Infant feeding strategies and other determinants of postnatal HIV-free survival rate in South Africa: parameter values for modeling postnatal HIV-free survival rate in South Africa

BY

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DECLARATION

I hereby declare that this dissertation represents my own works and has not been presented either wholly or in part for a degree at the University of Western Cape or any other university.

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Signature…………………………………..

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ABBREVIATIONS

AFASS	Affordable Feasible Acceptable Sustainable and Safe
ARV	Antiretroviral
ANC	Antenatal care
BF	Breast Feeding
EBF	Exclusive Breastfeeding
EFF	Exclusive Formula Feeding
FF	Formula Feeding
HIV	Human Immunodeficiency Virus
HST	Health Systems Trust
IATT	Inter Agency Task Team
MBF	Mixed Breast Feeding
MFF	Mixed Formula Feeding
MRC	Medical Research Council
MTCT	Mother to Child Transmission of HIV
NVP	Nevirapine
PMTCT	Prevention of Mother to Child Transmission of HIV
PNT	Postnatal Transmission
PBF	Predominantly Breastfeeding
pH	Potential of Hydrogen
WHO	World Health Organization
ZDV	Zidovudine
ZVITMBO	Zimbabwe vitamin A for mothers and babies

GLOSSARY AND DEFINITIONS

EBF

Feeding only breast milk, without any complementary liquid or solid foods (except medicines ordered by medical doctors)

MBF

Feeding infants with breast milk in addition to other solids and liquids

PBF

The infants' predominant source of food has been breast milk, however the infant also had non-nutritive liquids (e.g. water), but not solids or formula or other nutritive liquids (e.g. other milk).

EFF - Feeding infants only formula milk

MFF

Feeding infants formula milk with additional solids and liquids , but not breast milk

Late Postnatal Transmission

Postnatal transmission of HIV from mothers to child after 3 weeks of birth

Malnutrition

Weight for age Z score less than - 3.0

Underweight

Weight for age Z score less than - 2.0

Stunting

Height for age Z score less than - 2.0

Wasting

Weight for height Z score less than - 2.0

ABSTRACT

Background: Mother to child HIV transmission is a significant public health problem especially in Southern Africa. South Africa is the second highest burden country globally with 71 000 infants being HIV infected every year. The aim of this study was to identify and measure the influence of risk factors of late postnatal HIV transmission and/or mortality among infants born to HIV positive mothers.

Study design: Historical cohort data collected during 2002 – 2004 from 3 purposively selected PMTCT sites in South Africa (namely: Rietvlei, Umlzai and Paarl) is used. These three sites are purposively selected to reflect different HIV prevalence, socioeconomic and geographical locations. A total sample size of 469 mother–infant pairs were followed for 36 weeks.

Data Collection: Data were collected by trained field researchers and community health workers using semi-structured interviews including: infant feeding practices, infant and maternal mortality, disclosure of HIV status, basic knowledge of HIV/AIDS and MTCT and sociodemographic information. Dried blood spots were collected by heel prick in the baby at 3, 24 and 36 weeks, whilst in the mother finger prick was taken at 3 and 36 week visits.

Data Analysis: Data from all questionnaires were coded, captured and cleaned. STATA version 10 is used to analyze and measure the independent influence of risk factors of HIV-free survival rate. Variables found having significant association in the bivariate analysis were analyzed using Cox-proportional hazard model.

Result: Our study shows that early mixed feeding is a common practice in South Africa. Overall, 83% (as high as 90.26% in Rietvlei) of mothers were either mixed breast feeding or mixed formula feeding before the infant is at age 5 weeks. MBF at 7 weeks was associated with 3.5 fold increased risk of transmission and/or mortality as compared to EBF (p-value=0.22), while PBF had a 2 fold less hazard of transmission and mortality compared to MBF (p-value=0.1). In this study, failure to disclose, poor counselling and lack of close support by health facilities were major factors that contributed to inappropriate feeding choice and non-compliance to exclusive feeding. Poor counseling (below the average of expected level) had an associated 55% increased risk of transmission and/or mortality. A substantial proportion (70.61%) of women in our study didn't disclose their status to anyone. Failure to disclose was associated with 44% of increased risk of transmission and mortality. The study also showed households who had shortage of food were at increased risk (adjusted hazard ratio 1.7) of HIV transmission and/or mortality of infants. Maternal and infant factors such as premature birth, maternal viral load, poor weight gain during pregnancy and low birth weight were significant influential factors of HIV-free survival rate.

Conclusion: In general, this study has given us an idea that postnatal HIV-free survival is determined by the interrelated effect of multilevel co-factors. Therefore, comprehensive multi-sectorial approach is needed to address the MTCT and child mortality problem in South Africa. The health sector should take urgent action to improve the quality of counselling and health services given in health facilities. Government should give enough attention to reduce the bureaucratic hassles of receiving grant by HIV positive mothers.

CHAPTER ONE

1.0. INTRODUCTION

Mother to child transmission of HIV is a significant global public health problem. Despite the encouraging reduction in the burden of other infectious diseases in recent years, the pandemic continues to affect the lives of 2.3 million children living with HIV globally, of which 2 million were born in Sub-Saharan Africa[1]. South Africa ranks as the second of these highest burdened African countries with a 71 000 yearly incidence rate of HIV among children [2].

Providing a mother with antiretroviral prophylaxis, obstetric interventions and avoidance of breast feeding nearly eliminate the risk of transmission to less than 2%[3]. Nevertheless, in many developing countries, including South Africa, the question remains how to reduce MTCT in settings where not breast feeding poses significant risks to infants' survival. The risk of transmission without antiretroviral therapy or refraining from breast feeding ranges from 35-45% at 24 months, depending on the presence or absence of risk factors[4]. These factors may include health-seeking behaviour, health care system factors, maternal and infant factors, infant feeding modalities, and socio-economic status.

This study is the first part of an epidemiological modelling study under the Good Start project, which is funded by CDC and run by the MRC, UWC, HST and CADRE organisations. The first phase of this study, which is presented in this mini-thesis, identifies independent risk factors (parameters) that will be used to define the structure and dynamics of postnatal MTCT model to guide policy makers in examining locally appropriate interventions.

The paper is organised in 5 sections. The first chapter gives a general introduction to the problem, context and rationale of the study and presents the research questions, aims and objectives of the study. The second chapter reviews literature on risks of HIV-free survival and modeling the HIV-free survival rate. The following chapter elaborates on methods and materials used. Finally, chapters four and five present the results, discussion, conclusion and recommendations of the study.

1.1. BACKGROUND

The World Bank classifies South Africa as a middle-income country with a per capita income of US$11,110[5]. The country is known for its cultural and socio-economic diversity, with living standards ranging from shack living and < 1US$ /day earnings in black communities in rural and semi-urban areas to highly standardized and modern living styles. The health status trend of communities is closely linked with their socio-economic status and lifestyle. Water- and sanitation-related health problems are the main obstacles for the poor, making it difficult to choose alternative infant feeding options. The fertility rate is relatively high in the country due to cultural norms that foster teenage and premarital pregnancy. Current reports indicate that one in three pregnant women in South Africa is estimated to be HIV-positive[6]. The prevention of MTCT has become a high public health priority in South Africa since 2003. At the moment, almost all primary and secondary health facilities with antenatal care (ANC) clinics provide voluntary counseling and testing (VCT), infant feeding counseling, formula milk and Nevirapine to mother-child pairs as part of the national programme. The Anti-retroviral (ARV) coverage to mothers in the country has been doubled since the commitment at a UNIGASS meeting[7]. The antenatal care (ANC) utilization rate of the country is also one that can be mentioned as a good example in Africa, with the 2003 Demographic and Health Survey recording 92% of pregnant women with at least one antenatal visit[8].

The Good Start study, the source study for this data analysis, was conducted during the early stages (during October, 2002- September, 2004) of the PMTCT pilot programme implementation in South Africa. At this early stage, policy recommendations on prevention of postnatal transmission were vague and difficult to apply in operational settings. As a result, feeding counselling varied from site to site mostly influenced by opinions of local health authorities and health professionals. It was against this background that the Good Start study was initiated to evaluate the effectiveness of the PMTCT programme in increasing the HIV-free survival rate. Apart from the main study, several other sub-studies and secondary analysis have been completed. Some of these studies are reviewed in the literature review section of this mini-thesis. Further details of the study design and data collection methods are presented in the Methodology section.

1.2. PROBLEM STATMENT

South Africa is one of the hardest hit Sub-Saharan African countries with HIV in children (<15 yrs) and women together accounting for more than 60% of the total HIV prevalence[9, 10]. In the last 10 years, despite the huge decrease in the burden of other infectious diseases, the child mortality rate has increased from 65 deaths to 75 deaths/1000 births due to the increased rate of HIV-related deaths[11]. In spite of the availability of PMTCT services in most ANC clinics in the country, reducing mother-to-child transmission and mortality, particularly during the postnatal period, has been a major challenge. This is due to a lack of clear evidence and policy guidance on the different risk factors of postnatal transmission and death[1,2]. Developing a model that can be used to estimate the HIV-free survival rate based on locally identified risk factors can inform policy decisions to focus on the most appropriate interventions that are crucially needed for a given locality. However, in the literature, so far, most models are designed based on narrow assumptions that consider infant feeding patterns as the only risk factors of postnatal transmission and/or mortality, while no model could be found in the literature that includes broader risk factors of HIV-free survival. These factors can include maternal and infant factors, knowledge, health-seeking behaviour, the health care system and socio-economic factors. Part of the reason for this is a lack of data that provides good estimates of the above-mentioned parameter values in different settings. Therefore, this mini-thesis particularly aims to contribute toward filling this gap by identifying and measuring the influence of high-risk factors on HIV-free survival rates using cohort data collected from rural, urban and semi-urban areas in South Africa. The parameter values found from this study are envisaged for use to design a model that informs PMTCT policies and interventions at provincial level by predicting the possible transmission or mortality outcomes for a range of parameters (risk factors). This study also targets to inform questionnaire development for a six-week national PMTCT cross-sectional survey, which could be used to identify and measure the magnitude of risk factor for HIV-free survival of infants in each province for use in model development.

1.3. RESEARCH QUESTIONS

The longer term target of the project is to model the HIV-free survival rate of infants in each province. In order to do so, parameters that are predictors of HIV-free survival rate must be identified and quantified for each province of South Africa. As a first phase of this project, this thesis targets to answer two of the questions that are crucial to know about in order to develop the postnatal HIV-free survival model.

Question 1: What are the risk factors of postnatal HIV-free survival rate at 9 months among infants born from HIV-positive mothers in the three purposefully selected sites of South Africa?

Question 2: What are the baseline risk factors of postnatal HIV-free survival rate that data can be collected cross-sectionally from all provinces at the 6 weeks immunization visit?

After answering these two questions, in the longer term plan, the project targets to develop a model that can be used to estimate the HIV-free survival rate of infants in each province using risk factors identified from the three selected cohort study sites and the cross-sectional data.

1.4. AIMS AND OBJECTIVES

1.4.1. AIM

- The aim of this study is to identify and measure the strength of influence of different infant feeding patterns, socio-economic factors, maternal/infant factors, psycho-social factors and health care system factors on late postnatal HIV transmission and/or mortality of infants at 36 weeks.

1.4.2. OBJECTIVES

- To identify and measure the strength of influence of infant feeding patterns on late postnatal HIV transmission and/or mortality.
- To identify and measure the strength of influence of socio-economic factors on late postnatal HIV transmission and/or mortality.
- To identify and measure the strength of influence of maternal and infant health factors on late postnatal HIV transmission and/or mortality.
- To identify and measure the strength of influence of health care system factors on late postnatal HIV transmission and/or mortality.
- To identify and measure the strength of influence of knowledge and health seeking behaviour on late postnatal HIV transmission and/or mortality.
- To identify and measure the strength of influence of psycho-social factors on late postnatal HIV transmission and/or mortality.
- To identify baseline influential risk factors of late postnatal HIV transmission and/or mortality that can be collected at the 6 weeks immunization visit.
- To provide recommendations on influential baseline and long-term risk factors of postnatal HIV transmission.

1.5. SIGNIFICANCE OF THE STUDY

This study is beneficial in that it enables policy makers, researchers and implementers to understand how postnatal MTCT and infant mortality is determined by various socio-economic, psycho-social and health system factors in addition to feeding patterns that are known to have strong influence on the HIV transmission rate and mortality of HIV-exposed infants. In doing so, the study contributes toward the development of a PMTCT model by providing measures of risk factors of HIV-free survival rate. Also, this study contributes to a national PMTCT cross-sectional survey by informing the questionnaire development phase of the study.

CHAPTER TWO

2.0. LITERATURE REVIEW

In this chapter, relevant literature on HIV and infant feeding strategies are reviewed. The first section presents the paediatrics burden of HIV by comparing developing countries' burden with developed countries'. Following this, infant feeding modalities practiced in most places of South Africa will be presented. The next two sections discuss the prominent debates among researchers on the HIV and mortality risks of different infant feeding types and duration of breast feeding. In the next section, mathematical models that attempt to measure the risks and benefits of infant feeding strategies are presented. The last section provides a review of current research on postnatal Anti-retroviral (ARV) therapy trials and the summary recaps the chapter by reviewing current research needs.

2.1. THE PAEDIATRICS BURDEN OF HIV IN DEVELOPED AND DEVELOPING COUNTRIES

HIV is a major cause of death in children less than 15 years of age. It is estimated that 2.3 million children under the age of 15 years globally are living with HIV, with 1 800 new cases every day [2]. Every year, more than 700 000 children become HIV-infected and 570 000 of them die before they celebrate their fifth birthday[1]. 12% of the total new HIV infections globally occur in the paediatrics age group, predominantly due to MTCT [12, 13].

Africa accounts for 90% of the global new paediatrics age HIV-infections and 80% of the worlds HIV-positive pregnant women[9,12]. Western countries report that the MTCT rate has been controlled to a level below 2%[3]. By contrast, 75% of the world PMTCT burden occurs in 10 developing countries, namely, Nigeria, South Africa, Ethiopia, Tanzania, DRC, India, Zimbabwe, Kenya, Mozambique and Zambia. The first five countries, South Africa being the second of these, account for almost half (46%) of the global MTCT burden[2]. Cumulative mother-to-child HIV transmission rates in most of these resource-poor African settings run between 25% and 45% [14, 15]. Unlike Western countries, Africa has poor access to ART, medical services are not adequate to give planned caesarean section operations and, often, the means to safe formula feeds are non- existent. Currently, only about 220 000 of the more than 2 million pregnant women estimated to be living with HIV receive ARV prophylaxis for PMTCT, representing an estimated coverage rate of 11% (8%−16%) [7].

According to a recent UNICEF estimate, South Africa is the second highest burdened country in MTCT with 33.5% of babies born from HIV-positive women becoming infected every year [2]. The prevalence of HIV is high amongst women of age group 20-24years and 25-29years, who also experience a high fertility rate[16]. The ANC prevalence, which was less than 1% in 1990 has inclined to 35% in 2006[16]. Based on current birth rates and HIV prevalence amongst pregnant women, approximately 300 000 infants are exposed to HIV in South Africa each year [16].

As in other developing countries, the prevention of transmission through breast feeding is a major challenge in South Africa. Breast feeding accounts for one third to half of all mother-to-child transmission cases in predominantly breast feeding settings in the country[17]. In the absence of intervention, 5-20% of the transmission occurs during breast feeding[15]. According to a meta-analysis study, the cumulative post-natal risk is estimated to be 8.9 transmissions/100 child - years of breast-feeding (95% CI, 7.8–10.2 transmissions/100 child - years of breast-feeding) with a cumulative probability of 9.3% (95% CI 7.8 – 10.2 transmissions/100 child - years of breast feeding) after eighteen months of breast feeding[18]. The literature indicate that transmission through breast feeding is higher than the above reported rate in places where malnutrition and infectious diseases are common[19, 20].

