns have also been traced to contaminated powdered formula. Between 1982 and 1994 the FDA issued 22 significant recalls of infant formula in the United States. Phyto-oestrogens, found in soy formula have the same properties as the hormone oestrogen which could potentially reduce fertility in boys and bring about early puberty in girls (WHO, 2009). The WHO's and United Nations Children's Fund's (UNICEF) global recommendations (2009) for optimal infant feeding as set out in the Global Strategy are described in Appendix A.

A small number of health conditions in the infant or the mother, as described in Appendix B, may justify recommending that she not breastfeed temporarily or permanently. These conditions affect only a few infants and mothers. Whenever breastfeeding is stopped the benefits of breastfeeding should be weighed against the risks posed by the presence of certain conditions. Mothers who are affected by any of said conditions should receive treatment according to standard guidelines.

2.15. CURRENT FEEDING PRINCIPLES AND RECOMMENDATIONS

The Guideline Development Group identifies nine key principles that are summarized in Table 2.15 that should be read together with the seven evidence-based recommendations.

2.15.1. Principles

These values cannot be subjected to formal research but represent public health approaches and preferences (WHO, 2010).

Table 2.15 Summary of the nine key principles for feeding of infants by mothers who are HIV-infected

Key Principle 1 **Balancing HIV prevention with protection from other causes of child mortality**
Infant feeding practices of mothers who are HIV-infected should support the greatest likelihood of HIV-free survival of their children that do no harm the health of the mother. To achieve this, prevention of HIV transmission needs to be balanced with meeting the infant's nutritional requirements and protection against non-HIV morbidity and mortality.
Key Principle 2 **Integrating HIV interventions into maternal and child health services**
National authorities should aim to integrate HIV testing, care and treatment interventions for all women into maternal and child health services. This should include access to $CD4^+$ T cell count testing as well as antiretroviral therapy or prophylaxis for the women, in order to prevent MTCT of HIV.
Key Principle 3 **Setting national or sub-national recommendations for infant feeding in the context of HIV**
National or sub-national health authorities should decide whether health services will mainly counsel and support mothers known to be HIV-infected to either: o breastfeed and receive antiretroviral drugs, or o avoid all breastfeeding in order to give the infant the greatest chance of HIV-free survival.
International recommendations and the following should be taken into account: o the socio-economic and cultural context of the population that is served by the maternal and child health services; o the quality and availability of health services; o local epidemiology, including HIV prevalence among pregnant women; and o the main causes of maternal and child under-nutrition and child mortality.

Key Principle 4 **When antiretroviral drugs are not (immediately) available, breastfeeding may still provide infants born to HIV-infected mothers with a greater chance of HIV-free survival**
ARVs should be available to prevent HIV transmission to infants and for maternal health. While ARV interventions are being scaled up, national authorities should not be deterred from recommending that HIV-infected mothers breastfeed as the most suitable infant feeding practice in their setting. Unless environmental and social circumstances are safe for, and supportive of replacement feeding, mothers should be counselled to exclusively breastfeed in the first six months of life and continue breastfeeding thereafter even when ARVs are not available. Even in circumstances where ARVs are unlikely to be available the recommendation for HIV-exposed infants should also be breastfeeding.
Key Principle 5 ***Informing mothers known to be HIV-infected about infant feeding alternatives***
Women known to be HIV-infected should be informed about the infant feeding practices that are recommended by the national or sub-national authority to improve HIV-free survival of HIV-exposed infants and the health of HIV-infected mothers. They should be informed that there are alternatives.
Key Principle 6 **Providing services to specifically support mothers to appropriately feed their infants**
All pregnant women and mothers should have access to skilled counselling and support in appropriate infant feeding practice and ARV interventions to promote HIV-free survival of infants.
Key Principle 7 **Avoiding harm to infant feeding practices in the general population**
Counselling and support given to mothers known to be HIV-infected must be done in such a way as to not undermine optimal breastfeeding practices in the general population.
Key Principle 8 **Advising mothers who are HIV uninfected or whose HIV status is unknown:**
Mothers who are known to be HIV uninfected or whose HIV status is unknown should be counselled to exclusively breastfeed their infants for the first six months. Then complementary foods should be introduced while continuing breastfeeding for 24 months or beyond.
Mothers whose status is unknown should be offered HIV testing.
Mothers who are HIV uninfected should be counselled about ways to prevent HIV infection, and they should know that services such as family planning are available to help

them remain uninfected.
Key Principle 9 ***Investing in improvements in infant feeding practices in the context of HIV***
Resources and commitment should be increased by governments, other stakeholders and donors, for the implementation of the global strategy for infant and young child feeding, the United Nations HIV and infant feeding framework for priority action and the global scale-up of the prevention of mother-to-child transmission of HIV in order to effectively prevent postnatal HIV infections, improve HIV-free survival and achieve relevant United Nations General Assembly Special Session goals.

2.15.2. Recommendations

Recommendations are summarised in Table 2.16. These are directed towards policymakers, academics and health workers and are intended to inform and assist national technical groups, international and regional partners who provide HIV care and treatment services and/or maternal and child health services in countries affected by HIV, in formulating national or sub-national infant feeding recommendations in the context of HIV.

Table 2.16 Recommendations for feeding of infants by mothers who are HIV-infected

Recommendation 1 **Ensuring mothers receive the care they need**
Mothers known to be HIV-infected should be provided with lifelong Antiretroviral Therapy (ART) or Antiretroviral (ARV) prophylaxis interventions to reduce HIV transmission through breastfeeding, according to WHO recommendations.
Recommendation 2 **Which breastfeeding practices and for how long?**
In settings where national or sub-national authorities have decided that maternal, newborn and child health services will principally promote and support breastfeeding and ARV interventions, infants of mothers known to be HIV-infected (and whose infants are HIV uninfected or of unknown HIV status) should be exclusively breastfed for the first 6 months of life. Complementary foods should thereafter be introduced, and breastfeeding should be continued for the first 12 months of life.

Recommendation 3

When mothers decide to stop breastfeeding

In settings where national or sub-national authorities have decided that maternal, newborn and child health services will principally promote and support breastfeeding and ARV interventions, mothers known to be HIV-infected who decide to stop breastfeeding should stop gradually within one month. ARV prophylaxis should continue for one week after breastfeeding has fully stopped. Abrupt stopping of breastfeeding is not advisable.

Recommendation 4

What to feed infants when mothers stop breastfeeding

Infants of HIV-infected mothers who decide to stop breastfeeding should be provided with safe and adequate replacement feeds to enable normal growth and development. Alternatives to breastfeeding include:

For infants under six months of age*:*

- Commercial infant formula milk as long as home conditions outlined in Recommendation #5 are fulfilled;
- Expressed, heat-treated breast milk (see Recommendation #6).
- Home-modified animal milk is not recommended as a replacement food in the first six months of life.

For children over six months of age:

- Commercial infant formula milk as long as home conditions outlined in Recommendation #5 are fulfilled;
- Animal milk (boiled for infants under 12 months), as part of a diet that provides adequate micronutrient intake;
- Meals, including milk-only feeds, other foods and combination of milk feeds and other foods, should be provided four or five times per day. All children need complementary foods from six months of age.

Recommendation 5

Conditions needed to safely formula feed

Only when specific conditions are met can mothers, known to be HIV-infected, give commercial infant formula milk as a replacement feed to their HIV-uninfected infants or infants who are of unknown HIV status:

- ✓ safe water and sanitation are guaranteed at the house and in the community; and
- ✓ infant formula milk can constantly be provided to support normal growth and development of the infant; and
- ✓ it can be prepared cleanly and regularly enough so that it is safe and carries a low risk of diarrhoea and malnutrition; and

✓	infant formula milk can exclusively be given for the first six months; and
✓	the family is supportive of this practice; and
✓	healthcare that offers comprehensive child services is readily available.
Recommendation 6 **Heat-treated, expressed breast milk**	
An interim feeding strategy can be the expression and heat-treating of breast milk:	
•	in special circumstances such as a low birth weight or if the infant is ill in the neonatal period and unable to breastfeed; or
•	when the mother is unwell or has a breast health problem such as mastitis; or
•	to assist mothers to stop breastfeeding; or
•	ARV is temporarily not available.
Recommendation 7 **When the infant is HIV-infected**	
Infants that are known to be HIV-infected should be exclusively breastfed for the first six months of life and continue to be breastfed as per the recommendations for the general population - that is up to two years or beyond.	

2.16. MATERNAL NUTRITION AND BREAST MILK

The question of composition and volume of breast milk produced by mothers, on different planes of nutrition at different phases of lactation, is a major issue especially in resource poor countries, where food supply is limited. There are concerns about the adequacy of such milk for infants (Ettyang, van Marken, Lichtenbelt, Esamai, Saris and Westerterp, 2005; Jelliffe and Jelliffe, 1978).

During lactation, a mother's energy intake should increase by about 10% to cover the energy cost of breastfeeding, and if she is moderately or very active, up to 20%. In very ill-fed mothers, lactation can sometimes lead to weight loss that can be as much as 7kg over a year, leading to nutritional oedema in very poorly nourished women. The quantity of breast milk decreases and ultimately ceases in seriously malnourished women. Thus, malnourishment and the cessation of breast milk can have fatal consequences for the nursing baby (Jelliffe and Jelliffe, 1978). Women who are well nourished and follow a varied diet will usually retain enough milk (if enough was stored during her pregnancy) to cover for any extra needs. Even if a

woman is moderately malnourished the quality of breast milk is good (WHO, 2009). A study done by Jelliffe and Jelliffe(1978) showed that protein supplementation, to the lactating mother's diet, increased the volume of milk produced and the weight of the infant but not the protein content of the milk.

Studies done on fat content, in poorly fed women in developing countries, have demonstrated considerable variation, but recent work suggests that the fat content may be reduced to as low as 1g/100mℓ. The calories of this milk are associated with significant lessening of energy intake. The polyenoic fatty acids may be diminished in malnourished mothers' breast milk and this could have possible bad consequences in relation to brain growth (Emmett and Rogers, 1997; Jelliffe and Jelliffe, 1978).

Lactose is constant in concentration and shows no diurnal variation. Even in poorly nourished mothers lactose does not seem to vary much (Lönnerdal, 1986; Jelliffe and Jelliffe, 1978).

Various vitamins in breast milk are affected differently by malnourishment:

- Vitamin A is influenced by the adequacy of the diet of the mother during pregnancy and lactation. Intake is generally higher in spring and summer months due to greater availability of green leafy and yellow vegetables.
- Fat-soluble Vitamin D levels are low in human milk, but the recent discovery that breast milk contains a water-soluble conjugate of vitamin D explains the clinically well-recognized rarity of rickets in breastfed infants. Milk levels of vitamin D may be of minor physiological importance since it appears that sunlight may be the main contributor of antirachitic sterols in infants.
- Thiamine concentrations have been found to be low in regions with a high incidence of infantile beriberi. This is due to insufficient maternal stores and intake.
- Sufficient amounts of riboflavin are found in human milk, provided that the maternal diet is adequate.
- Niacin can be synthesized from tryptophan, thus human milk has a high potential niacin concentration.

- Low levels of vitamin B_{12} have been found in milk of poorer, vegetarian women. Vitamin B_6 is also affected by maternal intake.
- Vitamin C levels vary with the season. When fresh fruits and vegetables are readily available, poorly nourished women often show lower concentrations of ascorbic acid than well-nourished women.
- Calcium levels in poorly fed mothers range from normal to somewhat low concentrations.

(Lönnerdal, 1986; Jelliffe and Jelliffe, 1978)

The maternal diet seems to have no effect on the concentration of magnesium, sodium, potassium, chlorine, iron, zinc, copper, and the trace elements (Lönnerdal, 1986).

Human milk is all that is required to sustain growth and good nutrition in the infant for the first six months providing that the mother is well-nourished. These mothers will have produced foetuses with optimal stores, and will have built adequate nutritional reserves themselves, including subcutaneous fat. The volume and composition of milk in poorly nourished women is surprisingly good. Metabolic adaptations have been made because of their maternal depletion, but the quantity and quality is often suboptimal (Ettyang *et al*., 2005; Jelliffe and Jelliffe, 1978).

Having taken all of the above into consideration, it appears that breast milk is best for infants. That being the case, the question whether HIV disease progression will affect the nutrient composition of breast milk in HIV mothers, risking not only MTCT but also malnutrition, arises. As shown, mothers who breastfeed need an increase in their energy intake to provide sufficient nutrients for themselves and their infants, to sustain basic metabolic functions. That is so even before taking the increased energy needs of HIV-positive mothers into consideration.

CHAPTER 3

METHODOLOGY

3.1. STUDY DESIGN AND WORKPLAN

This study used a descriptive design. The breast milk of HIV-infected volunteers, who were lodging and day visiting mothers at the Paediatric and Neonatal wards of National; Pelonomi and Universitas Hospitals in Bloemfontein, the Mangaung University of the Free State Community Partnership Programme (MUCPP), Heidedal and Botshabelo clinics, was measured for its nutrient composition. Blood and breast milk samples as well as clinical data from the patient files were obtained from each participant, after permission was granted by the appropriate authorities. Laboratory measurements were taken and procedures performed in the laboratories of Bloemfontein and Cape Town PathCare, and at the Mowbray Maternity Hospital Milk Depot in Cape Town.

A summary layout of the data collection which included a consent form, demographic profile as well as blood and milk analysis is given in Figure 3.1 below.

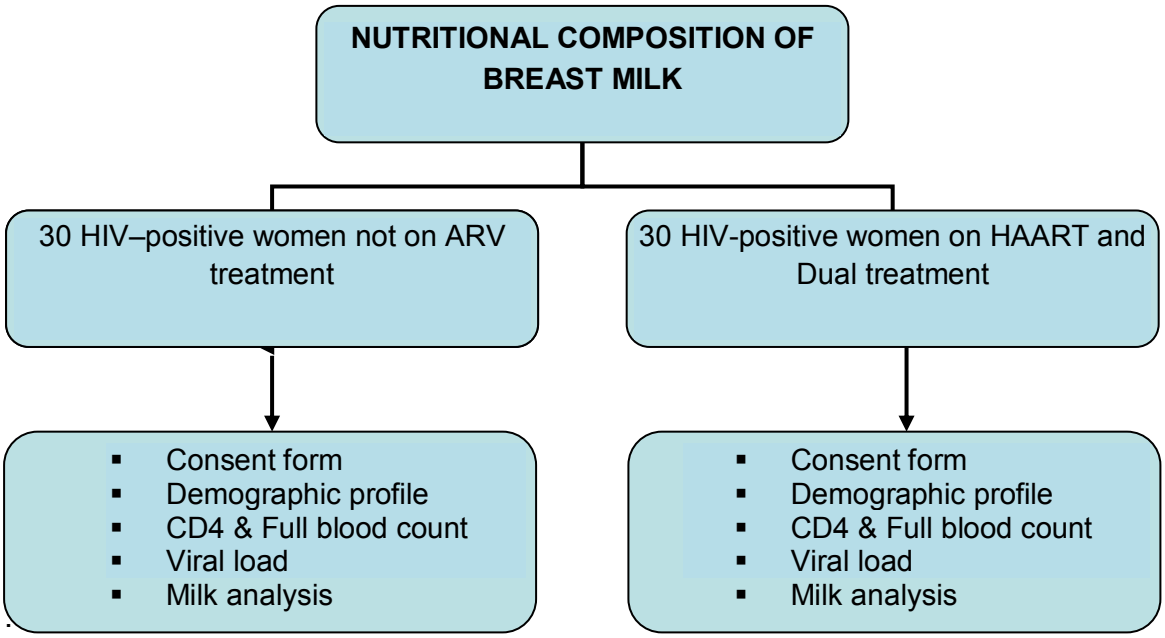


Figure 3.1 Summary layout of the data collection

3.2. STUDY POPULATION

The study population consisted of HIV-infected female volunteers who were lodging or day visiting mothers at the Paediatric and Neonatal wards of National, Pelonomi and Universitas Hospitals in Bloemfontein, the Mangaung University of the Free State Community Partnership Programme (MUCPP), Heidedal and Botshabelo clinics. Their HIV status and treatment regime were obtained from their files. Various $CD4^+$ T cell counts were included to accommodate the different stages of HIV. The population was divided into two groups:

Group 1: 30 HIV-positive patients who were not on ARV treatment were selected.

Group 2: 30 HIV-positive patients on ARV treatment were selected.

3.3. SAMPLE SIZE

Sixty patients, who gave written informed consent and met the inclusion criteria, were selected for this study.

3.4. INCLUSION AND EXCLUSION CRITERIA

The inclusion and exclusion criteria for the subjects were as follows:

3.4.1 Inclusion Criteria

- All lactating women who exclusively breastfed.
- Subjects had to have been post-partum at least 5 days to avoid the analysis of colostrum. The analysis had to be performed on mature milk. Colostrum is the deep yellow-colored milk secreted the first days after childbirth. Secretion thereof lasts about 5 days after birth and changes to mature milk gradually.
- Subjects should not have been breastfeeding for longer than 45 days.
  (Summary: Mothers who had been breastfeeding between 5-45 days).
- Subjects had to be between 18 – 40 years of age.
- Mothers who were not still breastfeeding a previous child.

3.4.2. Exclusion Criteria

- Evidence of psychiatric disorder, antagonistic personality, poor motivation to participate in this study or limited ability to comply with protocol requirements.
- History of, or current compulsive alcohol abuse (>10 drinks weekly), or regular exposure to other substance abuse.
- Participation in another study with an experimental drug within eight weeks of the first administration of study medication.
- Heavy smoking (i.e. more than 20 cigarettes per day).
- Diabetic HIV-positive individuals.

3.5. WITHDRAWAL CRITERIA

Subjects were informed of their right to withdraw from the study at any time, irrespective of the reason. None of the subjects withdrew from the study.

3.6. SUBJECT IDENTIFICATION

Each subject received a unique ten digit laboratory number and retained this number throughout the study. Initials and date of birth were obtained from the subjects' identification books.

3.7. SUBJECT INFORMED CONSENT

A written information sheet as well a verbal explanation concerning the purpose, nature and possible risk involved in taking part in this study was given to the subjects. They were also informed about the purpose, procedures, restrictions, obligations, remuneration and insurance coverage that were relevant to the study. The informed consent discussion and written patient's information were both included to provide adequate information and ascertain that the subjects understood all the ramifications. By signing and dating the informed consent form, the respondents voluntarily accepted the terms of the study and agreed to participate in

the study. The researcher who conducted the informed consent discussion also signed with the respective subjects.

The subject information sheets and informed consent forms were made available in English, Afrikaans and Sesotho. An interpreter was also present to afford the respondents the opportunity to use their preferred language. The subjects were provided with the subject information sheet and informed consent form in the language of their choice and they retained copies of both.

3.8. DATA AND SAMPLE COLLECTION

3.8.1. Questionnaire

After the consent was completed the patients were interviewed and asked to complete a questionnaire to gather socio-economic and breastfeeding information about the participants (see Appendix C).