2.2. INFANT FEEDING PRACTICES IN SOUTH AFRICA

Breast feeding is an integral part of womanhood in Africa. As in most other African countries, South Africa has strong cultural and social values attached to breast feeding. Mixed and prolonged feeding are the two predominantly practiced breast feeding types in the country. A baseline survey of infant feeding practices in the country indicate that mixed breast feeding is the most commonly practiced feeding type with a rate of 75.2% amongst infants less than 10 weeks of age and 87.4% amongst infants more than 10 weeks of age[21]. According to the 2003 demographic survey in South Africa, only 2% of infants were exclusively breast fed at 4-6 months[8]. An even lower rate of EBF has been reported at 1.2% among infants more than 10 weeks of age attending PMTCT and non-PMTCT clinics in South Africa[21]. This makes it a challenge for interventions targeting child infection and mortality among HIV-exposed children. Factors that are responsible for the low rate of EBF are lack of knowledge, cultural and social influence and low educational status of parents. Poor provision of proper counseling and support are some of the other obstacles mentioned in the literature[6]. In South Africa, provision of pre-lacteal feeds immediately after birth is a widely common practice[16]. Formula milk usage is relatively better in urban areas, even though most mothers do not exclusively formula feed. The survey by McCoy *et al* reported formula milk usage (either exclusively or simultaneously with breast milk) in 64.8% of urban infants aged less than ten weeks[21].

Interventions conducted to increase adherence to EBF have shown mixed results. A recent study which was done in South Africa focusing on rural areas came out with a high compliance rate among mothers after intensive counseling and support. In this study, compliance rate to EBF in the first 6 weeks, 3 months, and 6 months was 82%, 67%, and 40% respectively[22]. This study suggests that, with intensive counseling and support, it is quite possible to promote EBF even in rural settings of South Africa. Contrary to this, in Côte d'Ivoire, a study found that after constant nutritional counseling to EBF, only 18% of mothers exclusively breast fed from birth[23]. Factors such as quality of counseling, non-disclosure of status, and family influence contribute to low compliance.

2.3. INFANT FEEDING MODALITIES AND HIV INFECTION

2.3.1 FORMULA FEEDING

International child health guidelines are very conservative in recommending formula feeding to HIV-exposed infants born in developing countries. Recent updates made on the WHO infant feeding guidelines, which are based on the technical consultation held on behalf of the inter-agency task team (IATT), underpin the earlier recommendation that suggests avoidance of breast feeding unless, and only if, alternatives (such as formula milk) are acceptable, feasible, affordable, sustainable and safe (AFASS)[24]. When the AFASS criteria is fulfilled, mothers are encouraged to choose replacement feeding only under a condition that they have access to good health care and counseling services. Several thoughts underlie this highly conservative recommendation. Firstly, except in a few previously published research studies, almost all recent studies and practical experiences so far in developing countries have failed to demonstrate expected reductions of HIV infection and mortality by using formula milk[22,25,26]. A study by Coovadia and his colleagues in KwaZulu-Natal has found a two-fold increased risk of mortality in the first 3 months of infant life by using formula feeding (15%) compared to breast feeding (6%), despite the provision of intensive support and counseling services to mothers based on their choice either to exclusively formula feed or exclusively breast feed[22]. Similar results were found in Kenya[25] and Botswana[26]. In the Kenya study, mortality rates in the first 6 weeks were higher in the formula feeding group (but not statistically significant) than the breast feeding group, in spite of similar peripartum and pregnancy period history[25]. In Botswana, the mortality rate was higher in formula feeding infants at 7 months[26]. In actual interventions where mothers are responsible to make their own decisions, the impact of formula feeding could be bigger than reported in scientific studies, as mothers enrolled in trials usually have access to potable water, extensive education on safe preparation of formula, a reliable supply of formula, and medical care for their infants.

The other scenario which holds a much worse effect than formula feeding is the tendency to mix feed (with breast) among mothers who failed to carry on their decision to exclusively formula feed due to social stigma and financial shortages[25]. In such circumstances, shifting to mixed feeding (formula with breast) leads to a greater transmission and mortality risk than both EBF and EFF. For instance, among the Kenya RCT study participants, the compliance rate of exclusive formula feeding was only 75% and, as a result, 20.5% of infants in the formula feeding arm were infected with HIV[25].

Even though it is quite difficult to make comparisons between older and more recent published studies mainly due to the utilization of different statistical and data presentation methods, earlier studies disagree with the assertion that formula feeding has a higher risk. In fact, some of this research has shown a lower cumulative risk of mortality and HIV transmission by using formula feeding. However, earlier research studies have a number of methodological issues which may account for these differences. For instance, the first RCT study in Africa, which came out with a finding that EFF has a 16.2% lower transmission risk than EBF among rural Kenyan infants used no intensive support system to ensure the exclusivity of both feeding types[25]. As a result, the observed risk effect of EBF could likely be due to mothers who mixed breast fed their child instead of exclusively breast feeding. In addition, the study design (RCT) had limited follow-up data collection to identify those who switched to mixed breast feeding after assigned to exclusive formula feeding or exclusive breast feeding. RCT studies can only be advantageous, if used with an intensive and strong follow up and support system to assure compliance with the treatment arm among societies which have less cultural and social influence to adhere to exclusive feeding practice. In order to address this limitation, recent research has used nested cohort study designs within an already established RCT study to more accurately record the feeding practice, while benefiting from the advantage of already established RCT studies that control for confounding effects[27].

Similar to the Kenyan study, another early research study done by Bobat and his colleagues in Durban and a hospital-based study in Uganda recommend formula feeding, as the HIV transmission rate was less in the FF group (hazard ratio of 7.39 by using EBF in study by Bobat *et al*) compared to the exclusively breast feeding group [28,29]. However, it is noticeable that definition problems in classifying types of breast feeding have influenced the outcome of both studies. The Bobat *et al* study classified participants as EBF if they were feeding breast milk with solids and liquids (except formula milk), which is contradictory and totally unacceptable in the current definition of EBF (most studies only allow prescribed medicine and 1-3 lapses of water during the total study period). In addition to this, both (Bobats' and the Ugandan) studies suffer from confounding and modification effects, as stratified analysis was not possible due to small sample size and uneven sample size between the study arms[28,29]. The other limitation of the Bobats' study was the fact that the immediate outcome (transmission rate at 6 months) of the study could not be known as the study only tested blood samples after 9 months[28].

Another reason for the preference of avoidance of breast feeding in previously published studies relates to the limited understanding of the potential adverse effect of replacement feeding (such as risk of infection, stunting wasting and mortality) in which, as a result of this, many of the studies used transmission rate as the only outcome indicator for comparison of feeding patterns. For instance, both Bobat *et al*[28] and Michele *et al*[29] used transmission rate as the only outcome indicator for comparison of the formula arm with the breast feeding arm. Currently, however, after the findings by researchers such as Thior and his colleagues that any gains from reducing transmission by giving formula milk would be lost through increased mortality from diarrhoea and malnutrition, more consideration is given to child survival rather than just avoiding HIV infection[30]. Based on this, the composite indicator HIV-free survival rate (HIV transmission and/or mortality rather than only transmission) is recommended to be used as a primary outcome indicator for studies that measure the effectiveness of programmes targeting PMTCT [31, 32].

In general, with all the listed methodological weaknesses in previously published studies, the assertion that formula feeding is advantageous over breast feeding could not be accepted. No recent research could be found in the literature that supports formula feeding over exclusive breast feeding in low resource settings.

2.3.2. BREAST FEEDING

After the understanding that avoidance of breast feeding is a difficult option in the context of many poor socio-economic settings, the attention of researchers has been shifted to quantifying the risk that can be averted by modifying the pattern and duration of breast feeding. Most researchers agree that MBF has a higher risk of transmission and mortality than EBF. In South Africa, a Durban study was one of the early published studies that demonstrated this relationship between the type of breast feeding and HIV-free survival rate using a prospective follow-up of mother-child pairs who were on vitamin A trial for 15 months[33]. This study showed that EBF for 3 months, followed by rapid weaning, has an equal rate of HIV-free survival at 6 months to that of EFF, and has a lower risk compared to MBF. Likewise, the ZVITAMBO study, which was done in mother-child pairs from a vitamin A intervention in Zimbabwe showed that MBF had a four-fold increased risk of transmission and a three-fold increased risk of both mortality and transmission at 6 months[34].

As both the above studies were the first to support breast feeding, they were met with strong criticism. One of the criticisms was that, even though mothers were given a high dose of VITA supplement in both studies, the impact of breast feeding was not adjusted for the likely immuno-protective effect of VITA in breast fed infants[35]. In addition, in both studies there was no means to crosscheck whether the information given from mothers on EBF is correct or not. The Coutsoudis *et al* study used a comparison of the morbidity rate in EBF with MBF to crosscheck the reliability of the information given by mothers, but other methods such as diary books were not used to corroborate mothers' reports[33]. The analysis by Coutsoudis and his colleagues also did not consider the competing risk of breast feeding and the effect of having big time intervals between the blood samples collected.

The other most recent research in South Africa, which was the first to initiate a study with intensively supported counseling intervention and a data collection method to identify the risk of transmission through EBF, supplemented the earlier study finding in

Durban by showing that mixed feeding has an up to 11 times higher risk of HIV transmission than exclusive breast feeding[22]. This study assessed MTCT and infant mortality rate using 6 months follow-up data from 7 rural and 2 urban and semi-urban areas in Durban. This study also had limitations of classification bias, and unevenness of sample size. For instance, the study assumed those who switched from EBF to formula feeding (FF) before 14 weeks as a FF group in the analysis of HIV point prevalence, even if they had a history of EBF in the early weeks of child birth. The follow-up duration was also short (only for 6 months) limiting ability to examine long-term outcomes beyond 6 months. Unlike the Coutsoudis *et al*[33] and ZVITAMBO[34] studies, this study reduced recall bias through frequent visits and diary use by mothers. In this study, the estimated postnatal transmission risk was 10.72 per 100 child - years of exposure to exclusive breast feeding. While in the ZVITAMBO study, postnatal transmission rate was 5.1 per 100 child-years of EBF. The likely reasons for this difference could be due to the fact that the Coovadia *et al* [22] study was done in rural areas where the majority of mothers are home stay mothers who practiced EBF after 3 months, whereas the ZVITAMBO[34] study was conducted in an urban area and the rate of EBF after 3 months was lower.

In both the Coovadia *et al*[22] and ZVITAMBO[34] studies, the transmission and mortality hazard was lower in the predominantly breast feeding group (PBF) compared to the MBF group. The mechanism for this was explained in relation to the lower frequency of exposure to other foods in PBF groups as opposed to MBF which minimize the intestinal mucosal irritation. The impact of EBF in the context of other factors such as a high CD4 count was assessed in the ZVITAMBO study. The result shows that the protective effect of EBF becomes even greater among women in whom the CD4 count was less than 500 cells/ul[34].

Consecutive research done after correcting the methodological weaknesses of the earlier studies has confirmed that MBF is a strong risk factor of postnatal transmission and death. For instance, Becquet and his colleagues show in their study that MBF that begins in the first month of the infant's life has a six-fold increased risk of postnatal transmission, which is significantly higher than in the case of EBF[36]. A study in Zambia also demonstrated that MBF has a stronger (Hazard ratio 3.48) postnatal transmission risk than EBF[27]. The mechanism for getting protection from EBF is explained in these studies, namely, that EBF provides immunity and protects from easy invasion with HIV through keeping the integrity and pH of intestinal mucosal cells. Alternatively, it is explained that exclusive breast feeding reduces engorgement of the breast and associated mastitis, which is one of the known high risk factors for increased postnatal HIV transmission.

In general, current studies and policies agree that mixed breast feeding is highly risky, therefore EBF should be promoted among mothers who do not fulfill the AFASS criteria for replacement feeding. The updated guideline of the WHO on infant feeding also emphasise the need for proper counseling and support to avoid mixed feeding practice, especially in the first 6 months of the infant's life [24].

Nonetheless, the WHO updated guideline recommends mixed feeding after 6 months for mothers who are not able to afford adequate weaning food for their child[24]. This is one research question that is currently probed among researchers, namely, whether the starting time of mixed breast feeding and prolonged breast feeding have significant influence on the transmission and mortality rate of infants. Becquet *et al* show in their study that MBF that begins in the second and third month of infant life has no significant influence on increasing postnatal transmission; however, a significant increase (6.3 fold) in transmission occurs if breast feeding continues after 6 months[36]. This finding may suggest that mixed feeding in the first month of the child's life has a much higher risk than mixed feeding started in the 2nd and 3rd months, whereas prolonged breast feeding (breast feeding after 6 months) still has a worse impact. The Coovadia study in Durban shows an equal risk of transmission at 6 months among infants mixed breast fed before and after 3 months of age, but reports an increased risk of transmission at 18 months if breast feeding continued beyond 6 months[22,37]. The lack of association between mixed feeding in the 2nd and 3rd month of infection in the Becquet *et al* study might be associated with inadequacy of sample size to reach to significance level[36]. The ZVITAMBO[34] study supports the result from the Coovadia *et al* study[22] that starting MBF before or after 3 months has equal influence on transmission of HIV. In the ZVITAMBO study, infants who have been receiving exclusive breast feeding for three months and switched on to MBF after 3 months had an equally higher risk of postnatal transmission with infants that were mixed fed from birth[34]. Therefore, the ZVITAMBO study recommends rapid weaning after EBF for 4-6 months to avoid infections that occur through mixed feeding during weaning[34].

On a similar topic, preliminary results from the Kesho Bora study suggest the need for further investigation into the risk associated with breast feeding cessation. The Kesho

Bora study suggested this based on the outcome of their study, which showed that 4 out of a total 10 deaths occurred within 3 months of weaning [38]. Such deaths might be related to increased risk of infection due to mixed breast feeding during prolonged weaning. Alternative to this, a mother may have weaned the baby without having AFASS mechanism set in place to provide the baby with adequate and uncontaminated nutrition during the weaning period. In this regard, more studies are required to investigate reasons related to high transmission and mortality rates during the weaning period. Contrary to the above studies, another recent finding that has been used as the main evidence to update the WHO guideline indicates that breast feeding after 6 months has no greater effect on postnatal transmission[27].

In general, while issues such as the impact of the duration of EBF, the risk associated with MBF during weaning, and the relative benefit of prolonged breast feeding among economically poor people are issues that still need further research, a common agreement among most current studies is the need for urgent policy revision in developing countries for the promotion of early EBF.

2.4. RISK FACTORS FOR POSTNATAL BREAST FEEDING TRANSMISSION

2.4.1. MATERNAL FACTORS

HIV viral load, CD4 count, advanced maternal diseases, newly-acquired infection in the breast-feeding period, breast abscess, cracked nipples and associated factors such as mastitis that increase breast milk viral load are some of the known maternal related factors in the literature. According to a study in Malawi, breast milk viral load, mastitis, and HIV plasma load were detected as highly significant predictor variables of overall mother-to-child transmission in a multivariate analysis[39]. In this study, breast milk viral load was 700 copies/ml among women with HIV-l-infected infants, while it was at an undetectable (<200 copies/ml) level among those with uninfected infants. In the same study, the occurrence of mastitis was associated with increased postnatal transmission. Even though this study could not confirm that the above findings are true for postnatal transmission (as there was no test made to isolate infection occurring before and after delivery), assertive explanations are given on the mechanism of associations identified. For instance, the mechanism for the correlation between mastitis and infection is explained as follows: during mastitis, para-cellular pathways between mammary alveolar cells open up and allow inflammatory cells (such as HIV-1 infected lymphocytes) and extra cellular fluid entry into the milk, which raise the HIV-1 viral load in the milk and adds to the risk of HIV transmission through breast milk. Other studies that support these findings are widely in existence. For instance, a recent study by John *et al* confirmed that breast abscess and mastitis are strongly associated with postnatal transmission[40].