3.8.2. Breast milk samples

Mothers were asked to abstain from breastfeeding their babies for three hours before milk collection. The breast milk samples were collected just before the mothers began breastfeeding. The procedure was explained to each participating mother and they were asked to collect 5-10mℓ of their breast milk in sterile 50mℓ plastic containers, themselves.

3.8.3. Blood samples

A registered phlebotomist collected two 5mℓ ethylenediaminetetraacetic acid (EDTA) tubes of blood from each participant.

3.9. DATA FROM PATIENT FILES

The medical history of the patient was obtained from her patient files. If the patient had given consent to participate in the study, her $CD4^+$ T cell count as well as the treatment was obtained from her patient file – where available.

3.10. LABORATORY ANALYSIS

3.10.1 SAMPLE PREPARATION

3.10.1.1 Breast milk

Breast milk samples were divided into two sterile containers then frozen immediately after collection to ensure a homogenous sample for analysis at the two different laboratories (PathCare Bloemfontein and Mowbray Maternity Hospital Milk Depot, Cape Town). Samples were transported to Cape Town, within two weeks of sampling, on dry ice with an overnight courier to ensure that they stayed frozen until analysis.

Milk can be frozen in a freezer compartment for up to two weeks before analysis, but once thawed milk cannot be frozen again. It can, however, be refrigerated for up to 9 hours, before analysis, once thawed.

3.10.1.2 Blood

The two EDTA tubes of blood that were collected were processed according to the standard operating procedures (SOP) of PathCare. The full blood count and $CD4^+$ T cell count were done upon arrival at the laboratory. The viral load was sent to the PathCare laboratory in Cape Town for analysis. Plasma was separated from the red blood cells by centrifugation of the EDTA sample at 800g for 20 minutes and then the plasma was toppled into a polypropylene tube that was labelled, before analysis, with the appropriate barcode and subject number.

3.10.2 SAMPLE ANALYSIS

3.10.2.1 Breast milk analysis

The analysis of the biochemical variables were measured according to standard operating procedures of each laboratory. The following variables were included in the analysis of the breast milk:

Macro-nutrients:

- Lactose (%);
- Proteins (%);
- Fat (%);
- Total Solids (%) and
- Energy (kcal/100mℓ).

Micro-nutrients:

- Calcium (mmol/ℓ);
- Phosphate (mmol/ℓ).

The micro-nutrients were analyzed by PathCare in Bloemfontein and the macro-nutrients were analyzed at the Mowbray Maternity Hospital Milk Depot in Cape Town.

3.10.2.1.a Measurement of macro-nutrients

***Instrument*:** MIRIS Human milk analyser
***Manufactured by*:** MIRIS
***Principle and methodology*:** The MIRIS Human Milk Analyzer is based on a unique patented technique in combination with approved IR-technology (Infrared transmission spectroscopy). Fat, protein, lactose, energy and total solids are analyzed directly in one single run without any chemicals. Results are obtained within one minute (MIRIS, 2010).

***Quality assurance*:** On-board quality assurance

3.10.2.1.b <u>Measurement of micro-nutrients:</u>

***Instrument*:** DXC 800

***Manufactured by*:** *Beckman Coulter*

***Principle and methodology*:**

<u>Phosphate</u>: PHOSm reagent is used to measure the phosphorus concentration using a timed-rate method. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a coloured phosphomolybdate complex (BECKMAN COULTER, 2006).

<u>Calcium</u>: Total calcium concentration is measured through indirect potentiometry utilizing a calcium ion selective electrode in conjunction with a sodium reference electrode. A calcium ion selective electrode measures un-bound free calcium ions in solution (BECKMAN COULTER, 2005).

The DXC 800 is a fully automated analyzer, and after the samples were put into the sample racks, they and the reagent probes samples' exact volumes were placed into cuvettes and the respective reactions occurred. Reactions are measured against a calibration curve and the calcium and phosphate concentrations are taken.

***Quality assurance*:** Synchron 1, 2 and 3.

3.10.2.2 <u>Blood analysis</u>

The CD4/CD8^{+}T cell counts (cells/cells/mm^{3}), viral load (cps/mℓ) and the full blood count (consisting of red blood cells (RBC)(x10^{12}/ℓ); haemoglobin (HB)(g/dℓ); haematocrit (HCT)(ℓ/ℓ); mean corpuscular haemoglobin (MCH)(pg); mean corpuscular haemoglobin concentration (MCHC)(g/dℓ); mean corpuscular volume (MCV)(fℓ); platelets (PLT)(x10^{9}/ℓ) and white blood cells (WBC)(x10^{9}/ℓ) were measured in the EDTA blood of each patient.

3.10.2.2.a CD4/CD8^{+}T-cell counts

***Instrument*:** FACSCalibur

***Manufactured by*:** Beckton Dickenson

***Principle and methodology*:** MultiTEST 4-colour reagents use a time-saving lyse/no-wash method for direct immunofluorescence staining of human peripheral blood specimens. When whole blood is added to TruCOUNT tubes containing MultiTEST reagents, the fluorochrome-labelled antibodies bind specifically to antigens on the surface of lymphocytes. The FACSCalibur flow cytometer detects four fluorescent colours as well as forward scatter and side scatter (BECKTON DICKINSON, 2010).

Twenty microliter of MultiTEST CD3/CD8/CD45/CD4 reagent was pipetted at the bottom of a TruCOUNT tube. Then 50µℓ of well mixed blood from each patient was added to the bottom of the TruCOUNT tube. The tube containing the blood and reagent was vortexed gently, but thoroughly, to allow proper mixing of the reagent and EDTA blood. It was then incubated for 15 minutes in the dark, at room temperature. After incubation 450µℓ FACS Lysing Solution was added and again vortexed gently and left in the dark for another 15 minutes.

After preparation, the samples were analyzed on the BD FACSCalibur analyzer which uses flowcytometry to determine the CD4-, CD3-, and CD8 positive cells (BECKTON DICKINSON, 2010).

***Quality assurance**:* Commercial controls were prepared in accordance with SOP and run with each batch of patients' samples on either a low, medium or high Trucount bead control and another control was analyzed with each batch.

3.10.2.2.b Viral load

***Instrument*:** COBAS AmpliPrep and the COBAS TaqMan

Manufactured by: COBAS

Principle and methodology: The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test was used for the determination of the viral load. This is a nucleic acid amplification test for the quantification of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma, using the COBAS AmpliPrep instrument for automated specimen processing and the COBAS TaqMan analyzer for automated amplification and detection.

The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA); and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labelled oligonucleotide detection probes specific for the test.

The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armoured RNA. The Master Mix reagent contains a primer pair specific for both HIV-1 RNA and HIV-1 QS RNA. The Master Mix was developed to ensure comparable quantitation of group M subtypes of HIV-1. The detection of amplified DNA was performed using a target-specific and a QS-specific dual-labelled oligonucleotide probe that allowed independent identification of HIV-1 amplicon and HIV-1 QS amplicon. The quantitation of HIV-1 viral RNA was performed using the HIV-1 QS.

The HIV-1 QS is a non-infectious Armored RNA construct that contains HIV sequences with identical primer binding sites as the HIV-1 RNA target, and a unique probe binding region that allows HIV-1 QS amplicon to be distinguished from HIV-1 amplicon. The HIV-1 QS is added to each specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection of cleaved dual-labelled oligonucleotide detection probes. The COBAS TaqMan analyzer calculates the HIV-1 RNA concentrations by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control. The HIV-1 QS compensates for effects of inhibition and controls for the preparation and

amplification processes, allowing a more accurate quantitation of HIV-1 RNA in each specimen.

Selection of the target RNA sequence for HIV-1 depends on identification of regions within the HIV-1 genome that show maximum sequence conservation among the various HIV-1 group M subtypes, and HIV-1 group O specimens. In order to address the high genetic variability of the virus, two regions of HIV genome are simultaneously targeted for amplification and detection by the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test. Two target-specific and one QS-specific dual-labelled oligonucleotide probes permit independent identification of the HIV-1 amplicon and of the HIV-1 QS amplicon. Accordingly, the appropriate selection of the primers and the dual-labelled oligonucleotide probe is critical to 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 uses reverse transcription and PCR amplification primers that define sequences within the highly conserved regions of the HIV-1 *gag* gene and of the HIV-1 LTR region.

The viral load testing was performed by qualified Medical Technologist/Technicians, as per PathCare Laboratories' SOP for viral load testing. The test can quantify HIV-1 RNA over a range of 40 – 10 000 000 copies/mℓ (ROCHE, 2007).

***Quality assurance*:** HIV-1 L(+)C, HIV-1 H(+)C and CTM (-) C

3.10.2.2.c <u>Full blood count</u>

***Instrument*:** Sysmex XT2000*i*
***Manufactured by*:** Sysmex®
***Principle and methodology*:** The Sysmex XT2000i is an automated haematology analyzer for in-vitro diagnostic use in clinical laboratories. RBC and PLT are analyzed by the RBC detector using Hydro Dynamic Focusing. Haemoglobin is analyzed by the HB detector based on the sulfolyser (SLS) haemoglobin detection method. The HCT is a direct measurement on the red cell transducer. The MCV, MCH and MCHC are parameters calculated from the red cell parameters. The WBC

is analyzed with an optical block based on the flow cytometry method, using a semiconductor laser (SYSMEX, 2005).

Quality assurance: Three levels of commercial controls were used, namely e-Checks level 1, 2 and 3.

3.11. STATISTICAL ANALYSIS

Data from this study was analyzed by the STATISTICA® '98 Edition, Statsoft Inc. Software. Data was presented in both tabled and graphic format using descriptive statistics. Spearman correlation analysis was used to draw comparisons between the stage of disease (using the CD4 lymphocyte cell count as an indicator) and the nutrient composition of the breast milk. The chi-square non-parametric analysis was used to compare categorical data. A p-value of less than 0.5 was considered to be significant.