In the literature, high levels of breast milk sodium concentration (normal mature breast milk contains ~5-6 mmol/L of sodium) is reported in colostrum, during termination of weaning and during mastitis[41]. The explanation is that breast milk with elevated sodium levels has significantly fewer CD4 lymphocytes and a significantly higher plasma HIV-1 load, which might increase the rates of mother-to-child transmission[39]. Mastitis is usually unilateral, and whether the contra-lateral, unaffected breast has lower breast milk HIV load than the affected breast is not yet known. None of the above literature reported significant difference in maternal age, body mass index, CD8 lymphocyte count and CD4/CD8 ratio between infected and uninfected infants' mothers.

Low CD4 count and high RNA viral load are the other most commonly reported influential risk factors in postnatal transmission. In Coovadia's study, infants born to mothers with a CD4-cell count of 200–500 cells per μL were nearly twice as likely to have died (unadjusted HR 1·89, 1·16–3·08, p=0·011) than were those born to mothers with a CD4-cell count greater than 500 per μL[22]. In the same study, the risk of PNT in mothers with a CD4 count <200 cells/ul is 4 fold as opposed to those with a CD4 count >500 cells/ul. Similar to this, in a nested case control study, maternal viral RNA level >43000 copies/ml were associated with a 4 fold increase in the risk of transmission[40]. Another recent HAART-based RCT study[38] recommends early identification and active referral of pregnant women with advanced disease stages (CD4+ <=200 cells/m^3), as maternal illness and poor infant health were the two major reasons in the study that prevented initiation of breast feeding in 5 out of 11 infants that died among infants born from mothers with CD4+ count below or equal to 200 cells/m^3.

According to Dunna *et al*[42], the risk of transmission in mothers with a newly-acquired infection in the breast-feeding period also has the highest risk at 29 % as compared to 12% overall transmission in stabilized maternal infection (95 % CI 16, 42). In the ZVITAMBO study, maternal nutritional status was also found to be an influential factor[34]. Based on this study, each additional centimeter of maternal MUAC was associated with a 6–12% reduction in PNT, while infants with mothers of severe baseline anemia were at high risk of PNT and mortality in the first 6 month. In the

Coovadia and BHITS studies, maternal age and parity were less strongly associated with PNT [22,43].

2.4.2. INFANT FACTORS

Even though the mechanism is not yet known, being male is reported in many studies as an influential factor that increases late postnatal transmission [43]. Few arguments and explanations are given in the literature on the mechanism of the correlation. For instance, some literature argued that it could be simply due to the confounding effect of cultural values as males get more attention for complementary foods and prolonged breast feeding compared to female infants[44]. Other research argues that females might get infected at the early transmission period and this reduces the susceptible number of girls for the postnatal pool[45]. But, in the BHITS study, both arguments are opposed as no significant difference was seen either in duration of breast feeding or in early transmission susceptibility rate among girls[43]. Low birth weight was not an influential factor in the BHITS meta-analysis study. Another possible explanation is that, due to gender differences in immune functioning, girls are less susceptible to the infection after 4 weeks of age than are boys. These researchers substantiate their argument by citing other studies that have found the immune systems of infected male infants to be varied somewhat from the immune systems of infected female infants[46]. Most studies did not explicitly stratify MTCT analyses to show the independent contributions of in utero and postnatal transmission, and the lack of association with infant gender was made on the basis of overall MTCT rates at 4 weeks[41].

Oral thrush is another infant-related factor identified in the literature to increase postnatal transmission rate. According to a study in Côte d'Ivoire, late postnatal transmission was more common among children with oral thrush than other infants[23]. The literature indicates that oral thrush enables the passage of HIV-infected lymphocytes to the infant blood stream.

2.4.3. OTHER RISK FACTORS

Many studies usually associate the HIV transmission and mortality risk of HIV-exposed infants with factors related to the mother (maternal factors) [39, 41], the infant (infant factors) [43, 44], the media of transmission (feeding patterns) [47, 48] and the virus (HIV-1 vs HIV-2)[49, 50], whilst only a few studies partially describe the effect of broader risk factors (such as socio-economic status and health system factors) on HIV-free survival rates [37,51]. It is generally known that the effect of factors such as socio-economic status, health system factors, knowledge, health seeking behaviour and psycho-social factors has a paramount place on choice of feeding and subsequent exposure to the risk of HIV transmission and/or mortality, particularly in developing countries. However, studies that measure the independent influence of such factors on HIV-free survival rates are minimal.

The few studies that quantify the magnitude of the effect of broader risk factors show that socio-economic factors such as having toilets, access to electricity and the unemployment of mothers are influential factors of a HIV-free survival rate[37,51]. For instance, in the Mashi study, analysis of risk factors of mortality showed that having no latrine has a three-fold increased risk of mortality (after controlling for confounding factors) compared to having an indoor latrine[51]. In the same study, sharing latrines compared to having private latrines was associated with a 70% increased risk of mortality at a significance level of 0.16. In Botswana, the same study result shows that having electricity is associated with 90% less hazards on the HIV-free survival rate after adjusting for confounding factors[51].

Another study by Rollin and his colleagues reported a 90% increased risk of transmission and mortality of infants among unemployed mothers compared to employed mothers in the Durban study sites [37]. The explanation given to the effect of employment of mothers in this study is that employment of the mother could be a proxy indicator of maternal health and maternal autonomy in addition to the increased access to resources from which employed mothers can benefit.

On the other hand, most of the previous analysis was done using the Good Start data and, even though this did not quantify the independent influence of broader risk factors, they have findings implicative of possible association between broader risk factors and the HIV-free survival rate. The first survival analysis done by Jackson and her colleagues, using the Good Start study data, shows health system factors such as antenatal care and syphilis test services significantly describe the HIV-free survival rate differences between Rietvlei and Paarl sites, with Rietvlei having 81% (p-value = 0.08) increased HIV transmission and death which was significantly higher than in the case of Paarl due to low coverage of syphilis service provision [52]. However, as the main target of this study was not to analyse health system risk factors, the reported magnitude of the influences of antenatal care and syphilis test service do not show the independent influence of the antenatal care and syphilis test service, as the reported magnitude was not adjusted for the contribution/confounding effect of other factors such as health-seeking behaviour, knowledge and socio-economic indicators.

A community-based situational analysis made by a Good Start study group in the same (Good Start) study sites reports that transport and distance to health facilities, health-seeking behaviour and poor quality of care are the major influential factors of utilization of maternal and neonatal health services[53]. Also, a qualitative study by Doherty among Good Start study participants reported a strong belief in the benefits of breast feeding, ability to resist pressure from family to introduce other fluids, and a supportive home environment as key factors that enabled women to maintain exclusive breast feeding[54]. However, in both of these studies, the magnitude of the influence of such risk factors on HIV-free survival rate were not quantified due to the qualitative nature of the study.

To our knowledge, the magnitude of broader risk factors of HIV-free survival rate other than those mentioned above are not demonstrated in the literature and, therefore, the effect of such factors is poorly understood. Thus, with the aim to fill this gap, the primary target of our study is to measure the magnitude of the influence of broader risk factors such as socio-economic status, health system factors, knowledge, feeding pattern, health behaviour and psycho-social factors on HIV-free survival rate. Our study differs from previous analysis that used Good Start data in that we analyse the independent influence of multi-level immediate and broader risk factors of HIV-free survival rate with the aim to contribute parameter values to a PMTCT model that informs PMTCT policies and interventions at provincial level by predicting the possible transmission or mortality outcomes for a range of parameters (risk factors).

2.5. MODELING THE EFFECTS OF DIFFERENT INFANT FEEDING STRATEGIES

Several mathematical models have attempted to weigh the risks and benefits of infant feeding strategies in different settings. These studies have used varying measurements of parameters to provide policy guidance on the benefits and risks of different infant feeding modalities classified per site (urban and rural), age and infant mortality rate, essentially for the purpose of allocating adequate resources and emphasizing the safest infant feeding method. The major shortcoming of most of these studies is the fact that they base their model parameters only on infant feeding modalities and do not incorporate the impact of the underlying socio-economic factors, health system, maternal and infant risk factors that may have contributed to the rate of HIV-free survival.

For instance, Khun and Stein used infant feeding patterns as the only influential factors in the transmission and/or mortality rate of children[55]. Based on their result, the lowest adverse outcome occurs if all HIV-negative women optimally breast feed and all seropositive women avoid breast feeding, given IMR is below 100/1000 live births and the relative risk of adverse outcomes of avoiding breast feeding is set at 2.5 (compared to optimal breast feeding)[55]. However, other high risk factors of transmission and mortality (other than infant feeding types) were not included in the assumption. Such models, apart from their importance of showing the relatively advantageous infant feeding method per localities, have minimum contribution to the development of interventions that target high risk factors of transmission and/or mortality. High risk factors are major determinants of transmission in any feeding scenario; therefore, they should be included in such models.

The other limitation of most models relate to assumptions about the timing and pattern of breast feeding, and transmission rate. For instance, Piwoz suggested that exclusive breast feeding produces the best outcome in most low-income countries where IMR is >25/1000 live births[56]. However, this study assumed that the transmission rate of HIV is constant throughout the late postnatal (after the first week) breast feeding period. Some current research contradicts with this assumption, as transmission through breast feeding can vary depending on the pattern (EBF versus MBF and PBF) and duration of breast feeding. Models that account for transmission variations per feeding type and duration are needed.

Other than this, only a few modelers use urban-rural classifications to demonstrate the variation of transmission and/or mortality dynamics per localities. Piwoz and Ross, in their model, used urban and rural classifications using data from Ethiopia[56]. Such classifications are important as local circumstances between rural and urban areas are largely different. Adoption of Piwoz's model in South Africa gives that, in seven of the nine provinces, early exclusive breast feeding followed with rapid cessation results in the highest HIV-free survival rate at 12 months. In low IMR areas such as Western Cape and Gauteng, avoidance of breast feeding results in an equal HIV-free survival rate with safer breast feeding. However, in poorer settings such as the Oliver Tambo district in the Eastern Cape, where IMR is more than 80/1000 live births, universal adoption of formula feeding would lead to a lower HIV-free survival rate than no intervention (Unpublished results, Chopra *et al*, cited by Doherty, 2006)[6]. The infant feeding scenarios presented by Piwoz are more linked with the current interventions and use more recent studies on EBF than the study by Ross and Labbok who defined safer breast feeding in a different way from available interventions[57].

2.5.1. PARAMETER SELECTION FOR MODEL DEVELOPMENT

One advantage of mathematical models is that, as opposed to statistical models that rely on statistical significance, mathematical models are less strict on statistical significance levels. This is due to the reason that mathematical models permit manipulation of the parameter values for a range of values around the point estimate by which one can be able to know and compare the possible outcomes for different values of parameters. This unique nature of mathematical models is advantageous, especially when parameters are known to have evidence on their clinical importance but, due to sample size problems, one may fail to achieve the given statistical significance. For instance, Ross and Labbok, in their model that estimate the effect of different breast feeding patterns, have used the average breast feeding rate from three studies as an actual value for their estimate[57]. After doing so, sensitivity analysis was made for a range of values within the confidence interval and critical values were set to indicate which minimum and maximum values of breast feeding rate can change the direction of the effect. Often, mathematical models allow the use of parameters that are not significant at 0.05 level of significance, especially if the parameters are supported by strong evidence in the literature and if parameters were taken from a small sample size. Other sources of parameters in the literature include local records and evidence of clinical/public health importance in the literature[56, 57].

Commonly used parameters in the above studies include the following variables with varying measures: postnatal mother-to-child transmission rate in different breast feeding scenarios, relative risk (RR) of adverse outcome in the formula feeding group, breast feeding rate (B1), exclusive breast feeding rate (B2) and baseline infant mortality rate in breast feeding group (without including death due to the HIV pandemic), which are all used to weigh the risk and benefit of different infant feeding scenarios in HIV-positive mothers. The IMR estimates were taken from reports prior to the acceleration of the epidemic, or the non-HIV estimates of current IMR. Sensitivity analysis was made through setting critical values, and CI (confidence interval) ratios, and re-running of the data using these values. Most of the latest research includes age-specific transmission rates and relative risks, to give policy recommendation on the age trend of the HIV- and non-HIV-related risks of different infant feeding modalities.

2.6. ANTI-RETROVIRAL THERAPY IN BREAST FEEDING WOMEN

In resource-limited settings, due to the crucial necessity of breast feeding for nutritional, immunologica1, social and cultural benefits, the development of ART has become a current research priority to reduce transmission through breast feeding. This strategy, if it works, has a robust rationale of minimizing postnatal transmission through reducing the viral load in plasma and breast milk, and also as a prophylaxis to reduce seroconversion in uninfected infants during breast feeding. A number of investigations on animal models as well as in human beings are underway in several countries to explore the effect of single or multiple drugs, the appropriate timing (duration) to initiate and/or complete the drug, and on selection of appropriate target mothers based on their

CD4 count level .

Most of these studies designed their trial based on current knowledge. For instance, the following three timings are selected by ART studies found in different countries based on reported timing of high transmission rate: at ultra short regimen (only for 1st week after birth) to reduce the high rate of infection through colostrum; a trial that covers the first 6–12 weeks of breast-feeding (for early weaning infants); and a trial that provides antiretroviral prophylaxis to infants through the first 6 months of life accompanied by early weaning [17]. There are also interventions going on to provide ART throughout breast feeding time to avoid transmission through prolonged breast feeding.

The preliminary result from two separate studies in Malawi that aimed to assess the short-term impact of a mono antiretroviral drug (NVP once) compared with the dual ART (NVP + ZDV for the first week from birth) to the neonate suggest that dual antiretroviral prophylaxis may be more effective than the use of a single drug[58,59]. In Tanzania also, in the MITRA study, which non-randomly provided mothers with combivir (ZDV/3TC) from week 36 of pregnancy to 1 week after delivery and infants with combivir for 1 week, and daily 3TC (4 mg/kg) throughout the breast feeding period, has reported a 7% difference (reduction) in transmission rate from a historical control group[60].

There are also trials that are designed based on mothers' viral load level to reduce transmission due to high maternal viral load. The Kesho Bora multi-country study targets to assess the impact of ART in three groups: life long HAART in mothers with

CD4 counts < 200/mm³ or clinical AIDS; short course MTCT prophylaxis to mothers with CD4 counts > 500/mm³ who are at low risk of HIV transmission to infants and high risk of HAART toxicity and development of resistance. The third group are women with CD4 counts 200-500/mm³ where the risk-benefit balance between risks of HAART, reducing MTCT, and where the health benefits for mothers is not known will be randomized to receive either short-course MTCT prophylaxis or triple-ARV MTCT prophylaxis during late pregnancy and breast feeding[6].

Preliminary results from the Kesho Bora study[38] reports a low (5.8%) cumulative (1-year) risk of HIV-infection by using short-course MTCT prophylaxis (ZDV started at 34-36 weeks of pregnancy until the time of delivery plus single dose NVP to mother and baby immediately after delivery) among mothers with a CD4+ count >500 cells/m³. Based on this, the study suggests the need for further investigation into the potential additional benefit of prolonging ART prophylaxis during the lactation period. A similar random control trial in the Dream study has reported a reduction in late postnatal transmission after introducing HAART to mothers for the first six-month period of lactation with such effect being protective up to 12 months of age [61].