3.12. ETHICAL CONSIDERATIONS

3.12.1 Ethical approval

Ethical approval was obtained from the Ethics Committee of the University of the Free State (ETOVS nr 108/08) (See Appendix D). Subjects were informed of the relevance of the study and they were requested to participate voluntarily. Their right to withdraw from the study without having to explain their reasons for doing so was explained to each participant before she was requested to sign an informed consent contract.

3.13. SAFETY VARIABLES

3.13.1 Project safety

Blood was collected by registered medical personnel and the breast milk samples by the patients themselves. The samples were not handled by anybody else. This was a routine medical procedure and did not involve any risk to the patient.

3.13.2 Patient safety

Blood and breast milk samples were collected by registered medical personnel and sent to PathCare and Mowbray Milk Depot laboratories.

CHAPTER 4

RESULTS

The socio-economic, breastfeeding, blood and milk analysis data are reflected in this chapter by means of figures and tables. A Spearman correlation coefficient was used to determine whether there was a correlation between the immunological data (HIV disease progression) and data of milk composition.

4.1 TREATMENT REGIME OF THE STUDY POPULATION

Sixty HIV-infected participants who met the inclusion criteria were selected and divided into two groups. Group one consisted of 30 participants who did not receive any treatment for HIV (HIV-infected without treatment group). The second group consisted of 30 participants who did receive treatment for HIV (HIV-infected with treatment group). Figure 4.1 reflects the treatment regime of the selected study population. Fifty percent of the participants did not receive any ARV treatment, 10% received dual treatment and 17% was on HAART. The ARV regime could not be established for 23% of the participants.

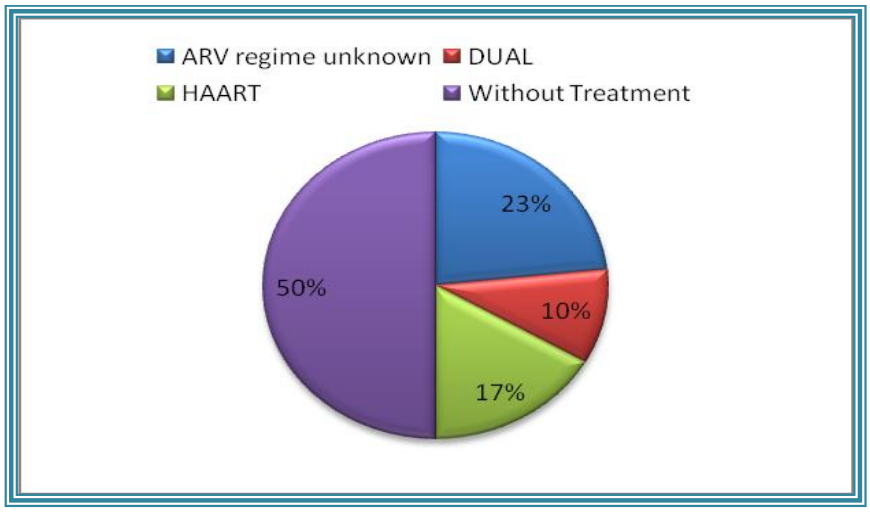


Figure 4.1 The treatment regime for the selected study population (n=60)

4.2 SOCIO-ECONOMIC RESULTS

The information gathered was based on the participants' socio-economic status, the highest education that the babies' caregivers had received (Table 4.1), the number of people contributing to the household income (Table 4.2), the employment status of the participants (Table 4.3) and whether the participants had access to a refridgerator, water and electricity (Figure 4.2). Based on the above information, the following was established:

The table below summarizes the highest education of the baby's caregiver by group.

Table 4.1 Highest education level of the baby's caregiver (n=60)

Highest education level	HIV-infected without treatment group (n=30)	HIV-infected with treatment group (n=30)
None	2 (6.67%)	1 (3.33%)
Primary	4 (13.33%)	4 (13.33%)
Secondary	24 (80%)	25 (83.33%)
Tertiary	0 (0%)	0 (0%)

When comparing the highest education level of the caregivers of the babies, there was no significant statistical difference detected between the HIV-infected without treatment mothers' caregivers and the HIV-infected with treatment mothers' caregivers ($p=1.000$), where a p-value of <0.05 is considered to be significant. The majority of the groups had a secondary education, 80% and 83% respectively, but none of the caregivers had any tertiary education, with only a small percentage that had no educational training at all (6.67% and 3.33% respectively).

The number of people who contributed to the household income at the time of the interview is shown in Table 4.2.

Table 4.2 Number of people contributing to the household income (n=60)

Number of people contributing to household	HIV-infected without treatment group(n=30)	HIV-infected with treatment group (n=30)
0	7 (23.33%)	6 (20%)
1	17 (56.67%)	22 (73.33%)
2	6 (20%)	2 (6.67%)

No significant difference between the two groups was found when comparing the number of people contributing to the household income (p=0.4363). Twenty three percent and 20% of the study group did not have anyone contributing to the household at that stage, where the majority of the study population (76.67% and 80% respectively) had somebody that contributed to the household income.

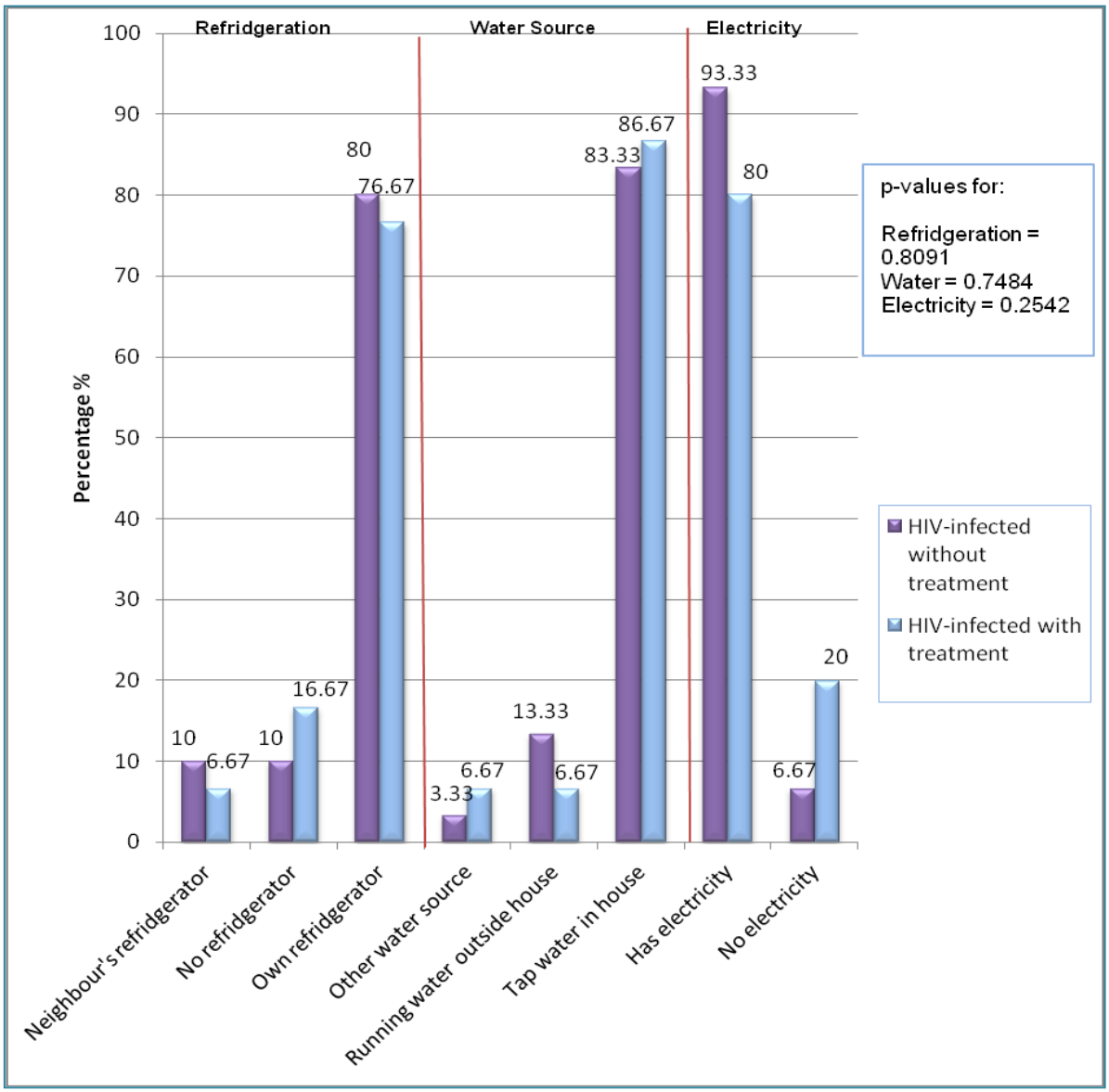
Table 4.3 indicates the employment status of the participants at the time of the interview.

Table 4.3 The employment status of the two groups (n=60)

Employment status	HIV-infected without treatment group (n=30)	HIV-infected with treatment group (n=30)
Housewife by choice	9 (30%)	8 (26.67%)
Self employed	1 (3.33%)	0 (0%)
Unemployed	16 (53.33%)	21 (70%)
Wage/Salary earner	4 (13.33%)	1 (3.33%)

The majority of both groups were unemployed at the time of the interview, 30% and 27% of the mothers were a housewife by choice and 13% and 3% earned a salary/wages. No significant statistical difference was detected between the two groups (p=0.2645).

The availibility of certain household appliances and needs is reflected in Figure 4.2.