2.7. CURRENT RESEARCH NEEDS

In the literature, it is known that complete avoidance of breast feeding can fully eliminate postnatal HIV transmission. However, the operational effectiveness of this strategy in developing countries is under question because it has potential adverse effects such as risks of infections, wasting, stunting and a related increased rate of mortality. Studies done so far in South Africa to assess the impact of different feeding patterns and interventions have various limitations. Few cases in the literature analyse duration of breast feeding as a factor of transmission risk. The majority of these studies are solely based either on urban hospitals or have short follow-up periods. They are also not free from major limitations as their primary target was not investigation of the impact of different infant feeding practices. Policy guidance is needed, especially on feeding strategies that could prevent the risk of postnatal transmission with minimized non-HIV-related risks. EBF is the most scientifically feasible nutrition for the growth and development of the child. Additional studies that investigate the effect of duration of EBF, transmission risk of MBF during weaning, and the benefit of prolonged weaning are needed. On the other hand, other high risk factors of transmission and/or mortality are not investigated thoroughly. Therefore, there is a need to conduct a longitudinal study in representative areas from urban, semi-urban, and rural parts of the country using the appropriate data collection and analysis methods. Mathematical models that incorporate a range of these high-risk factors can inform policy development on locally appropriate interventions.

CHAPTER THREE

3.0. METHODOLOGY

3.1. STUDY DESIGN

This is a secondary analysis of data from a cohort study of women and infants participating in three South African National PMTCT programme sites – The Good Start Study. The aim of the Good Start study was "evaluation of the routine operational effectiveness of the South African National PMTCT Programme"[52].

The Good Start study recruited women from those attending one of three (out of 18) selected South African National PMTCT pilot antenatal clinics. Women were recruited from 28 weeks of pregnancy, following an explanation about the study. The PMTCT programme at these sites was implemented according to the South African National PMTCT Protocol which, during the study period (2002-2004), consisted of antenatal counselling and HIV-testing, infant-feeding counselling once or twice antenatally, free infant formula to women who chose not to breastfeed and single-dose Nevirapine for the mother and infant. The infant-feeding counselling was based on the WHO/UNICEF recommendations that women should be given information about the risks of different feeding options and then helped to make an appropriate choice.

3.2. STUDY POPULATION

Paarl, Rietvlei and Umlazi are the three study sites deliberately selected from the original 18 national pilot sites to reflect different socio-economic contexts, rural–urban locations and HIV prevalence rates. Paarl (Western Cape Province) is a peri-urban/ rural area, with a relatively higher socio-economic profile, a relatively well-functioning public health system, with antenatal HIV-prevalence of 9% during the study period. Rietvlei (Eastern Cape Province) is a rural area in one of the poorest regions of South Africa, with 28% antenatal HIV-prevalence. Umlazi (KwaZulu-Natal Province), a peri-urban area with formal and informal housing, is considered to be intermediate with regard to health resources compared with the other two sites. The antenatal HIV-prevalence in Umlazi was 47% during the study period[62].

3.3. INCLUSION AND EXCLUSION CRITERIA

The target participants of this analysis are infants born from HIV-positive mothers who were not infected during pregnancy, labour and delivery. Infants who were positive at the 3-week PCR test stage and infants that died before or at 3 weeks were excluded from the study to rule out HIV transmissions occurring during pregnancy and the intrapartum period. Infants who did not have the PCR test at 3 weeks were included in this analysis if one of their 24- or 36-week PCR tests was negative. Infants who missed the 3-week PCR test and who were positive at the 24-week visit test were excluded as we were not able to be sure whether they were infected at the intrapartum or postpartum period. Twin infants were excluded from the study to avoid subject dependency.

3.4. SAMPLING AND SAMPLE SIZE

The sample size for each site was estimated to provide a precision in the HIV transmission (or death) rate at 9 months of 4.3% in Umlazi, 6.5% in Rietvlei and 7.5% in Paarl. Patient volumes and HIV rates varied across sites. Study length was determined to achieve the desired minimum precision in the smallest site (Paarl), resulting in better estimated precision in the two larger sites. HIV-positive mothers were recruited from the local hospital or clinic offering the PMTCT programme by a qualified field researcher. Refusal rates, both for the PMTCT programme (0–19%) and study participation, were low in all sites. The total sample size of the Good Start study participants were 665 mother–infant pairs which were divided among the three sites as follows: Paarl - 149, Rietvlei - 192 and Umlazi - 324. From these 665 infants, 469 mother-child pairs are included for this study after excluding infants that were infected or died before 3 weeks. Loss to follow-up at 36 weeks overall was lower than expected, at 21%, and was not statistically different across sites[28]. The findings from this result can be generalized to districts which have similar socio-demographic and HIV prevalence rates[52].

3.5. DATA COLLECTION

Data were collected during the period October 2002 to November 2004 from the above three selected PMTCT sites by either trained field researchers (postpartum in the delivery facility, and in the home at 3weeks to measure early feeding and HIV transmission; at 24 weeks as the age by which breast feeding should have been discontinued; and at 36 weeks, which was the original PMTCT programme exit point) or trained community health workers (in the home at 5, 7, 9, 12, 16, 20, 28 and 32 weeks) from HIV-positive mothers recruited around the time of delivery. Data on infant feeding practices, infant and maternal health status, disclosure of HIV status, basic knowledge of HIV/AIDS and MTCT, socio-demographic and other relevant information are collected using the semi-structured interview method. In addition, dried blood spots were collected by heel prick in the baby at 3, 24 and 36 weeks to determine HIV infection whilst, in the mother, finger prick was taken to measure the viral load at 3 and 36 weeks. Interviews were in the preferred language of the subject (Xhosa, Zulu, Afrikaans or English). The field researcher also reviewed the perinatal medical record of both mother and infant to extract data on antenatal, intrapartum and postpartum care, PMTCT programme care and the occurrence of pregnancy or newborn complications[52].

3.6. VALIDITY AND RELIABLITY OF THE STUDY

Intensive training was given to interviewers to ensure that a standard method is followed. Practice interviews were also undertaken to ensure that all interviewers understood and followed the standard method. Questionnaires were developed after consulting existing validated tools on infant feeding and all questionnaires were piloted. All study activities were described in standard operating procedure (SOP) guides that were kept at all three sites and an external quality control specialist performed two study audits during the course of the study to ensure that field staff was adhering to the SOPs of the study. To ensure reliability of the anthropometric measurements, electronic scales were used and all scales were checked regularly for accuracy with a standard weight. To ensure accurate measurement of the main outcome, DNA PCR Assay that detects pro-viral cell-associated DNA with a sensitivity of 95-99% and a specificity of 98% was used[62].

The selection of indicator variables and categorisation of continuous variables into dummy variables considered both clinical meanings and statistical test (martingale test).

The latter test is used to ensure that continuous variables are categorised in their best functional form. Confounding variables were identified and adjusted for their effect on the independent risk factors, if they had 10% or more effect on the independent variable under investigation[63].

3.7. DATA MANAGEMENT AND DATA ANALYSIS

3.7.1. DATA MANAGEMENT

Data from all questionnaires were coded, double data entered in EpiData and cleaned in SAS version 9.1. Data were transferred to STATA version 10 for multivariate analysis. Data was transformed to a format suitable for survival analysis. Character variables were recoded into numeric variables. Dates that were stored in text variables were converted to time durations that are suitable for survival analysis. New dummy variables were generated for continuous variables and multiple level factors. SAS version 9.1 was used for descriptive analysis, because the Chi-square command of this software gives both relative risks and confidence intervals, while the Chi-square test from STATA only gives p-values.

This is the first study from Good Start studies that looks at a broader range of predictor variables of a HIV-free survival rate. For the purpose of various previous studies published from Good Start Data, data was cleaned partially by different parties (researchers) in which each of them cleaned part of the data separately by concentrating on variables useful for their own research. These separately cleaned datasets were combined together into a master dataset and further data cleaning was made on variables that were not cleaned before.

Also, additional dummy variables were created from continuous and multiple level factors when it was found necessary for our study. Categorisation of such variables was based on biological meaning, commonly used categorisations in the literature and the distribution of the data. The suitability of these categorisations was assessed using the martingale test which examines that the variables are in their best functional form. Anthropometric scores were calculated using the WHO Anthro-2005 software. Categories of malnutrition, stunting and wasting were created from the anthropometric scores based on the WHO recommended cut-off points (see glossary and definitions). Socio-economic scores were created from variables such as availability of working items, access to water, toilet and other variables with weightings that reflect their strength of influence.

Variables were grouped into seven categories (Table 1), namely: knowledge and health-seeking behaviour, health system factors, socio-economic factors, psycho-social factors, feeding patterns, and maternal and infant factors (see the full list of variables used in Annex II). Such categorisations were created after consulting literature on commonly used indicators of the seven categories mentioned above. Under each of these categories, variables were again sub-grouped based on proximity/correlation to each other. This was done to avoid the use of two dependent variables in a model which would create a multi-colinearity effect and violation of the Cox Proportional Hazard model assumption for subject independence. For instance, under infant factors, the sub-category weight contains all weight measurements of infants from birth until 36 weeks and premature birth. As weight at the 24-weeks or 36-weeks visit can be influenced by weight at birth and weight at 3 weeks, they are sub-grouped into one sub-category to indicate that they cannot appear together in a model.

Table 1: List of variables assessed for any significant influence on HIV-free survival rate

Category	Variables
Infant factors	
Weight	Infant weight at birth
	Infant weight at 3 week
	Infant weight at 24 week
	Infant weight at 36 week
Delivery outcome	Premature birth
Gender	male
Child illness	child has been sick at 24 week
	Mother changed the way she feed at 24 week due to child illness
	child been sick at 36 week
	Mother changed the way she feed at 36 week due to child illness
Treatment/illness during birth	Child given oxygen at birth
	new born respiratory complication
Immunization	Child didn't receive measles at 36 week
	Child fully immunized for 24 weeks vaccines
Maternal factors	
Breast problem	Mother suffered from engorged breast at 36 week
	Mother suffered from inflamed breast at 36 week
Weight gain during pregnancy	Weight loss or poor weight gain during pregnancy
Mother's illness	Mother has been admitted to hospital at 24 week
	Mother has been admitted to hospital at 36 week
	Changed feeding pattern due to mother's illness
Socioeconomic factors	
	Don't have piped water inside house
	Don't use piped water at all

	have flush toilet
	Run out of food in the past one year
	Mother main income provider of the family
	Mother unemployed
	Paarl site
	Rietvlei site
	Socioeconomic scores
Psychosocial factors	
	Non-disclosure of status
	Response of the community to disclosure
Health system factors	
	ANC visit less than or equal to three
	Postnatal visit less than 4
	Counselling score below half
	Syphilis test taken
Knowledge and health seeking behaviour	
	Mom knows that HIV transmits during pregnancy
	Knowing all 3 transmission routes vs not knowing all or knowing some or having incorrect knowledge
	Mom didn't discuss anyone at the clinic on the best way of feeding
	Mothers tendency to go to health professionals first when they get difficulty of feeding
	Using at least boiled water to wash formula bottle
	Mother hasn't yet discussed with professionals how to prevent future pregnancy
Infant feeding type	
	Mixed Formula Feeding vs Exclusive Formula Feeding
	Mixed Breast Feeding vs Exclusive Breast Feeding

3.7.2. DATA ANALYSIS

The following three steps have been followed to establish the strength of influence of independent risk factors of a HIV-free survival rate.

In the first step, a bivariate analysis was carried out to identify significant influential variables (see Annex II for selected variables list) of HIV-free survival rate. Variables found statistically significant at significance level 0.10, those that had a large relative risk value, or strong evidence in the literature of their significant importance were selected and included for multivariate analysis using Cox Proportional Hazard.

In the second step, Cox Proportional Hazard was used to identify and measure the independent influence of predictor variables identified from the first step. Two kinds of models were built based on the study questions. The first model built analyses the hazard of all independent factors (from recruitment to 36 weeks) selected from the bivariate analysis. In addition to this, a baseline model was fitted using the Cox Proportional Hazards model only on variables that can be collected up to the 6-week immunisation visit to inform questionnaire development for a national cross-sectional survey.

Based on the Ghent recommendation [31, 32], the primary outcome assessed in this study is HIV transmission and/or death of an infant between 3 weeks and 36 weeks. We calculated the duration of follow-up for non-infected children from recruitment to the date of the last follow-up, or for infected children to the mid-point between the date of the last negative and first positive results. Similarly, the duration of follow-up for infants who died with unknown status is from recruitment to date of death.

The hazard ratio from the Cox Proportional Hazards model is used to measure the relative strength of independent variables. A p-value of 0.2 (20% chance of random error) is used as a cut-off point for significance level to identify independent factors. The model building process was made by adding one variable at a time in a stepwise fashion to a univariate model containing the independent risk factor under investigation. A variable(s) that has 10% or more effect on the hazard of the independent variable under investigation remains in the model. Such adjusting factors were selected prior to the start of the model building process based on the criteria that they have to be statistically/biologically related with the independent factor and the outcome variable, with no intermediary effect between the independent variable and the outcome. In all model development processes, the log likelihood ratio and AIC (Aikaki) criteria were used to select the best-fit model with the smallest loss of information.

Multi-colinearity effect was avoided by building alternative models for a sub-set of variables that are not indicators from the same sub-group (see previous section, e.g., a model would not include both prematurity and low birth weight). Breast feeding pattern was treated as a time-dependent variable and Cox-regression model was fitted for analysing the effect of MBF and MFF at 5-week and 7-week time durations.

Model checking was made for continuous variables using the Martingale residual test to check whether the appropriate set of explanatory variables are included with their best functional form. The Cox-Snell test was used to check the overall model fit to the data. Outliers and influential observations were checked using the deviance residuals test and score residuals. The Proportional Hazard assumption was tested using the Schonfeld residuals test.

3.8. PMTCT MODEL STRUCTURE AND SELECTION OF PARAMETERS

The future target of the Good Start project is to develop a mathematical model that estimates the HIV-free survival rate of infants using data (parameter values) from this mini-thesis and other literature. In this section, we have illustrated a prototype mathematical model equation (see equation 1-8 below) that will give us a preliminary idea on the structures of the model and parameter values needed.

PMTCT Model equations

Let S_1 be the total susceptible infants born from HIV-positive mothers at time t_0. It follows that, in the first 3 weeks, I_1 of these infants get infected with HIV at a rate, γ_1, and the remaining uninfected S_2 infants still continue being susceptible to HIV infection. Therefore, S_1, S_2 and S_3 are the number of susceptible infants in each time interval from birth – 3weeks, 3 weeks-24weeks and 24 weeks-36weeks, in the given order (the time periods are selected in order to suit with our data). I_1, I_2 and I_3 of these susceptible infants get infected in each of the above time intervals, at a rate γ_1, γ_2, and γ_3 in their respective order. It is assumed that these infected infants die (D1) at a constant rate σ that is considered to be greater compared to the baseline (mortality among HIV-unexposed children) mortality rate. Then, the ordinary differential equation of a mathematical model that estimates the HIV transmission and/or mortality rate among HIV infants can be given as follows:

$$S_1 = \pi S_1 - S_1 (\mu + \alpha_1 + \gamma) \qquad (1)$$

$$I_1 = \gamma_1 S_1 - I_1 (\mu + \sigma) \qquad (2)$$

$$S_2 = S_1 [1 - (\gamma 1 + \mu + \alpha_1)] - [S_2 (\mu + \alpha_2 + \gamma)] \quad (3)$$

$$I_2 = \gamma_2 S_2 - I_2 (\mu + \sigma) \quad (4)$$

$$S_3 = S_2 [1 - (\gamma_2 + \mu + \alpha_2)] - [S_3 (\mu + \alpha_3 + \gamma)] \quad (5)$$

$$I_3 = \gamma_3 S_3 - I_3 (\mu + \sigma) \quad (6)$$

$$D_1 = \sigma (I_1 + I_2 + I_3) \quad (7)$$

$$D_2 = \alpha_1 S_1 + \alpha_2 S_2 + \alpha_3 S_3 \quad (8)$$

Where, π is birth rate among HIV-positive mothers and μ is mortality due to other natural causes. D2 is deaths with unknown causes among HIV-exposed infants that might be as a consequence of formula feeding (not breast feeding) in each time interval given above. It is assumed that the rates ($\alpha_1, \alpha_2, \alpha_3$) of death with unknown causes vary between time intervals.

This model will use the hazard ratios of parameters (independent influential factors) identified from this mini-thesis using the Cox Proportional Hazard model with a significance level of 0.20. The reason that we used 20% significance level as oppose to the usual 0.05 significance level is that developing a mathematical model (which is the main purpose of this study) allows such flexibilities, as sensitivity analysis would be made later in the model development stage to show the possible outcomes if the parameter values vary around the point estimates observed in this study.

3.9. STUDY LIMITATIONS

The fact that our data is a secondary data limits our influence in ensuring quality of data collection and recruitment procedures. However, the data was collected with good internal and external quality control. In addition, the limitations of any cohort study is confounding effect and pre (self) selection bias, as exposures are not randomized. In order to reduce confounding effect, we have used a suitable statistical model (Cox Proportional Hazard Model) which takes into account the confounding effect of variables. The other limitation of our study approach could be the complete reliance of our study on mothers' reports on breast feeding compliance. However, in order to reduce recall bias, frequent visits were made by community health workers. This study cannot assess any impact related to Anti-retroviral drugs, as postnatal ARV services were not rendered during the study period.

Our data was collected during the time when EFF was more promoted and the advantages of EBF were not recognized by health professionals. As a result, we have a lower number of EBF groups as compared to the other infant feeding types, and this caused a lack of power to detect statistical significance. Due to the small sample size of EBF mothers, the study could not also deeply engage in analysing the HIV-free survival rate per different duration of EBF and starting time of mixed breast feeding. Also, another limitation in this study is the large time gaps between the blood sample collection durations (3, 24 and 36 weeks). Based on recommended methods, the sero-conversion time is taken as the time mid-way between the last negative and first positive result [31,32]. As the majority of the mothers might have stopped breast feeding at 9 months, the study did not acount for the competing risk of timing of breast feeding cessation. Finally, these study sites were not randomly selected; however, they could be considered reasonably representative of similar settings found throughout South Africa.

3.10. ETHICAL CONSIDERATIONS

This is a secondary analysis of data from the Good Start cohort study. Ethical approval for the source data was obtained from the University of KwaZulu-Natal. I have obtained permission from the principal investigators to work on the source data. During the collection of the data, mothers were told that their status was confidential and that they could withdraw from the study at any stage, if they wanted to. Compensation for participating in the study was given based on local norms in the sites. Mothers were provided with the necessary information about the study before they signed informed consent at recruitment stage. All interviews were private and confidential. During data capturing, study ID was used instead of names to keep confidentiality. Log sheets were kept at the study site under the control of site co-ordinators. As a secondary analysis, this author does not have access to the original log books and all data being used is only coded by the anonymous study ID.

CHAPTER FOUR

4.0. RESULTS

4.1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

The Good Start data were collected from 665 infants born from HIV-positive mothers. For this study, 469 infants of the total 665 infants met the inclusion criteria of infants, namely, that they should be alive and HIV-negative at 3 weeks. Based on this, 149 infants that were dead or HIV-positive or whose status was unknown at 3 weeks were excluded to avoid infants infected during the intrapartum / peripartum period. From the remaining 516 infants that were eligible for this study, 47 infants had no HIV test in any of the visits after 3 weeks. These infants had unknown status and therefore could not be included in the analysis. This gives a remaining sample size of 469 infants for this analysis.

In the overall sample, the number of female infants (52.03%) is slightly higher than male infants (47.97%). The majority (83.29%) of infants were already introduced with solid and liquid foods before 5 weeks. The highest EFF and MFF rate is reported in Paarl, while Umlazi has the highest EBF and MBF rate. Comparison of the sites by exclusivity of feeding shows that Rietvlei has poor compliance to exclusive feeding with 90.26% of the infants being on either mixed breastfeeding or mixed formula feeding by 5 weeks (see Table 1).

Most of the mothers are in the inter-quartile age range of 22-29 years. The majority

(56.33%) of the mothers had at least passed standard 8. Nonetheless, mixed feeding patterns are practiced in both groups in spite of this relatively high educational status. Only 36.29%

Variables	*Overall*	*EBF*	*MBF*	*EFF*	*MFF*

of the mothers get their drinking water from piped water in the house. 15.77% of the mothers use either borehole or river/stream water for drinking. The remaining 48.08% mothers get their drinking water from piped water in public or at a yard. Half (9) of the mothers who claimed that they do not have any kind of toilet were on formula feeding, even though this was not recommended for such mothers. Similarly, 30 (41%) out of the total 73 mothers who used borehole and rain/stream water had been formula feeding their child up until 5 weeks, even though this is highly risky in their circumstances. The other 24 mothers who use borehole or rain/stream water were mixed breast feeding their child before 5 weeks (see Table 2).

The mean log viral load of mothers who used exclusive formula feeding (3.97) is slightly higher than mothers who were exclusively breast feeding (mean=3.6), mixed breast feeding (mean=3.7), and mixed formula feeding (mean=3.74).

There was, overall, 22 (4.69%) infant deaths during the postnatal period, of which 6 (27.27%) died of known HIV-positive results and the remaining 16 (72.73%) died with unknown HIV-status at the time of death. An additional 55 infants were HIV infected after 3 weeks (first positive test for 31 infants at 24 weeks and 23 infants at 36 weeks) and stayed alive until the end of the study (36 weeks). Only 2 children died from the EBF group.

Total	469	33 (8.62%)	167(43.60%)	31 (8.09%)	152(39.69%)
Median maternal age(years)	25 (15-42)	26(17-42)	24(15-41)	26(18-35)	25(15-41)
Site					
Paarl	119 (25.37%)	8 (7.14%)	20(17.86%)	24(21.43%)	60 (53.57%)
Rietvlei	126 (26.87%)	5 (4.42%)	57 (50.44%)	6 (5.31%)	45 (39.82%)
Umlazi	224 (47.76%)	20 (12.66%)	90 (56.96%)	1 (0.63%)	47 (29.75%)
Last standard passed					
Below standard 8	200 (43.67%)	13 (7.51%)	75 (43.35%)	18 (10.40%)	67 (38.73%)
Standard 8 and above	258 (56.33%)	19 (9.45%)	87 (43.28%)	12(5.97%)	83 (41.29%)
Toilet					
Flush toilet	223(48.16%)	17 (9.50%)	60 (33.52%)	21 (1.73%)	81 (45.25%)
Pit latrine	178 (38.44%)	11(7.28%)	84 (55.63%)	3 (1.99%)	53 (35.1%)
VIP	29 (6.26%)	2(10.0%)	13(65.0%)	0(0.0%)	5(25.0%)
None	17 (3.67%)	2(12.50%)	6 (37.50%)	4 (25.0%)	4 (25.0%)
Source of drinking water					
piped - in house	168 (36.29%)	11 (8.53%)	50 (38.76%)	14(10.85%)	54 (41.86%)
piped – in yard	124 (26.78%)	13(12.62%)	42 (40.78%)	7 (6.80%)	41 (39.81%)
piped - in public	96 (20.73%)	6 (7.50%)	39 (48.75%)	6 (7.50%)	29 (36.25%)
Borehole	31 (6.70%)	2 (7.69%)	13 (50.0%)	2 (7.69%)	9 (34.62%)
River/stream	42 (9.07%)	1 (2.44%)	21 (51.2%)	1 (2.44%)	18 (43.90%)
Main income provider of the family					
Mother of the child	48 (10.62%)	2 (4.76%)	19 (45.24%)	2 (4.76%)	19 (45.24%)
Father of the child	143 (31.64%)	20(17.24%)	43 (37.07%)	10(8.62%)	43 (37.07%)
House hold (HH) member living at home	153 (33.85%)	6 (4.88%)	50 (40.65%)	12(9.76%)	55 (44.72%)
HH member living away	38 (8.41%)	2 (5.71%)	20 (57.14%)	2 (5.71%)	11 (31.43%)
More than one source	27 (5.97%)	0 (0%)	12 (52.17%)	3 (13.04%)	8(34.78%)
Child sex					
Male	225 (47.97%)	13 (7.30%)	73 (41.01%)	16 (8.99%)	76 (42.7%)
Female	244 (52.03%)	20 (9.76%)	94 (45.85%)	15(7.32%)	76 (37.07%)
Average log viral load	3.71 (2.7-5.9)	3.6 (2.8-4.9)	3.7 (2.7-5.4)	3.97 (2.9-5.8)	3.74 (2.7-5.9)
Infant Death	22 (4.69%)	2(11.11%)	10 (55.56%)	0 (0%)	6 (33.33%)
HIV infection and/or death	70 (14.93%)	6 (10.0%)	29 (48.33%)	1 (1.67%)	24 (40.0%)

Table 2 Characteristics of live born infants and their HIV-infected mothers by infant feeding practice at 5 weeks

Note: row percentages are used

4.2. MULTI-COLINEARITY EFFECT WITHIN VARIABLES

In order to avoid multi-colinearity effect between variables, we used statistical tests (correlation test and chi-square test) and clinical knowledge to identify and sub-categorise variables with political multi-colinearity effect. The following correlation tests are examples of what was done to identify multi-colinearity effect. In the table below, within the category of infant factors, weight measurements were categorised under one sub-group because the correlation test shows that the weight measurements taken at the 3 consecutive visits have within subject correlation in a decreasing order. Therefore, these variables cannot be used together in one model as using them together would create a multi-colinearity effect. Also, use of these variables in one model would violate the Cox Proportional Hazard model assumption for within subject independency.

Table 3: Correlation coefficients comparing weight measurements at 3, 24 and 36 weeks visit

Z scores	Weight for age Z score at 3 weeks	Weight for age Z score at 24 weeks	Weight for age Z score at 36 weeks
Weight for age Z score at 3 weeks	1.000		
Weight for age Z score at 24 weeks	0.5558	1.000	
Weight for age Z score at 36 weeks	0.4687	0.8818	1.000

Socioeconomic factors such as availability of piped water and flush toilets that had correlation to each other were grouped in one sub-category. This is because, for instance, it is likely to find piped water in houses where flush toilets are available. The chi-square test that was done using the data (Table 4) confirms this by showing that the majority (92.56%) of those who have flush toilets also had piped water in the house. Therefore, these two variables were assigned under one sub-category (socio-economic risk factors) to indicate that the use of one can represent the other, while using both variables in one model would result in multi-colinearity effect.

Table 4: Correlation between flush toilets and availability of piped water in the house

Variables	Piped water in the house (%)	No piped water in the house (%)
No flush toilet	32.74	67.26
Have flush toilet	92.56	7.44
Total	63.87	36.13

Chi-square = 180.0 p-value=0.0000

The variables maternal illness and changing the way the mother fed the infant due to maternal illness are sub-grouped together because the later variable (changing the way the mother feeds due to maternal illness) is the direct consequence of the former variable (maternal illness) and this makes them correlated. Therefore, these two variables were assigned in one sub-group to use only one of the variables at a time as this will avoid both multi-colinearity effect and use of intermediate variables in a model.

Sometimes, variables which are not from the same sub-category, such as poor or no weight gain during pregnancy (a maternal group factor) and low birth weight (an infant group factor), were used separately during model development as they had correlation with each other (see Table 5). In Table 5, 33.33% of mothers who had poor weight gain during pregnancy gave birth to low birth weight children and this was significant with a p-value of 0.025. It is expected to have low birth weight infants by mothers who had poor weight gain (or loss of weight) during pregnancy. Therefore, we avoided using these two variables together.

Table 5: Correlation between low birth weight and poor maternal weight gain

Variables	Low birth weight (%)	Normal birth weight (%)
Mother had poor weight gain during pregnancy	33.33	66.67
Mother gained weight during pregnancy	13.11	86.89
Total	13.76	86.24

Pearson chi 2(1) = 5.0014 Pr = 0.025

4.3. BIVARIATE ANALYSIS

Data was analysed using Pearson Chi-square tests to screen variables that are influential on HIV-free survival rates at 36 weeks. Variables that are influential at p-value cut off point 0.1 were considered as significant influential factors and were included in the multivariate analysis. The table below presents variables considered for multivariate analysis with their statistical significance. In addition to statistical significance, variables have been selected based on large relative risk value (even with wide confidence intervals) and having strong evidence in the literature on their clinical/public health importance. For instance, variables such as log viral load and mixed breast feeding were considered for multivariate analysis, even though they were not significant at p-value cut off point 0.1 (note: log viral load was a significant predictor variable of overall HIV-free survival rate in previous studies' bivariate analysis, while the same analysis on postnatal HIV-free survival rate is not significant in this study), because of strong evidence of their importance in the literature. Interpretations of the results are presented within the table.

Table 6: Variables influential on chi-square test

Independent variables[i]	*Chi-square test (p-value)*	*Relative Risk*	*Remarks*
Knowledge and health seeking behaviour			
Knowing all 3 transmission routes	0.0924	0.46(0.18-1.21)	Protective effect of knowing all transmission routes
Didn't discuss with anyone at the clinic on the best way of feeding	0.055	1.53 (1.0-2.35)	Risk
Who would you go to first if you get difficulty with feeding	0.056	1.51 (0.98-2.32)	Risk of not going to health workers first
Maternal factors			
Had weight loss or poor weight gain during pregnancy[ii]	0.05		Significantly influential on HIV transmission/death
Average log viral load	0.5[iii]	Mean difference = -0.058	Viral load is slightly lower in HIV-free and alive infants

		(-0.228-0.111)[iv]	
Socio-economic factors			
Doesn't have piped water inside house	0.096	1.44 (0.93-2.24)	Risk
Uses piped water in public/bore hole/river, rain water tanker/other vs who use piped water in yard or inside house	0.0075	1.68 (1.14 -2.46)	Risk
Don't use piped water	0.0004	2.01 (1.37- 2.955)	Risk
Out of food	0.098	1.448 (0.93- 2.26)	Risk
Mother unemployed	0.0571	0.529 (0.26-1.06)	Protective
Being in Rietvlei	0.0029	1.78(1.2- 2.6)	Risk
Socio-economic scores	0.099[iii]	Mean difference = 0.33 (-0.06 0.73)[iv]	Mean socio-economic scores is greater in HIV-free and alive infants
Psychosocial factors			
Didn't discuss feeding decision with anyone else apart from health staff	0.0027	2.25(1.28-3.93)	Risk
Infant factors			
Not immunized up to 6months	0.0043	1.8(1.2-2.8)	Risk
Changed their feeding pattern due to child illness [ii]	0.05		Significantly influential on HIV transmission/death
Not receiving 9mth measles vaccine	0.039	3.05 (1.0-9.64)	Risk
Premature birth	0.01	1.79 (1.16- 2.77)	Risk
Oxygen treatment given at birth	0.074	1.49 (1.0-2.29)	Risk – proxy for sick baby
Had resp. problem at birth (proxy for sickness of baby at birth)	0.0978	1.53 (0.93-2.5)	Resp problem at birth is risk for HIV/death

Weight at birth	0.065 [iii]	Mean difference 0.177 (0.05 - 0.3) [iv]	HIV-free and alive children have higher birth weight
WAZ score at 24 weeks (measurement of malnutrition)	0.0003 [iii]	Mean difference 0.66 (0. 30-1.02) [iv]	The mean WAZ score of HIV-free & alive children is significantly higher
WAZ score at 36 weeks (measurement of malnutrition)	0.03 [iii]	Mean difference 0.45 (0.04 – 0.9) [iv]	The mean WAZ score of HIV-free & alive children is significantly higher
WHZ score at 24 weeks (measurement of wasting)	0.0026 [iii]	Mean difference 0.71 (0.25- 1.2) [iv]	HIV-free & alive children have significantly higher WHZ score
stunting at 24 weeks	<0.0001	2.21(1.5-3.2)	Risk
stunting at 36 weeks	<0.0001	3.3(2.2-4.9)	Risk
Health system factors			
Counselling score below average	0.048	1.47 (1.003- 2.16)	Poor counselling score is risk
Syphilis test taken	0.01	0.61(0.4-0.9)	Protective
ANC visit <=3	0.07	1.427 0.97-2.1	ANC visit <=3 is risk
<4 postnatal visit	0.127	1.67 (0.87-3.2)	<4 postnatal visit is risk
Feeding patterns			
EFF vs |MFF at 5 weeks	0.06 [ii]		MFF is risk
PBF vs MBF	0.16 [ii]		MBF is risk

[i] Definitions of all variables found in Annex II

[ii] The p-value is from Fisher's Exact Test

[iii] The p-value is from Anova T-test

[iv] note that the values in bracket are lower and upper CI of the mean

4.4. MULTIVARIATE ANALYSIS

4.4.1. FEEDING PATTERNS

The analysis to identify the influence of feeding patterns on HIV-free survival rate is made based on what the child has been fed until 5 and 7 weeks. This time duration was chosen because, after 5 weeks, the number of infants in the EBF and EFF group hugely declines to a level that cannot be used for analysis (only 8 infants were on EBF at 12 weeks). In addition, for the purpose of informing the 6 weeks cross-sectional study questionnaire development, the 5 and 7 weeks feeding pattern data should be indicators to represent early feeding patterns.