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

Figure 4.2 The availibility of refridgeration, water source and electricity supply to the study population

More than three quarters of both study groups had access to their own refridgerators (80% & 76.67%), tap water in the house (83.33% & 86.67%) and electricity (93.33% & 80%) (Figure 4.2). No significant difference (p=0.8091 for refridgeration, p=0.7484 for water and p=0.2542 for electricity) was detected between the two study groups pertaining to any of the resources indicated in Figure 4.2.

4.3 BREASTFEEDING RESULTS

In both the HIV-infected without treatment group as well as the HIV-infected with treatment group there were 29 mothers who breastfed exclusively and only 1 in each group who did not breastfeed exclusively. The fact that this woman did not exclusively breastfeed will not have any effect on the nutrient composition of the breast milk. The table below shows the number of times the mothers breastfed their baby during a 24 hour period. The whole group indicated that the best nutrition for their babies was breast milk.

Table 4.4 Frequency of breastfeeding intervals per 24 hour period (n=58)

Group	N	Median	Lower Quartile	Upper Quartile	Mean	SD	Minimum	Maximum
HIV-infected without treatment group	29	7	7	7	6.8	0.9	3	7
HIV-infected with treatment group	29	7	7	7	6.8	0.8	3	7

SD=standard deviation

The median (7), minimum (3) and maximum (7) in both of the study groups were the same and no significant difference between the two groups was detected for the number of times the mothers breastfed their babies (p=0.6779).

Although vitamin supplementation is recommended for pregnant woman only 12% of the participants were drinking a vitamin supplementation (Figure 4.3).

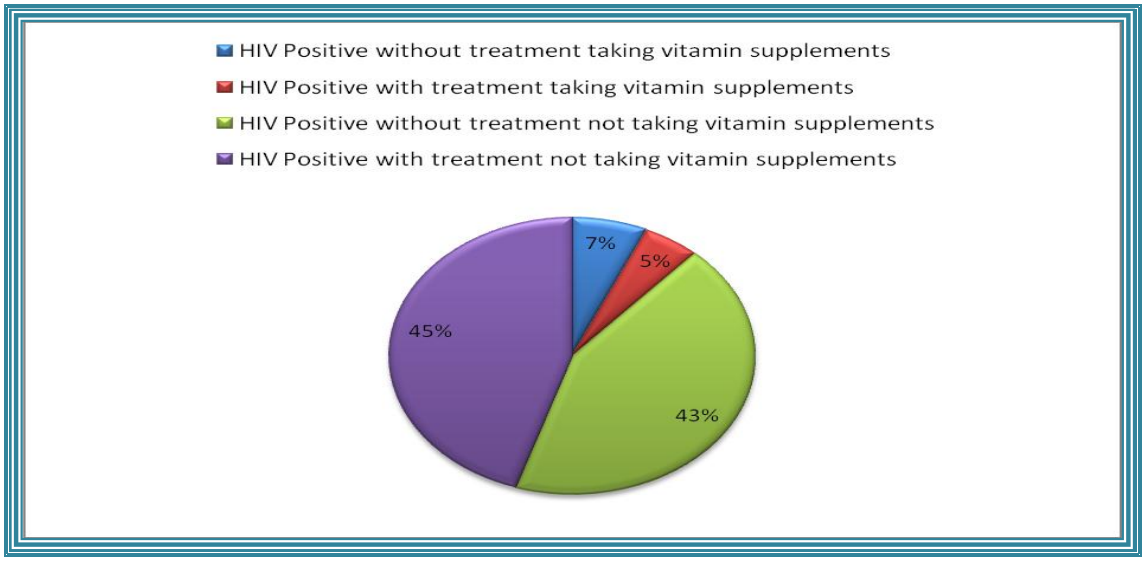


Figure 4.3 Vitamin intake of the study population during the study (n=60)

4.4 HAEMATOLOGICAL AND IMMUNOLOGICAL ANALYSIS

Table 4.5 on the next page represents the haematological and immunological parameters of the study population.

Table 4.5 Haematological and immunological parameters of the study population (n=60)

Variable	Group n=30	Normal range	Median	Mean	Standard Deviation	Minimum	Maximum	p-value*
White blood	NT		6.59	6.78	2.31	3.48	13.50	
cells (10^3/µℓ)	T	4.0 - 11.0	8.38	8.66	3.25	4.35	14.68	0.0364*
Red blood	NT		4.00	3.88	0.54	2.83	4.97	
cells (10^6/µℓ)	T	3.7 - 5.3	3.47	3.55	0.75	2.10	5.24	0.0467*
Hemoglobin	NT		11.60	11.42	1.82	6.80	14.70	
(g/dℓ)	T	12.0-16.0	10.35	10.71	2.00	7.70	15.30	0.1352
Hematocrit	NT		0.36	0.35	0.05	0.21	0.44	
(ℓ/ℓ)	T	0.35-0.45	0.33	0.33	0.06	0.21	0.45	0.1302
Mean Corposcular	NT		90.00	91.43	10.88	67.00	123.00	
Volume (fℓ)	T	81 - 100	92.50	94.60	10.51	74.00	116.00	0.2932
Mean Corposcular	NT		29.00	29.63	3.93	21.00	38.00	
Hemoglobin (pg)	T	28 - 35	30.00	30.67	4.42	22.00	39.00	0.3336
Mean Corposcular	NT		32.00	32.47	1.66	29.00	37.00	
Hemoglobin Concen-tration(g/dℓ)	T	32 - 37	32.00	32.03	2.25	25.00	36.00	0.5261
Platelets	NT		346.00	350.83	111.32	150.00	656.00	
(10^3/µℓ)	T	140 - 420	310.50	320.70	90.84	124.00	512.00	0.3516
Neutrophils	NT		3.29	3.89	1.96	1.34	9.46	
(10^3/µℓ)	T	2.0 - 7.5	4.44	5.36	2.90	1.44	10.82	0.0594
Lymphocytes	NT		2.04	2.14	0.80	0.92	4.27	
(10^3/µℓ)	T	1.0 - 4.0	2.37	2.36	0.76	1.10	3.88	0.2675
Monocytes	NT		0.49	0.51	0.18	0.24	0.98	
(10^3/µℓ)	T	0.0 - 0.8	0.63	0.71	0.36	0.23	1.94	0.0117*
Eosinophils	NT		0.16	0.21	0.23	0.02	1.07	
(10^3/µℓ)	T	0.0 - 0.4	0.13	0.20	0.20	0.01	0.91	0.9116
Basophils	NT		0.30	0.03	0.01	0.01	0.07	
(10^3/µℓ)	T	0.0 - 0.1	0.02	0.03	0.03	0.01	0.06	0.8725
CD3 cell count	NT		1756.00	1770.93	735.25	712.00	3742.00	
(cells/mm^3)	T	1100-1700	1855.50	1919.23	641.95	700.00	3100.00	0.2739
$CD4^+$ T cell count	NT		430.50	577.97	377.72	138.00	1447.00	
(cells/mm^3)	T	700 - 1100	540.00	565.40	287.61	94.00	1528.00	0.5692
CD8 cell count	NT		1081.50	1138.57	467.13	498.00	2452.00	
(cells/mm^3)	T	500-900	1209.50	1285.67	480.51	349.00	2521.00	0.1882
CD4:CD8 ratio	NT		0.42	0.53	0.31	0.10	1.27	
ratio	T	1.0 - 1.5	0.44	0.47	0.24	0.08	1.18	0.7170
HIV-1 viral load	NT		676.50	37812.07	95462.32	40.00	478135.00	
(cps/mℓ)	T	<40	788.00	63565.60	191016.80	40.00	777115.00	0.6883

p-value *-shows a significant difference with a value <0.05
NT – HIV-infected without treatment group
T – HIV-infected with treatment group
Red values indicates values that fell outside the normal reference values

The median for the haemoglobin, $CD4^+$ T cell count and the CD4:CD8 ratio for the HIV-infected group receiving treatment was lower than the reference range. For the HIV-infected with treatment group the red blood cells, haemoglobin, haematocrit, $CD4^+$ T cell count and the CD4:CD8 ratio was lower than the reference range (Table 4.5).

The median value for the basophils, $CD3^+$ T cell count, $CD8^+$ T cell count and viral load was higher than the reference range in both of the study groups.

The median $CD4^+$ T cell count was 430.5 with the lowest count being 138 and the highest 1447 for the HIV-infected without treatment group. For the HIV-infected with treatment group the median was 540 with the lowest $CD4^+$ T cell count being 94 and the highest value 1528 (Table 4.5).

The median viral load for the HIV-infected without treatment group was 676.50 and for the HIV-infected with treatment group the median was 788.

The only variables that showed a significant statistical difference between the HIV-infected without treatment group and the HIV-infected with treatment group was WBC (p=0.0364), RBC (p=0.0467) and monocytes (p=0.0117).

4.5 MILK COMPOSITION ANALYSIS

The data of the milk composition analysis of the study population is summarized in Table 4.6.