Five definitions were used to classify feeding pattern. Exclusive Breast Feeding (EBF) - defined as exclusively breast feeding the baby without giving any liquid or solid except prescribed medicine. Mixed Breast Feeding (MBF) – feeding infant with breast milk in addition to solids and liquids. Predominant Breast Feeding (PBF) - the predominant source of infant feeding was breast milk, but water, glucose, and other prescribed medicines were also given, but no formula milk. Exclusive Formula Feeding (EFF) - exclusively feeding the child with formula with no liquids and breast milk allowed and Mixed Formula Feeding (MFF) - when a child is given solids and liquids with formula, but no breast milk. Based on this, the following sections present comparisons of HIV and/or death status among infants in different feeding groups.

Exclusive Breast Feeding vs. Mixed Breast Feeding

The impact of mixed breastfeeding was compared at 5 and 7 weeks visits. At the 5 weeks visit, the comparison between EBF and MBF had a similar influence on HIV-free survival rate (RR=0.97, CI= 0.37-2.58 & P-value= 0.957). In the 7 weeks feeding pattern data, the crude hazard ratio shows that MBF has a 3.6-fold increased risk of HIV infection and death at a p-value of 0.21. After adjusting for known predictors of HIV-free survival rate (viral load, infant sickness at birth, discussing feeding choice with health professionals, mothers' employment and socio-economic scores), the hazard of MBF only reduces slightly to 3.5 with p-value 0.22. In both models, the reason why statistical significance is not achieved could be due to small sample size, as the confidence interval is wide and the sub-arm sample size shows only 17 infants were in EBF groups. The attempt to investigate the influence of EBF vs MBF at 12 weeks failed due to a huge decline of participants in the EBF group - only 8 participants were on EBF at 12 weeks. Baby sickness was chosen as an adjusting factor to control for reverse causality – i.e. sickness of infant that causes feeding pattern change.

Table 7: Comparison of HIV-free survival rate among EBF vs MBF groups

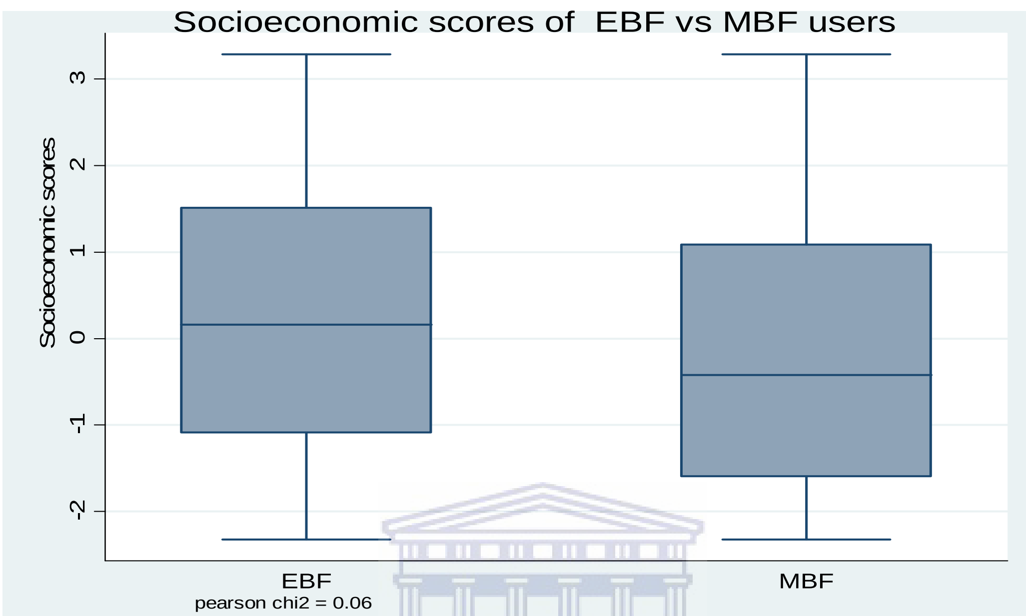
Feeding pattern	**Crude Hazard ratio**	**Adjusted hazard ratio ***
EBF vs MBF until 7weeks	3.6 (CI: 0.4- 27.4) (p-value = 0.25)	3.5 (CI: 0.4- 26.0) (p-value=0.22)

* adjusted for viral load, baby sickness at birth, discussing feeding choice with health professionals, mothers' employment and socio-economic scores

The t-test analysis made between socio-economic score and feeding pattern shows that socio-economic score significantly influences the difference between MBF and EBF. Those who were on MBF at 5 weeks had mean a socio-economic score 2-fold below mothers that

EBF their baby (see Figure 1).

Figure 1: Socioeconomic status of MBF users vs EBF users



In order to investigate the influence of intensity of mixed breastfeeding on HIV transmission and death, the effect of PBF was compared with MBF at 5 weeks. After adjusting for the same factors used to adjust the feeding pattern above, MBF causes a 2-fold increased hazard of infection and/or death compared to predominant breast feeding although, once again, these differences are not statistically significant.

Table 8 : The effect of mixed breast feeding vs PBF (predominant breast feeding)

Feeding pattern	Crude hazard ratio	Adjusted hazard ratio
PBF vs MBF until 5weeks	1.6 (CI: 0.85- 2.84) (p-value = 0.15)	2.00 (CI: 0.86- 4.6) (p-value=0.10)

* adjusted for viral load, baby sickness at birth, discussing feeding choice with health professionals, mothers' employment and socio-economic scores

Exclusive Formula Feeding vs Mixed Formula Feeding

In the univariate analysis result presented below, the crude hazard ratio of mixed formula feeding from birth to 5 weeks has a 5.24-fold increased risk of transmission and mortality compared to exclusive formula feeding with a p-value of 0.104. However, after adjustment for variables correlated with MFF, this association declines to a non-significant 50% increased risk of HIV infection and mortality by mixed formula feeding compared to EFF.

Table 9: The effect of mixed formula feeding at 5 weeks

Feeding pattern	**Crude hazard ratio**	**Adjusted hazard ratio***
MFF vs EFF at 5 weeks	5.24 (CI: 0.7-38.7) (p-value =0.104)	1.5 (CI: 0.19-11.45) (p-value = 0.704)

* adjusted for disclosure of status at baseline, proper cleaning of formula bottles (at least with boiled water) and geographical location, which explains the socio-economic and health service variations between the three sites.

The fact that the crude hazard ratio of MFF significantly reduced after adjustment may indicate that most of the influence of MFF is explainable with the association between the adjusting factors and exclusive formula feeding. Variables used for adjusting the EFF vs MFF model are disclosure of status at baseline, proper cleaning of formula bottles (at least with boiled water) and geographical location, which explains the socio-economic and health service variations between the three sites. Disclosure of status among the MFF group was 32% compared to 62% among EFF mothers (chi2 = 0.009). Practice of proper cleaning of formula bottle (by using at least boiled water) was better among EFF mothers (83%) compared to MFF mothers (66%) (chi2=0.07).

A comparison could not be made between exclusive breast feeding and exclusive formula feeding due to the small sub-arm sample size.

4.4.2. OTHER RISK FACTORS

The analysis to identify other risk factors of HIV-free survival rate has been made at two levels. Firstly, a model was built for all variables (recruitment-36 weeks) that were found influential in the chi-square test analysis. The result from this is presented in the first seven consecutive sections below. Next to this, a baseline model was developed from variables collected at baseline as well as the 3 weeks and 5 weeks visits. The later model is restricted to variables that can be collected before or at 6 weeks from birth for the purpose of informing questionnaire development for the 6 weeks national cross-sectional survey, which aims to identify the baseline risk factors of HIV-free survival rate in each of the provinces in South Africa.

4.4.2.1. HEALTH SYSTEM FACTORS

From the crude hazard ratio of syphilis test in the table below, participants that were not provided with the syphilis test service are associated with a 77% increased risk of HIV infection and/or mortality of infants. This was adjusted for factors that had statistical and biological (intermediate factors and health system indicators are not included) correlation with both syphilis test and HIV-free survival rate. Based on this, after adjusting for 24 weeks' immunization history, disclosure of status, 36 weeks log viral load, mothers' history of illness and socio-economic scores, the adjusted hazard ratio increases to 2.18-fold higher risk of HIV-infection and/or mortality due to a missed opportunity of syphilis test service. A bivariate analysis made between the syphilis test service and some other risk factors indicate that most of those who did not get the syphilis test have a poor 24 weeks infant immunization history and never discussed infant feeding options. Most mothers who were sick or who had a high average viral load were given the syphilis test compared to healthy mothers with a viral load relatively lower than those who got the syphilis test.

Table 10: Health system related predictor factors of HIV-free survival rate

Risk factors	**Crude hazard ratio**	**Adjusted hazard ratio**
Syphilis test service not given (missed opportunity)	1.77 (CI: 1.1-2.9) (p-value=0.020)	[a]2.18 (CI: 1.1 – 4.5) (p-value= 0.033)
Counselling score below average	1.39 (CI: 0.87-2.2) (p-value=0.17)	[b]1.55 (0.91 - 2.6) (p-value=0.087)
Postnatal visit at least 4 times	0.58 (0.29-1.15) (p-value=0.118)	[c]0.52 (0.24-1.1) (p-value=0.085)

[a] adjusted for disclosure of status, log viral load, immunization at 24 weeks, history of mothers' sickness and socio-economic scores

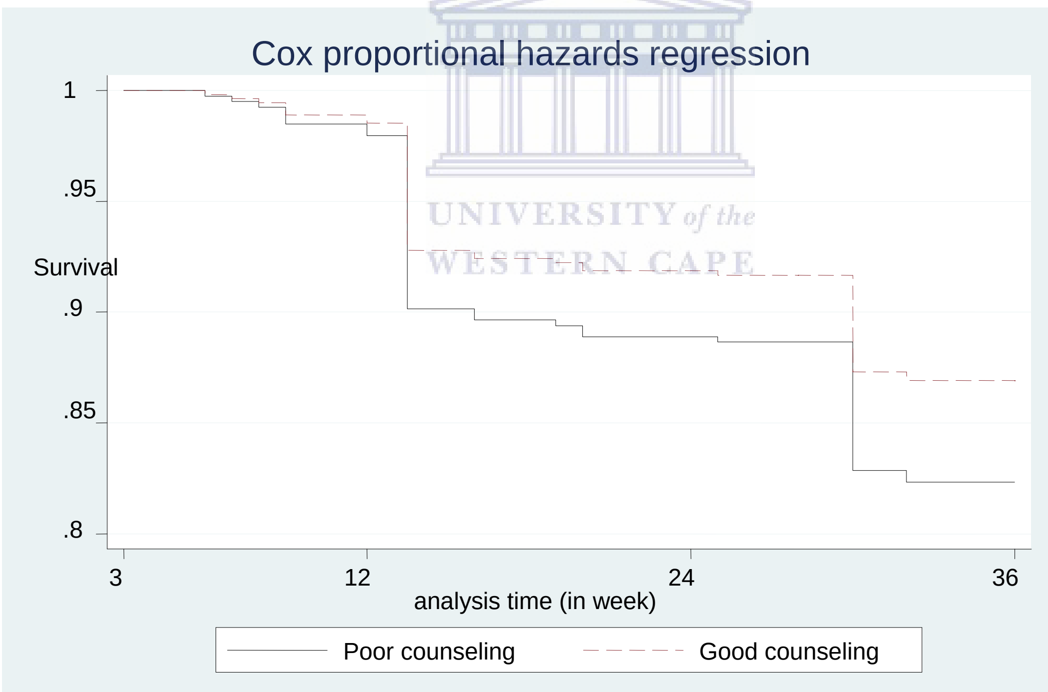
[b] adjusted for disclosure of status, unemployment of mother, child sickness at birth and premature birth

[c] adjusted for disclosure of status, log viral load, child sickness at birth, knowledge of the three transmission routes

Poor counselling service, after adjusting for disclosure of status, mothers' employment status, sick baby at birth and premature birth, show a 55% increased risk of HIV infection and/or mortality among infants whose mothers did not get good quality counselling (see definition of good counselling score in Annex1).

As shown in the survival curve below, mothers that received good counselling (as measured by a counselling score above average of the expected level) had better HIV-free infant survival rates across the study period than mothers who were poorly counselled. Much of the difference appears in the period after 12 weeks.

Figure 2: HIV-free survival of infants by counselling score



In the univariate model, infants who had frequent (>=4) postnatal visits were protected from HIV transmission and mortality by 42%. After adjusting for factors such as child sickness, log viral load at 36 weeks, knowledge of transmission routes, and disclosure of status, the protective effect of postnatal visit increases to 48% (see Table 10).

4.4.2.2. KNOWLEDGE AND HEALTH-SEEKING BEHAVIOUR OF MOTHERS

Health-seeking behaviour was measured using a variable (discussing feeding option with health professionals) that is also partially an indicator for health service performance. Based on the finding, infants of mothers who seek health professionals' explanation for infant feeding options were significantly at lower risk of HIV infection and/or mortality (adj. HR=0.49) than infants whose mothers did not seek professionals' advice for feeding options. Similar to this, knowledge of the three mother-to-child transmission routes of HIV (after adjusting for health system and socio-economic factors) was associated with 57% lower transmission and/or mortality.

Table 11: Mothers' knowledge and health-seeking behaviour

Risk factors	Crude hazard	Adjusted hazard
Seeking professional explanation for options of feeding	0.55 (CI: 0.32 -0.94) (p-value=0.029)	[a] 0.49 (CI: 0.27-0.9) (p-value=0.024)
Not knowing transmission routes	0.50 (CI: 0.18- 1.39) (p-value=0.19)	[b] 0.44 (CI: 0.14-1.41) (p-value=0.16)

[a] adjusted for premature birth, infant sickness at birth, immunization at 24weeks, average log viral load, no hunger, mother, and main income provider of the family

[b] adjusted for average log viral load, history of syphilis test, mother main income provider of the family

4.4.2.3. INFANT RELATED FACTORS

Malnourished children at the 36 weeks and 24 weeks visist had a significantly higher risk of HIV infection and/or mortality. In order to avoid reverse causality between HIV infection and malnutrition, the multivariate analysis was restricted on HIV-negative infants at 24 weeks. The adjusted hazard ratio result shows that malnourished infants have a 3.8-fold increased risk of contracting HIV and/or mortality compared to normal weight infants at the 24 weeks visit. Child sickness that caused feeding change was associated with an 8.8-fold increase of HIV infection and mortality.

Table 12: Infant related predicting factors of HIV-free survival rate

Infant related factors	**Crude hazard ratio**	**Adjusted hazard ratio**
Malnutrition at 36 weeks	7.48 (CI: 4.6-12.1) (p-value=0.000)	[a]6.36 (CI:0.94- 83.3) (p-value=0.057)
Malnutrition at 24 weeks	5.0 (CI: 2.9- 8.5) (p-value=0.000)	[a]3.8 (CI: 2.1-6.8) (p-value=0.000)
Child illness during 24-36 weeks that caused feeding change	3.142 (CI:1.2-9.57) (p-value=0.046)	[b]8.8 (CI: 2.0 -37.1) (p-value= 0.003)
Received 9 month measles immunization?	0.33 (CI: 0.10 -1.0) (p-value=0.06)	[c]0.26 (CI: 0.06-1.09) (p-value=0.066)
Premature birth	1.76 (CI: 1.0- 3.08) (p-value=0.045)	[d]1.84 (CI: 0.96-3.53) (p-value=0.07)
Low birth weight (<2.5kg)	1.33 (CI: 0.7-2.5) (p-value = 0.2)	[d]1.52 (CI: 0.8 – 2.85) (p-value = 0.19)

[a] Adjusted for average log viral load, seeking professional help during difficulty with feeding, disclosure

[b] Adjusted for average log viral load, seeking professional help during difficulty with feeding, knowledge of all three transmission routes, postnatal visit

[c] Adjusted for average log viral load, seeking professional help during difficulty with feeding, disclosure, discussing with professionals on feeding options (deaths occurring before 9 month immunization visit excluded)

[d] Adjusted for average log viral load, disclosure, discussing with professionals on feeding options, unemployment of mother, syphilis test service, child sickness at birth

Immunization for measles was associated with an increased HIV-free survival rate. Infants who completed the 9-month measles immunization had a 74% decreased risk compared to those who did not receive the measles immunization. Premature birth was associated with an 84% increased risk of HIV infection and/or mortality. Low birth weight infants had a 52% increased risk of infection and/or mortality than normal weight infants.

4.4.2.4. MATERNAL FACTORS

After adjusting for feeding pattern, a 10 unit increase in viral load was associated with a 2.19-fold increased risk of HIV infection and/or mortality. Mothers who had poor weight gain (or weight loss) during pregnancy were associated with a 2.51-fold increased risk of infant infection and/or mortality after adjusting for different socio-economic, health system and infant related factors.

Table 13: Maternal related predicting factors of HIV-free survival rate

Maternal factors	Crude hazard ratio	Adjusted hazard ratio
Weight loss or poor weight gain	2.67 (CI: 1.07- 6.6) (p-value=0.034)	[a]2.51(CI: 0.98- 6.42) (p-value=0.055)
Log viral load (average of 3 and 36 weeks viral load)	1.08 (CI: 0.76 -1.6) (p-value=0.646)	[b]2.19 (CI: 1.19- 4.0) (p-value=0.012)

[a] adjusted for counselling score, discussing feeding option, counselling score and socio-economic scores

[b] adjusted for mixed breast feeding

4.4.2.5. SOCIOECONOMIC AND PSYCHO-SOCIAL FACTORS

Disclosure of status is associated with a 56% low risk of HIV infection and/or mortality. From the crude hazard, infants from unemployed mothers have 46% low risk of infection and/or death. This could be due to the advantage that unemployed mothers have to exclusively breast feed their child compared to working mothers. In order to prove this, adjustment was made for feeding pattern. Once adjusted for the positive contribution unemployment could give to feeding pattern, unemployment becomes an economic indicator causing a 78% increased risk of infection and/or death among unemployed mothers. Families who experienced food insecurity (ran out of food anytime in the last 12 months) had a significantly higher risk (adj HR= 1.71) of infant HIV infection and death.

Table 14: Socio-economic and psycho-social predictors of HIV-free survival

Socioeconomic and psycho-social indicators	Crude hazard ratio	Adjusted hazard ratio
Disclosure	0.40 (CI: 0.21 -0 .80) (p-value=0.009)	[a]0.49 (CI: 0.21-0.9) (p-value=0.025)
Food insecure (ran out of food anytime in the last 12 months)	1.5 (CI: 0.9- 1.41) (p-value=0.1)	[b]1.71 (CI: 1.01- 2.89) (p-value=0.046)
Mother unemployed	0.54 (CI: 0.25 -1.19) (p-value=0.124)	[c] 1.78 (CI: 0.68-4.68) (p-value=0.23)

[a] adjusted for premature birth, counselling score, mother unemployment, child illness at birth, average log viral load; [b] adjusted for average log viral load, child illness at birth and discussing feeding option with professionals; [c] adjusted for mixed breast feeding

4.4.3. BASELINE RISK FACTORS

A model was fitted with baseline risk factors (risk factors which can be collected up to 6 weeks from birth) to inform the questionnaire development of the cross-sectional survey. The result from this analysis is presented in the tables below. In order to adjust for the effect of feeding patterns on the baseline indicators, two models were fitted: one with mixed breast feeding (Table 15) and the other with mixed formula feeding (Table 16). Building two models was essentially done to avoid multi-colinearity effect if both mixed breast feeding and mixed formula feeding variables were used as adjusting factors in the same model. For the purpose of the project (development of mathematical model), we have considered parameters that are significant at a cut-off point 0.20 as important predictor variables of HIV-free survival rate. Both models are selected after comparing their likelihood ratio test with other models fitted with baseline indicators and these two models were the best fit models.

Table 15: Model 1 - Baseline predictor factors that can be collected up to 6 weeks visit

Risk factors	Adjusted hazard ratio	P-value	Conf. Interval
Counselling score	2.1	0.08	0.91-4.8
Weight loss or poor weight gain during pregnancy	2.38	0.185	0.67-8.57
Sickness of child at birth	2.73	0.027	1.12- 6.68
Log viral load at 3 weeks	1.9	0.027	1.06-3.3

Most of these variables, except the variable being male, which is associated with a 2.14-fold increased risk of infection and/or mortality, the rest of the variables were also found influential in the previous models developed for risk factors from recruitment to 36 weeks. In addition, the model above shows that viral load at 3 weeks can be used as an alternative to average viral load (3 and 36 weeks).

Table 16: Model 2- Baseline predictor factors that can be collected up to 6 weeks visit

Risk factors	**Adjusted hazard ratio**	**P-value**	**Conf. Interval**
Male	2.14	0.113	0.84- 5.49
Seeking professional explanation for options of feeding	0.45	0.087	0.18-1.1
Disclosure	0.17	0.020	0.23-1.2
Child been sick at birth	2.19	0.157	0.73-6.53

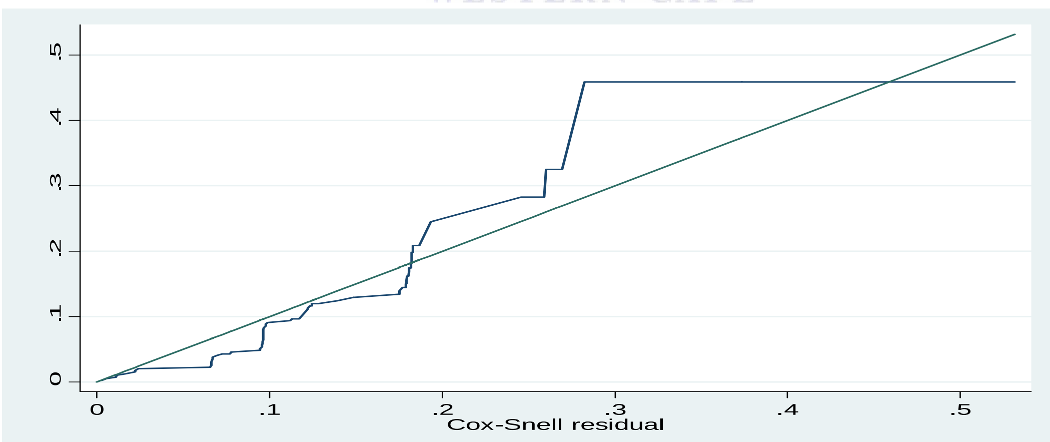
4.5. MODEL CHECKING

4.5.1. OVERALL MODEL FIT

All models were checked for overall model adequacy, using the Cox-Snell residual test. Under this section, two examples of the procedure, done to test overall model fit, are presented.

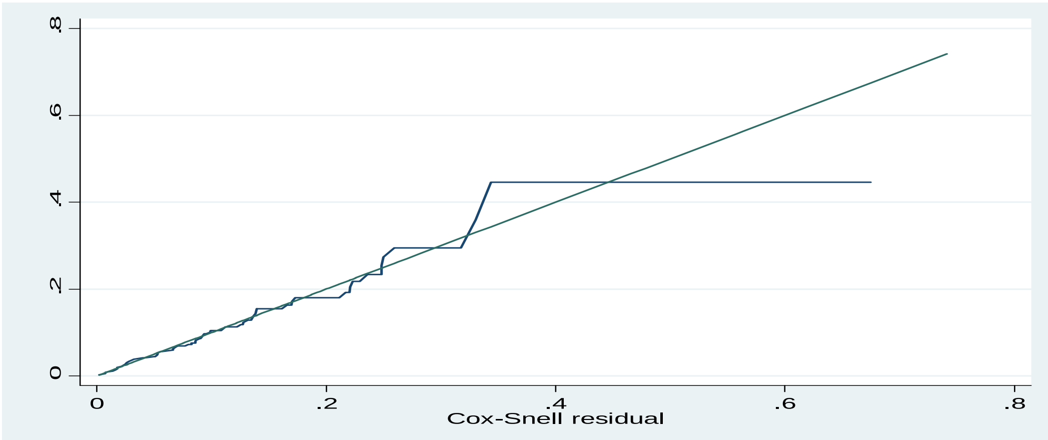
The graph below shows the Cox-Snell residual test made to check the overall model fit of baseline model 1. Based on this, the graph shows that the cumulative hazard function versus the Cox-Snell residuals plot is fairly close to a straight line through the origin with a unit slope. This suggests that the model fitted to the data is satisfactory.

Figure 3: Cox-Snell residual test for baseline model 1



Same as for the above, in the graph below, the Cox-Snell residual test made for baseline model 2 shows that the line does not deviate much from the reference line. Therefore, the second baseline model fitted on baseline risk factors is satisfactory.

Figure 4: Cox-Snell residual test for baseline model 2

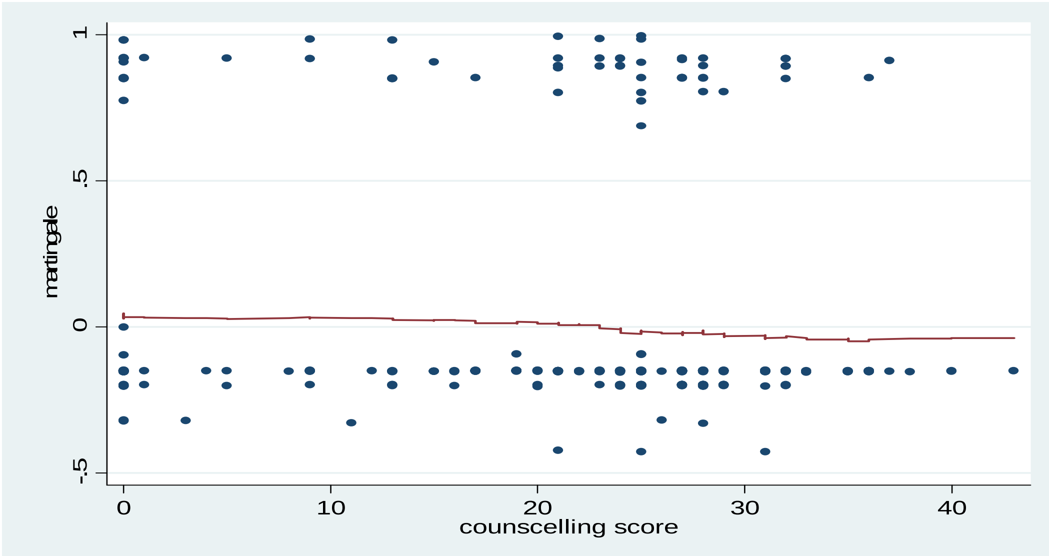


4.5.2. MARTINGALE RESIDUAL TEST TO CHECK THE FORM OF VARIABLES

The Martingale residual test was used to check the form of the continuous variables used in the model. The following are two examples of the Martingale test result which shows the variables are in their best functional form. The same procedure has been done to all continuous variables in the model and found a non-horizontal slope, showing that the variables can be used in their current form.

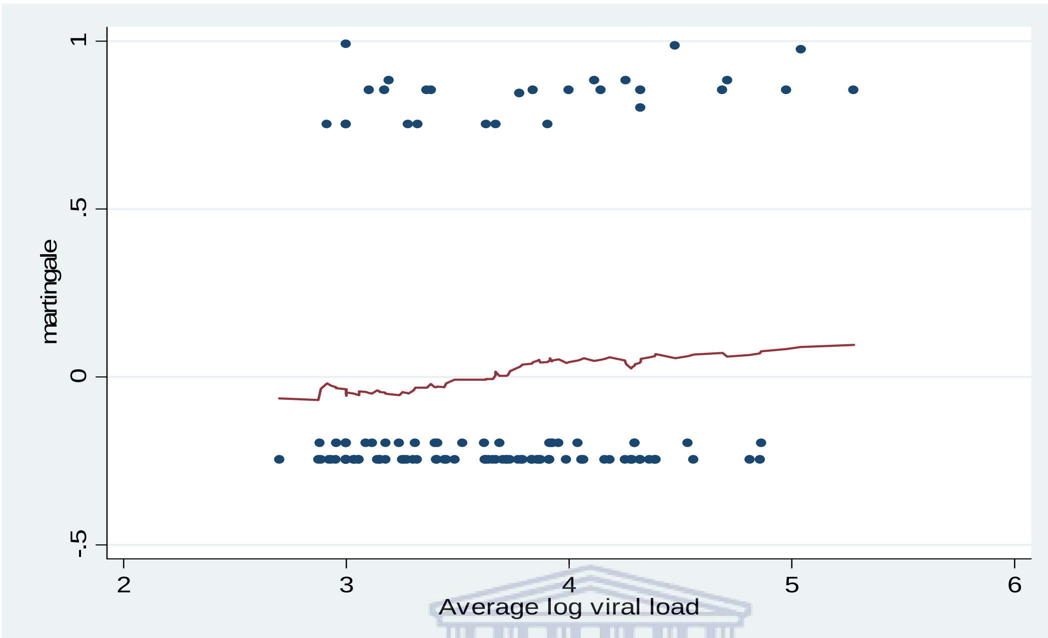
In the graph below, the smoothening curve for the Martingale residual versus counselling score is roughly linear and it is not horizontal. Therefore, the covariate counselling score can be included in the model in its current form.

Figure 5: Martingale residual test for counselling score



In the graph below, the smoothening curve for the Martingale residual versus average log viral load is roughly linear. Therefore,, the covariate average log viral load can be included in the model in its current form.

Figure 6: Martingale residual test for average log viral load



4.5.3. TESTING THE PROPORTIONAL HAZARD ASSUMPTION

Each of the models developed was tested for the proportional hazard assumption using the Schonfeld residuals test. One example from the baseline model is presented below. In this procedure, we have tested for non-zero slope in a regression of Schonfeld residuals on functions of time. We did so, because the test of zero slope is the same as testing that the log-hazard ratio function is constant over time. A chi-square probability value of less than 0.05 shows the null hypothesis that the slope is zero is rejected, which subsequently results in accepting the alternative hypothesis that the proportional hazard assumption is not met. While, with a p-value above 0.05, the null hypothesis that log-hazard ratio function is constant over time could not be rejected. Therefore, the proportional hazard assumption is not violated.