Table 4.6 Milk composition data of the study population (n=60)

Variable	Group n=30	Median	Mean	Standard Deviation	Minimum	Maximum	p-value*	Normal
Fat (%)	NT	3.10	4.32	4.54	1.30	26.90	0.9117	4.5[b]
	T	3.25	4.24	3.79	0.40	21.20		
Proteiens (%)	NT	1.80	2.04	1.50	0.50	7.50	0.3359	1.1[b]
	T	2.00	2.76	2.65	0.30	11.10		
Lactose (%)	NT	5.45	5.46	1.52	1.90	7.80	0.1759	6.8[b]
	T	4.90	5.14	1.45	1.80	7.60		
Total Solids (%)	NT	10.95	11.96	3.68	6.10	29.10	0.5296	12.6[b]
	T	11.10	11.10	3.65	2.30	20.20		
Energy (kcal/100mℓ)	NT	61.50	70.20	39.19	33.00	263.00	0.4285	60–75[a]
	T	57.50	63.57	23.84	20.00	132.00		
Calcium (mmol/ℓ)	NT	4.43	4.49	1.57	0.65	8.15	0.6572	2.5–3.0[a]
	T	4.53	4.60	1.40	0.50	7.05		
Phosphate (mmol/ℓ)	NT	1.18	1.34	0.72	0.50	2.95	0.8997	1.3–1.6[a]
	T	1.20	1.42	0.97	0.50	4.00		

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

NT – HIV-infected without treatment group

T – HIV-infected with treatment group

a – Values obtained from Jenness, 1979.

b - Values obtained from Mcgill University, 2012

The fat percentage of the HIV-infected group without treatment had a median value of 3.10 with a minimum of 1.30 and a maximum of 26.90. The HIV-infected with treatment group had a median of 3.25 with a minimum value of 0.40 and a maximum value of 21.20.

The percentage protein for the HIV-infected without treatment group was 1.80 with a minimum protein value of 0.50 and a maximum value of 7.50, for the HIV-infected with treatment group the median value was 2.00 with a minimum value of 0.30 and a maximum value of 11.10.

The median of the percentage lactose for the HIV-infected without treatment group was 5.45 with a minimum value of 1.90 and a maximum value of 7.80. For the HIV-infected with treatment group the median was 4.90 with a minimum of 1.80 and maximum of 7.60 (Table 4.6).

The percentage total solids median for the HIV-infected without treatment group was 10.95 with the minimum being 6.10 and the maximum 29.10 and for the HIV-infected with treatment group the median was 11.10 with a minimum value of 2.30 and a maximum value of 20.20.

The energy content of the HIV-infected without treatment group had a median value of 61.50 with a minimum of 33 and a maximum of 263. For the HIV-infected with treatment group the median was 57.50 and here the minimum was 20 and the maximum was 132 (Table 4.6).

The median for the calcium content of the milk for the HIV-infected without treatment group was 4.43 with a minimum value of 0.65 and a maximum of 8.15, were as the content for the HIV-infected with treatment group was 4.53 with a minimum of 0.50 and a maximum of 7.05.

The median phosphate content was 1.18 for the HIV-infected without treatment group, with a minimum of 0.50 and a maximum of 2.95. For the HIV-infected with treatment group the median was 1.20 with a minimum value of 0.50 and a maximum value of 4.00 (Table 4.6). There was no significant difference detected in the composition of the milk between the two groups (see Table 4.6).

4.6 CORRELATION BETWEEN THE IMMUNOLOGICAL PARAMETERS AND BREAST MILK DATA

Table 4.7 reflects the Spearman Correlation Coefficient (r) and the p-values between the immunological parameters and the breast milk of the HIV-infected group without treatment.

- If p <0.05: then there is a significant correlation between the two variables.
- If p ≥ 0.05: then there is no significant correlation between the two variables.
- A (-) r-value implies a weak negative relationship.
- A (+) r-value near 1 implies a strong relationship.

Table 4.7 Spearman correlation coefficient and p-values between the immunological parameters and breast milk of the HIV-infected without treatment (n=30)

	Fat (%)	Proteiens (%)	Lactose (%)	Total Solids (%)	Energy (kcal/100mℓ)	Calcium (mmol/ℓ)	Phos-phate (mmol/ℓ)
$CD4^+$ T cell count							
r-value	0.17979	-0.09472	0.09122	0.15961	0.13426	0.27051	0.35349
p-value	0.3418	0.6186	0.6316	0.3995	0.4794	0.1482	0.0553
CD4:CD8 ratio							
r-value	-0.0135	0.15942	0.05153	0.06674	0.00101	0.17401	0.19558
p-value	0.9436	0.4001	0.7868	0.726	0.9958	0.3578	0.3003
HIV-1 viral load							
r-value	0.21235	0.04592	-0.06731	0.36787	0.29869	-0.0818	-0.1263
p-value	0.2599	0.8096	0.7238	0.0455*	0.1089	0.6674	0.506

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

The only statistically significant difference between the immunological parameters and breast milk was found between the HIV-1 viral load and percentage total solids in breast milk for the HIV-infected without treatment group (p=0.0455).

Negative correlations were found between the $CD4^+$ T cell count and percentage protein, CD4:CD8 ratio and the percentage fat of the milk, HIV-1 viral load and percentage lactose, calcium (mmol/ℓ) as well as phosphate (mmol/ℓ). This implies that when the one parameter goes up the other parameter will decline and vice versa. However, these were all weak negative relationships. A value near 1 is regarded as having a strong correlation, 0.5 is regarded as having a moderate correlation and nearer to 0 as having a weak correlation. If the value is negative a value near -1 will have a strong negative correlation and nearer to 0 will have a weak negative correlation. None of the positive correlations were strong correlations (Table 4.7).

Table 4.8 reflects the Spearman Correlation Coefficient (r) and the p-values between the immunological parameters and the breast milk of the HIV-infected without treatment group.

Table 4.8 Spearman correlation coefficient and p-values between the immunological parameters and breast milk of the HIV-infected with treatment (n=30)

	Fat (%)	Proteiens (%)	Lactose (%)	Total Solids (%)	Energy (kcal/100mℓ)	Calcium (mmol/ℓ)	Phos-phate (mmol/ℓ)
$CD4^+$ T cell count							
r-value	-0.22426	-0.17972	-0.119	-0.39223	-0.39349	0.20946	0.21529
p-value	0.2335	0.342	0.5311	0.032*	0.0315*	0.2666	0.2532
CD4:CD8 ratio							
r-value	0.22592	0.08438	-0.09285	0.05132	0.07657	0.0697	0.13032
p-value	0.23	0.6575	0.6255	0.7877	0.6876	0.7144	0.4924
HIV-1 viral load							
r-value	0.03942	-0.11435	0.1083	0.09673	0.09304	0.06204	-0.02653
p-value	0.8362	0.5474	0.5689	0.6111	0.6248	0.7447	0.8893

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

The only statistically significant p-value between the immunological parameters and breast milk of the HIV-infected with treatment group was found between the $CD4^+$ T cell count and percentage total solids (p=0.032) as well as the energy (kcal/100mℓ) content of the milk (p=0.0315).

For the HIV-infected with treatment group negative correlations where found for the $CD4^+$ T cell count with percentage fat, percentage protein, percentage lactose, percentage total solids and energy (kcal/100mℓ) as well as for the CD4:CD8 ratio with percentage lactose and the HIV-1 viral load with percentage protein and phosphate (mmol/ℓ). Again, these were all weak positive correlations. None of the positive correlations had a strong relationship (Table 4.8).

CHAPTER 5
DISCUSSION

An HIV-infected mother can transmit the virus to her child during pregnancy, labour, delivery and through breast milk. When infants are not breastfed it is very difficult to balance the threat of infants acquiring HIV through breastfeeding versus the higher risk of death from other causes other than HIV like malnutrition and illnesses such as diarrhoea and pneumonia (WHO, 2010d). One of the most significant ways to reduce infant mortality rates is early and exclusive breastfeeding.

The possibility of transmission of HIV through breastfeeding can be reduced greatly by giving antiretroviral drugs to the HIV-infected mother or the HIV-exposed infant. A benefit that is closely associated with breastfeeding is that these mothers can offer their infants the same protection against the most common causes of child deaths (WHO, 2010d).

This chapter will discuss the socio-economic factors, breastfeeding data, blood and milk analysis data as well as correlation between the immunological and breast milk data.

5.1 SOCIO-ECONOMIC FACTORS

If the sole consideration was to prevent HIV-infection through breast milk, infected mothers would be advised not to breastfeed but to give their baby formula milk. However, HIV-infection is not the only consideration. It is important for infected mothers living in poor households in developing countries to consider risks related with not breastfeeding carefully. In terms of serious morbidity and mortality the risks are much higher in poor households with inadequate sanitation, unsafe and scarce water supplies, no refrigeration, poor health services, and little knowledge of hygiene (Latham and Preple, 2000).

Socio-economically disadvantaged people have more restricted educational opportunities, and are at an increased risk for HIV infection (Klatt, 2011). Young women from lower quintiles and rural areas are less likely to have truthful knowledge of HIV and AIDS (UNICEF, 2010). They may often not have access to HIV testing and often lack access to antiretroviral therapy following infection (Klatt, 2011). The majority of this sample group had a secondary education with no difference between the two groups (Table 4.1). Most of the women had one individual who contributed to the household income with nearly a fifth of the participating women having no one that contributed to the household income (Table 4.2). More than 50% of the women were unemployed, this being higher than South Africa's unemployment rate of 25.5% for the years 2000 to 2012 (Trading economics, 2012). Only a small percentage was housewives by choice (Table 4.3). Child mortality is higher among children living in rural areas, in less educated communities and in the poorest households (WHO, 2012). The risk of a child dying before five years of age in a low-income country is about 18 times higher than that of one from a high-income country (WHO, 2012).

With no difference between the two groups, the majority of the participating women had electricity, tap water and a refrigerator in their homes (Figure 4.2). The presence and availability of these resources makes it more feasible for the women to formula feed if contamination, education and household income are not taken into account. Clinics often cannot supply formula, and with more than 50% of these women being unemployed and only having one individual that contributed to the household income, mothers often need to mix feed if the option to formula feed is chosen, increasing the infants risk to become HIV-infected.

5.2 BREASTFEEDING

Infant formula is not equivalent in nutrition to breast milk. It does not contain all the essential nutrients or antibodies to protect children from diarrhoea, pneumonia or malnutrition (Bloemen, 2012).

Malnutrition contributes to more than one-third of all under-five deaths (WHO, 2012). Globally, 30% of children under five are estimated to be stunted and 18% have low weight-for-height, mostly as a consequence of poor feeding and repeated infections (WHO, 2010d). According to the Convention on the Rights of the Child (WHO, 2010d), every infant and child has the right to good quality nutrition. The challenge is to balance out the risk of deaths from causes other than HIV, in particular malnutrition and serious illnesses such as diarrhoea and pneumonia when choosing not to breastfeed (WHO, 2010d).

South Africa is one of the 12 countries in the world where infant mortality has been on the increase. That is why the South African policy on breastfeeding has changed to adopt the WHO recommendations for a single infant feeding strategy, namely exclusive breastfeeding also for HIV-infected mothers as enshrined in the Tshwane Declaration, August 2011 (Goga *et al*., 2012). One of those recommendations is that the provision of milk formula through hospitals and clinics will no longer be practiced except when recommended by an authorised health practitioner (Motsoaledi, 2011).

In the past, the most important aspect was to prevent MTCT of HIV through breastfeeding and the low percentage – 8% – of exclusive breastfeeding in South Africa can be ascribed to this (Bloemen, 2012). There was a high prevalence of breastfeeding in both groups of women - this could likely be due to the fact that a high percentage of the individuals had a secondary education (Table 4.1). The fact that all the mothers indicated that breast milk was the best feeding option for their infants is a good indication that they had were well informed at the hospital/clinic about breastfeeding and the advantages of breast milk. Breastfeeding mothers who strive to exclusively breastfeed are faced with multiple challenges (Agunbiade and Ogunleye, 2012) and health system support of breastfeeding is an important factor for success. It is important that hospitals develop ways to promote breastfeeding and support programs as well as closely monitor outcomes from these services on an ongoing basis (Kuan *et al*., 1999).

Mothers need to be supported for their children to be optimally breastfed. Some of the actions that help protect, promote and support breastfeeding include:

- adoption of policies such as the International Labour Organization Maternity Protection Convention 183 and the International Code of Marketing of Breast-milk Substitutes;
- implementation of the Ten Steps to successful breastfeeding specified in the Baby-friendly Hospital Initiative, including:
    - Skin-to-skin contact between mother and baby immediately after birth and initiation of breastfeeding within the first hour of life
    - Breastfeeding on demand (that is, as often as the child wants, day and night)
    - Babies should not be given additional food or drink, not even water.

    (WHO, 2010d)

Giving ARVs to either the HIV-infected mother or the HIV-exposed infant significantly reduces the risk of transmitting HIV through breastfeeding. This enables HIV-infected mothers to breastfeed, with low risk (1-2%) of transmission (WHO, 2010d).

5.3 HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS

HIV-infected populations are burdened with malnutrition and numerous studies have reported that these deficiencies impair immune response and are associated with accelerated HIV disease progression (Dreyfuss and Fawzi, 2002). It is not clear whether HIV disease progression and its metabolic impact on the mother will affect the nutrient composition of breast milk in these immune-compromised individuals.

When comparing the haematological variables that were analysed, the white blood cells indicated a significant difference ($p=0.0364$) between the HIV-infected without treatment group and the HIV-infected with treatment group (Table 4.5). The HIV-infected with treatment group had a higher total white blood cell count (Table 4.5). This could be attributed to the fact that the treatment aided in the strengthening of the immune system. Although the neutrophil and lymphocyte count did not have a

significant p-value (p=0.0594 & p=0.2675 respectively) the treatment group had a slightly higher absolute neutrophil and lymphocyte count, giving the body better protection against infections. HIV attacks white blood cells and a condition called leukopenia can develop which makes the body more prone to infections (PDR Health Physicians Desk reference, 2011). Advanced HIV disease can cause neutropenia which could then result in a lower total white blood cell count (AIDS.org, 2007).

The red blood cells also indicated a significant p-value (p=0.0467) between the two groups. The HIV-infected with treatment group had a lower red blood cell count. This is likely be ascribed to the fact that drugs used to treat HIV and to combat other infections often cause blood disorders. They impair the production of leucocytes, red blood cells and/or platelets in the bone marrow (PDR Health Physicians Desk reference, 2011).

Both the groups were anemic with hemoglobin levels below normal. Anemia is common in pregnant women, particularly HIV-infected women in resource limited settings (WHO, 2010b). Bone marrow suppression is common in people with advanced HIV infection and this causes anemia to be a chronic disease. This impairment is due to the release of inhibitory substances and low levels of the hormone erythropoietin (PDR Health Physicians Desk reference, 2011) as well as the fact that HIV causes disturbances in iron metabolism (WHO, 2003). The HIV-infected with treatment group had a slightly decreased heamatocrit which can also be attributed to the anemia (AIDS.org, 2007).

Iron-folate supplementation is a standard component of antenatal care for preventing anemia and improving fetal iron stores. The WHO recommends that daily iron-folate supplements must be taken for six months during pregnancy to prevent anemia, and twice-daily to treat severe anemia (WHO, 2003). Only 12% of all the participants were taking vitamin supplements and this could account for the fact that the women were anemic.

The cluster of differentiation 3 positive ($CD3^+$) T cell count had increased above the normal reference value in both groups. The $CD3^+$ T cell count is the number of $CD8^+$ T cells plus the number of $CD4^+$ T cells and the natural killer cells. The $CD3^+$ T cell count increases because the $CD8^+$ T cells proliferate exponentially as $CD4^+$ T cells are depleted. The $CD8^+$ T cell proliferation is driven mainly by HIV RNA levels (Catalfamo *et al*., 2011).

The $CD4^+$ T cell count was lower than the reference range in both groups although there was no significant difference detected in the p-value (p=0.5692). The pathogenesis of HIV infection is largely attributed to the decrease in the number of T cells that have the CD4 receptor. The immune status of a person living with HIV can be assessed by measuring the absolute number of $CD4^+$ T cells. This is also the way to assess the severity of HIV-related immunodeficiency. The progressive decline in the number of $CD4^+$ T cells is associated with progression of HIV disease (Hargreaves, 2007; WHO, 2007). The $CD4^+$ T cell count had a higher median value in the HIV-infected with treatment group which correlates with the literature that the $CD4^+$ T cell count usually increases in response to an effective ART combination (WHO, 2007) (see Table 4.5). A possible explanation for the low $CD4^+$ T cell count of 94 in the HIV-infected with treatment group could be that the patients had just commenced with ART (unfortunately the duration of the treatment was not recorded in the current patient files) or the patient could have had a resistance against the treatment due to mutations (WHO, 2013; Sen, 2007). The lowest $CD4^+$ T cell count in both groups fell in the severe immune compromised category. When categorizing the two groups according to the immunological classification, the HIV-infected without treatment group fell in the “mild immunodeficiency” group and the HIV with treatment group in the “not significant” group (WHO, 2007) (Table 4.5).

During the course of the illness, the virus particles increase and the number of $CD4^+$ T cells decline (Department for work and pensions, 2009). The best laboratory measurement for determining the progression of AIDS is the viral load. Prior to any immune response the HIV-1 RNA typically exceeds 10,000 copies/mℓ. According to Klatt (2011), the initial viral load in females, following HIV infection, is 15,103

copies/mℓ. The median HIV-1 viral load for the HIV with treatment group was higher than the HIV-infected without treatment group. This pattern could have been explained better if the duration of ART had been established, since literature suggests that individuals receiving ART should have a lower HIV-1 viral load (Hargreaves, 2007) (Table 4.5). Persons starting therapy with high plasma levels of HIV (>100,000 copies/mℓ) may take longer to suppress. The failure to suppress viremia to <50 copies/mℓ by 16 to 24 weeks of therapy suggests poor adherence, inadequate drug absorption or drug resistance (Klatt, 2011; Hargreaves, 2007). The viral load can also be useful in predicting clinical progression (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2011).

5.4 MILK COMPOSITION

The analyzed milk data of the study population yielded no p-value of great significance, suggesting that there was no statistically significant difference recorded of the measured nutrients between mothers receiving treatment and those who did not receive any treatment for HIV. When looking at the measured nutrients from this study there was a difference between the minimum and maximum values that were obtained for the nutrients and this can be seen in the standard deviation values (Table 4.6). Previous studies suggest that race, age, parity, and/or diet fail to have a great effect on milk composition (Jenness, 1979) but that milk does evolve to meet the changing needs of the baby during growth and maturation (Lönnerdal, 1986). The milk expressed during the first minutes of feeding also has a lower fat content than that in the later stage of breastfeeding (Wagner, 2010). Other studies suggest that although breast milk composition is remarkably stable around the world, it can change slightly with different maternal diets and under different environmental conditions and that the fat content can vary with the mother's diet (Katz, 2006).

Variation in fat content affects the energy content of the breast milk, calories being associated with significant lessening of energy intake for the infant (Jelliffe and Jelliffe, 1978). A minimum fat value of 0.40% with only 20kcal/100mℓ was recorded. These values are less than the normal values for breast milk and these infants'

calorie intake will not be sufficient for proper growth and development. Cholesterol, triglycerides, short-chain fatty acids and long chain fatty acids are present in fat. Long chain polyunsaturated fatty acids - docosahexanoic acid (DHA) - and arachidonic acid (ARA) are important for the neurological development of a child, therefore these two fatty acids may be considered essential fatty acids (Wagner, 2010; WHO, 2009; Hamosh, 2001; Jenness, 1979).

Previous studies show that the lactose content does not seem to vary a lot – even in poorly nourished mothers (Lönnerdal, 1986; Jelliffe and Jelliffe, 1978). This study, however, showed a difference in the minimum and maximum values that were obtained for lactose (see Table 4.6) with the minimum value less than the normal value for lactose. Carbohydrates aids in providing protection against infections (WHO, 2009) and infants need constant protection against exposure to possible pathogens that could cause various infections increasing their mortality risk.

Calcium levels in poorly fed mothers range from normal to somewhat low concentrations (Lönnerdal, 1986). Calcium levels for this study ranged from low to high with the median value of both groups above normal. (See Table 4.6). Adequate calcium intake is essential for infant health and cases of rickets have been associated with low concentrations of breast milk calcium (Kent, Arthur, Mitoulas, Hartman, 2009).

The Spearman Correlation Coefficient was used to determine if HIV disease progression would have an influence on the nutrients that were measured in this research (Table 4.7 & Table 4.8). For the HIV-infected without treatment group, a significant correlation ($p=0.0455$) was found between the HIV-1 viral load and percentage total solids in breast milk. Total Solids comprises of fat, protein, lactose, minerals, vitamins and enzymes (McGill University, 2012). Since no correlation could be shown in the percentage of fat, percentage of protein, percentage of lactose, calcium (mmol/ℓ) or phosphate (mmol/ℓ) the difference could be in other milk components that were not measured in my study.

There was a negative correlation between the $CD4^+$ T cell count and the protein (%) content of the breast milk of the HIV-infected without treatment group, but it was a weak negative correlation (Table 4.7). This implies that when the $CD4^+$ T cell count increases the protein (%) content of milk will decrease and vice versa. The CD4:CD8 ratio and the fat (%) content also delivered a weak negative correlation as well as HIV-1 viral load (copies/mℓ) with percentage lactose, calcium (mmol/ℓ) and phosphate (mmol/ℓ) in the breast milk of the HIV-infected without treatment group.

For the HIV-infected with treatment group the only positive correlation was between the $CD4^+$ T cell count and the percentage total solids and energy (kcal/100mℓ) content of the breast milk (Table 4.8). A weak negative correlation between these parameters could also be shown. Furthermore, there was also a weak negative correlation between the $CD4^+$ T cell count (cells/mm^3) and percentage fat, percentage protein and percentage lactose. Lactose and the CD4:CD8 ratio also had a weak negative correlation. The HIV-1 viral load had a weak negative correlation between percentage protein and phosphate (mmol/ℓ).

CHAPTER 6
CONCLUSION

When a mother decides to exclusively breastfeed for the first six months it holds benefits for both the mother and for the infant, with one of those benefits being the protection against gastro-intestinal infections. The earlier breastfeeding is initiated the lower the risk for newborn mortality due to diarrhoea and other infections (WHO, 2010d).

In developing countries breastfeeding is very important for child survival but may also result in HIV infections in infants. Thus, the same economic and development inequities that make breastfeeding so critical to infant survival make formula feeding inaccessible, unfeasible, unaffordable, unsustainable and unsafe (not AFASS) for most families (Humhrey, 2010).

When deciding on the method of feeding the infant in HIV-positive mothers the risk of transmission of HIV to the infant and the risk of acquiring other potential fatal diseases from contaminated foods and malnutrition must be weighed. There are no known studies on the effect of HIV disease progression on the nutrient composition of breast milk.

No significant difference in the nutrients that were analysed could be revealed between HIV-infected mothers who received treatment and HIV-infected mothers who did not receive any treatment. HIV disease progression did not have an influence on the nutrients that were analysed and no strong positive correlation could be established between the immunological markers of HIV disease progression and the analysed nutrients in the breast milk. Taking this into consideration, HIV mothers can breastfeed their babies even if their HIV is at a more advance phase, but the emphasis should be placed on exclusive breastfeeding and getting the needed support to breastfeed.

Breastfeeding women should get support from their family, the community and healthcare workers in their decision to breastfeed their infant, but with South Africa's changed policy on breastfeeding the percentage of women who exclusive breastfeed should increase. This study showed that there was no correlation between the nutrient composition of breast milk and HIV disease progression, but that compliance in using ARV treatment is of utmost importance to reduce the HIV RNA levels in breast milk, minimising the transmission of the HIV through breast milk. Infant formula does not contain all the essential nutrients or antibodies to protect children from diarrhoea, pneumonia or malnutrition. Even severe immune compromised mothers can breastfeed their infants – and when this is done in conjunction with the current ARV recommendations for mothers and infants – breast is best.

Only a limited number of milk constituents could be analyzed due to the difficulty of analysing the milk and the high cost of the milk analysis. To get mothers to participate in the study voluntarily was also very difficult – this could have been because of the stigmatization that still exists around HIV. Although the whole process was handled with complete confidentiality, mothers were reluctant to participate and this yielded a small study group. This study would have been more informative had the study population been larger.

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APPENDIXES

APPENDIX A

RECOMMENDED INFANT AND YOUNG CHILD FEEDING PRACTICES NOT TAKING HIV/AIDS INTO CONSIDERATION.

WHO and the United Nations Children's Fund (UNICEF) global recommendations (2009) for optimal infant feeding as set out in the Global Strategy are:

- Exclusively breastfeeding for 6 months (180 days)
- Nutritionally adequate and safe complimentary feeding starting from the age of 6 months with continued breastfeeding to up to 2 years of age or beyond (WHO, 2009).

Exclusive breastfeeding means that an infant receives only breast milk from his or her mother or a wet nurse, or expressed breast milk 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, 2009).

Complementary feeding is defined as the process starting when breast milk is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk. The target range for complimentary feeding is generally taken to be 6 to 23 months of age, even though breastfeeding may continue beyond two years (WHO, 2009).

APPENDIX B

ACCEPTABLE MEDICAL REASONS FOR USING BREAST MILK SUBSTITUTES

(WHO, 2009)

Infants who should not receive breast milk or any other milk except specialized formula
➢ Infants with classic galactosemia: a special galactose-free formula is needed. ➢ Infants with maple syrup urine disease: a special formula free of leucine, isoleucine and valine is needed. ➢ Infants with phenylketonuria: a special phenylalanine-free formula is needed (some breastfeeding is possible, under careful monitoring).
Infants for whom breast milk remains the best feeding option but who may need other food in addition to breast milk for a limited period:
➢ Infants born weighing less than 1500 g (very low birth weight). ➢ Infants born at less than 32 weeks of gestational age (very pre-term). ➢ Newborn infants who are at risk of hypoglycaemia by virtue of impaired metabolic adaptation or increased glucose demand (such as those who are preterm, small for gestational age or who have experienced significant intrapartum hypoxic/ischemic stress, those who are ill and those whose mothers are diabetic); if their blood sugar fails to respond to optimal breastfeeding or breast-milk feeding.
Maternal conditions that may justify permanent avoidance of breastfeeding:
➢ HIV infection: if replacement feeding is acceptable, feasible, affordable, sustainable and safe.

Maternal conditions that may justify temporary avoidance of breastfeeding:

- Severe illness that prevents a mother from caring for her infant, for example: sepsis.
- Herpes simplex virus type 1 (HSV-1): direct contact between lesions on the mother's breasts and the infant's mouth should be avoided until all active lesions have resolved.
- Maternal medication:
  - sedating psychotherapeutic drugs, anti-epileptic drugs and opiates and their combinations may cause side effects such as drowsiness and respiratory depression and are better avoided if a safer alternative is available ;
  - radioactive iodine-131 is better avoided given that safer alternatives are available - a mother can resume breastfeeding about two months after receiving this substance;
  - excessive use of topical iodine or iodophors (e.g., povidone-iodine), especially on open wounds or mucous membranes, can result in thyroid suppression or electrolyte abnormalities in the breastfed infant and should be avoided;
  - cytotoxic chemotherapy requires that a mother stops breastfeeding during therapy.

Maternal conditions during which breastfeeding can still continue, although health problems may be of concern:

- Breast abscess: breastfeeding should continue on the unaffected breast; feeding from the affected breast can resume once treatment has started
- Hepatitis B: infants should be given hepatitis B vaccine, within the first 48 hours or as soon as possible thereafter
- Hepatitis C.
- Mastitis: if breastfeeding is very painful, milk must be removed by expression to prevent progression of the condition.

- Tuberculosis: mother and baby should be managed according to national tuberculosis guidelines
- Substance use:
  - maternal use of nicotine, alcohol, ecstasy, amphetamines, cocaine and related stimulants has been demonstrated to have harmful effects on breastfed babies;
  - alcohol, opioids, benzodiazepines and cannabis can cause sedation in both the mother and the baby.

APPENDIX C

Questionaire

NUTRIENT COMPOSITION 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 - 19

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 - 24

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	1	2	☐	28
Other source of water Specify _____			☐☐	29 - 30
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

13. 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

11. How many days per week do you work:

_____ ☐ 36

12. 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

APPENDIX D

Ethical approval

UNIVERSITEIT VAN DIE VRYSTAAT
UNIVERSITY OF THE FREE STATE
YUNIVESITHI YA FREISTATA

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Ms H Strauss

2008-07-25

MS G DE WET
C/O PROF FJ VELDMAN
SCHOOL OF HEALTH TECHNOLOGY
PRIVATE BAG X20539
CENTRAL UNIVERSITY OF TECHNOLOGY
BLOEMFONTEIN
9300

Dear Ms de Wet

ETOVS NR 108/08
MS G DE WET **DEPT OF BIOTECHNOLOGY, CUT**
PROJECT TITLE: THE POSSIBLE ASSOCIATION BETWEEN STAGE OF HIV DISEASE AND THE NUTRIENT COMPOSITION OF BREAST MILK.

- 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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