Based on this, the table below shows all of the variables in baseline model 1 have chi-square probability above 0.05. Therefore, the null hypothesis that the slope is zero (equivalently, the log-hazard ratio function is constant over time) could not be rejected. Therefore, none of the variables tested below did violate the assumption of proportionality of hazard ratio over time.

Table 17: Test of proportional-hazards assumption of baseline model 1

Variables	Chi2	df	Prob>chi2
Counselling score	0.30	1	0.58
Log viral load at 3 wks	1.81	1	0.16
Child been seek at birth	1.34	1	0.25
Poor weight gain (weight loss) during pregnancy	0.07	1	0.79
Global test	4.99	4	0.28

4.6 CONCLUSION

This chapter presented the results of this analysis. The next chapter discusses the implications of these findings and recommendations for programmes and future research.

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

This study has shown that HIV-free survival rate is influenced by multiple interrelated co-factors. These factors include both immediate and broader risk factors of HIV-free survival rate such as knowledge, health system factors, socio-economic factors, psycho-social factors, feeding patterns, and maternal and infant factors. The following section elaborates the findings of the study by means of a comparison with current research.

5.1. FEEDING PATTERNS

This study shows that early mixed feeding is a common practice in South Africa. Overall, 83% (as high as 90.26% in Rietvlei) of mothers were either mixed breast feeding or mixed formula feeding before the infant was at age 5 weeks. After excluding predominant breastfeeding from mixed breast feeders and allowing some water for formula feeding mothers, still more than half of infants were on mixed feeding. This finding is consistent with the findings of McCoy *et al*, which showed that more than three quarters of mothers in South Africa start mixed feeding before 10 weeks of age[21]. In the literature, major factors that are responsible for early introduction of mixed feeding include lack of knowledge, cultural and social influences (such as the practice of pre-lacteal feeds), low educational status of parents, unsuitable work circumstances, and poor provision of proper counselling and support services[6].

On the other hand, even though this study has a small number of infants in the EBF and EFF group, causing loss of statistical power to make comparisons of feeding patterns across different time durations, MBF at 7 weeks was found to have a 3.5-fold increased risk of transmission and/or mortality as compared to EBF, although the confidence intervals were wide. In addition, at a significance level of 0.1, PBF had a two-fold lower hazard of transmission and mortality compared to MBF. Even though these comparisons were not significant at 5% level of significance, the hazard ratio of MBF in our study is similar to the ZIVITMBO study, which reports a three-fold increased risk of postnatal transmission and/or death at 6 months by using early MBF[34]. Other research results on the hazards of early mixed breast feeding ranges from 4-fold to 11-fold depending on the outcome variable used (post-natal transmission only or HIV-free survival) and the time duration chosen[33, 36]. For instance, Coovadia et.al report an 11 times higher risk of postnatal transmission at 6 months by mixed breastfeeding for the first four months of an infant's life[22].

On the other hand, in the univariate Cox analysis, our study reports a 5.24-fold increased risk of transmission and/or mortality by mixed formula feeding compared to exclusive formula feeding in the first 5 weeks. However, such association disappears after adjusting for factors such as disclosure, and proper cleaning of bottles and sites (explains socio-economic and health service variation). The lack of association after adjustment might be due to the reason that the adjusting factors were stronger explanatory factors of HIV-free survival than MFF. On the other hand, this might also indicate that the impact of mixed formula feeding may be mediated by circumstances such as non-disclosure, poor socio-economic status and health service variations, which lead to the practice of mixed formula feeding ultimately resulting in increased risk of morbidity and mortality.

In general, our definition for exclusive breastfeeding and exclusive formula feeding was strict (allowing only for the use of prescribed medicine) considering that there should be no exposure to any liquid or solid food at early (5 and 7 weeks) weeks of an infant's life. Although we did this, we also have compared the HIV-free survival rate in the predominantly breast feeding group and the result shows that significant risk (at 10% significance level) is associated with mixed breast feeding as compared to predominant breast feeding. This leads to a similar conclusion made by other studies which found that, when compared to predominant breast feeding, mixed breast feeding has a higher transmission and mortality rate [33, 34].

In the data, it is observed that 9 of the mothers formula fed their babies in hospital and switched to exclusive breast feeding after they reached home, and 8 others exclusively breast fed at hospital and exclusively formula fed when they reached home. This has been one factor that made the feeding classification very difficult as all of these mothers were classified as mixed breast feeding even though the only lapse they had was during the hospital feeding. Counselling services that are tailored to mothers' circumstances would have helped these mothers to make decisions that they could continue to practice at home. Studies repeatedly show the existence of constant tension between the social risk and medical risk of not breast feeding and that health professionals should equally understand these factors in order to help mothers in making informed decisions[64, 65].

This study is also limited by the fact that we have a small sample size in the exclusive breast feeding group. As a result, the risk of HIV transmission and/or mortality could not be calculated by looking at the duration of the exclusive breast feeding and weaning period.

Available studies are in consensus with Miotti and his colleagues that the longer the duration of breastfeeding, the greater the risk of transmission [41, 66, 67]. The risk of transmission per day (24 hours) as a result of exposure to breast milk was reported by Richardson et al as 0.00028[68]. In general, while it is recognised that prolonged breast feeding increases the cumulative risk of transmission, in some circumstances (such as households who do not have adequate food), the WHO updated guideline recommends mixed breast feeding after six months as the risk of transmission may be less than the risk of severe malnutrition from stopping breast feeding completely[24].

Available research does not give much concrete evidence on the risk of transmission and/or mortality during weaning. A recent preliminary finding from the Kesho Bora study, however, suggests the risk during weaning might be higher as 4 out of 10 of the infants in their study died within three months of weaning[38]. Further studies are necessary to verify whether mixed breast feeding during breast feeding cessation (weaning) holds a much higher risk or not.

5.2. KNOWLEDGE / HEALTH SEEKING BEHAVIOUR AND HEALTH SYSTEM FACTORS

There is a strong relationship between health system factors and HIV-free survival. In our study, poor counselling (below the average expected level) had an associated 55% increased risk of transmission and/or mortality after adjustment for confounding factors (see annex 1 for definition of poor counselling). Indeed, studies in South Africa indicate that poor counselling and lack of close support by health facilities are major factors contributing to inappropriate feeding choice and non-compliance with exclusive feeding[53,64].

A qualitative study conducted in conjunction with this study found that, in spite of the high level of VCT and PMTCT uptake in South Africa, mothers are not given adequate information on essential conditions for safe formula feeding and appropriate use of EBF[64]. It could be due to this reason that, in this study, half (50.82%) of the mothers chose formula feeding, even though it was not recommended in their circumstances to use formula feeding. As a consequence of this, 87.10% of the participants who inappropriately chose formula feeding were found to be mixed feeding by 5 weeks, and 35.71% of them had an under-weight child at the 24 weeks visit. With the known morbidity and mortality risk attached to the use of formula feeding, counselling services should ensure that mothers have all essential conditions in place before choosing alternative feeding methods[69]. In this study, even though they are not many, mothers who received appropriate feeding option advice from health professionals and those who know all the transmission routes of HIV were at a significantly reduced risk of transmission and mortality suggesting a protective effect of knowledge and good quality counselling.

Other than counselling services, postnatal visits and syphilis test services were strong predictors of HIV-free survival. The study shows that not receiving syphilis testing has more than two-fold increased risk of HIV transmission and mortality. In order to confirm that syphilis testing can be a proxy for health system performance, mothers who were not given a syphilis test were checked as to whether they had ever discussed feeding options and whether their baby received immunizations at 24 weeks. Both analyses showed that most of the mothers who did not get a syphilis test also did not receive a 24-week immunization and proper feeding advice. In this regard, although poor health-seeking behaviour of participants could contribute to low utilization of such services, not all can be blamed on mothers, especially in this particular study where mothers have been at ANC/PMTCT clinics at least once. Such opportunities could be used to give all the necessary services and to encourage mothers for on-going counselling and support.

Another variable that potentially represents both health-seeking behaviour and health service provision, a variable that indicates the mother has discussed with health professionals on feeding options, was associated with a 61% reduction in transmission and mortality. This shows that efforts of mothers to seek professionals' advice have as important a contribution as health service quality in increasing HIV-free survival rate.

5.3. DISCLOSURE OF STATUS

Disclosure of status was another important predictor of HIV-free survival rate. A substantial proportion (70.61%) of women in our study did not disclose their status to anyone due to fear of stigma, discrimination and violence. Failure to disclose was associated with a 44% increased risk of transmission and mortality. By contrast, continued utilization of PMTCT services were observed among mothers who disclosed their status. A study in Botswana shows that women often choose to avoid the potential disclosure of their status by feeding according to traditional practices [70]. In our study, this may have been the reason for the majority of mothers' decisions to switch to mixed breast feeding after exclusively breast feeding in hospital. 78.40% of mothers who did not disclose their status switched to MBF when they returned home after exclusively breast feeding their babies in hospital. By contrast, more than three quarters of mothers who disclosed were able to discuss feeding options with professionals and also had properly followed immunization visits up to 24 weeks. The literature indicates that failure to disclose is one major reason why drop out rates from an on-going counselling and support programme is high, ultimately resulting in an inability to comply with feeding choices [71].

In general, in order to improve the adherence of women to an appropriate feeding choice, psycho-social counselling and support should be provided during the key moments of disclosure of HIV status. These key time periods could be during and at the end of pregnancy, in the first couple of weeks after birth, and during weaning. In some areas, initiatives such as continued integration of partners into PMTCT programmes show encouraging results in making disclosure easier for the women, ultimately contributing to compliance to appropriate feeding patterns and utilization of health services[70, 73, 74].

5.4. SOCIOECONOMIC FACTORS

In many sub-Saharan African countries, including South Africa, extreme poverty and unemployment often leave mothers with only limited options. In this regard, although factors such as knowledge, proper counselling, disclosure of status and health-seeking behaviour contribute to improving informed decisions, this may sometimes not help much if the socio-economic status of mothers is so bad that it prevents them from practicing their choice of feeding option. In our study, 50.96% of the participants had a history of running out of food in the past one year. Among these households, only 50.88% were receiving a grant/pension from the government. The remaining 49.12% (24% of the total sample), who had a shortage of food, did not have any grant supplements.

In relation to this, the study shows a history of lack of food in the past one year is associated with a 70% increased risk of HIV transmission and/or mortality of infants. In very low socio-economic circumstances, the likelihood of resources not being enough to adequately feed both the infant and the mother is a common phenomenon. As a result, this exposes children to risks of malnutrition and diarrhoeal diseases. Statistics South Africa indicates that, in areas like the Eastern Cape, 60-80% of the household still live below the R800 per month income line[72]. In such households, the use of formula feeding and/or early cessation of breast feeding are not appropriate choices. Despite this fact, our study shows that 49.6% of households that had a shortage of food were formula feeding their baby even though this was not a sustainable choice. This could have been avoided with proper counselling and continuous support by health professionals.

In addition, a big question to the government is the 24% of mothers that experience a shortage of food but have not had any kind of support from the government. These households face more of a food shortage problem when the child reaches the age of 6 months, requiring additional complementary feeding. It is due to this realisation that the WHO updated guideline recommends prolonged breast feeding, as the benefit may outweigh its risk in households with food shortages[24]. On the other hand, government has to give attention to such mothers so they can get the necessary help (grants) without complicated procedures. In addition, long-term plans should be designed for poverty alleviation and unemployment reduction among the majority in the black community of

South Africa[75].

On the other hand, our study has showed socio-economic score was a significant explanatory variable for the difference observed between exclusive breast feeding and mixed breast feeding, showing that MBF mothers were in a two-fold lower socio-economic status compared to EBF mothers. This confirms the general public health thought that lifestyle characteristics and living circumstances hugely affect healthy practices. Indeed, well-educated and resourceful households can be more cognisant and protective of infant health than households where living circumstances and education levels are bad.

In this study, when compared to working mothers, unemployed mothers had a better opportunity to EBF, resulting in better HIV-free survival rates among unemployed mothers who EBF their children. 42.86% of unemployed mothers were EBF at the 5 weeks visit compared to only 22.32% of the rest of the participants. However, among unemployed mothers who use MBF, unemployment was a risk with 78% increased transmission and mortality. This might be due to the additional economic burden that compromises child survival if the mother is both unemployed and mixed breast feeding. This result is consistent with the Rollins et al result, which found a 90% increased risk of HIV transmission in infants borne by unemployed mothers[37]. Employed mothers can benefit from relatively better access of resources and information. Other studies also associate maternal employment with maternal health, increased access to resources and maternal autonomy[37].

5.5. INFANT AND MATERNAL FACTORS

In our study, high viral load in the mother and poor weight gain during pregnancy were significantly associated with HIV transmission and mortality. Other studies in South Africa and elsewhere support the fact that maternal factors such as high viral load can increase the chance of transmission of HIV by increasing the level of viral load in breast milk[24,39, 43]. In addition, among mothers with a high viral load, frequent illness, admission to hospital and increased probability of maternal death usually result in neglect of the child and switching to mixed feeding [24,39, 43].

Poor maternal weight gain during pregnancy had a 2.51-fold increased risk of HIV transmission and/or mortality in our study. Even though the influence of such indicators has not been shown in the literature, other indicators of maternal health such as viral load are reported as high risk factors of HIV-free survival rate[43, 39]. Our study shows that poor maternal weight gain during pregnancy might be a proxy indicator for viral load and maternal health status. In the study, mothers with poor weight gain had a significantly higher average viral load compared to mothers with normal weight gain (ttest: mean diff= 0.56; p-value=0.0009). Also, poor weight gain during pregnancy can result in a low birth-weight infant, which consequently exposes children to an increased risk of HIV transmission and/or mortality.

While most other studies report breast inflammation as a risk for increased chance of transmission, in our study such association was not significant due to a lack of use of clinical investigations to detect sub-clinical mastitis[39, 40]. Our study simply used mothers' reports of signs and symptoms of breast inflammation. Therefore, a possible underdiagnosis of sub-clinical mastitis might result in under-estimation of the effect of breast inflammation. In addition, there were only a few reports of breast inflammation development during the study period which caused a lack of statistical power to detect any association that may exist between breast inflammation and transmission.

Premature birth and low birth-weight were found as significant influential factors of HIV transmission and mortality. Low birth-weight infants had a 52% increased risk of transmission and/or mortality. This finding was closer to the findings in Rollins et al who reported a 50% increased risk of transmission and/or death among low birth-weight infants[37]. Infant weight at 24 weeks was also found to have a 3.8-fold increased risk for contracting HIV and/or mortality after excluding premature birth and reverse causality effects of HIV-infection. Analyses of maternal and infant factors suggest interventions to reduce postnatal transmission and mortality should be started in early pregnancy in order to ensure proper child growth and maternal weight increase.

5.6. BASELINE RISK FACTORS

The PMTCT mathematical model, that is envisaged to be developed as the long-term objective of the project, plans to use parameters from this mini-thesis and the cross-sectional survey that will be collected from each province of South Africa at the 6 weeks immunization visit. In developing the PMTCT model, baseline risk factors that are found influential in both the baseline models and recruitment to 36 weeks models will be used as possible parameters.

For instance, among infant factors, being male and sickness of child at birth are found to be influential risk factors of HIV-free survival rate in the baseline model. Sickness of child at birth was also influential in the model fitted for recruitment to 36 weeks visit variables. The fact that this variable remained significant after adjusting for both baseline and long-term indicators suggests that it can be a good predictor of HIV-free survival rate that data can be collected at the 6 weeks immunization visit.

Similar to this, the result from the two baseline models indicate that weight loss /poor weight gain during pregnancy and log viral load at 3 weeks, taken from the maternal factors investigated in this study, have a significant influence on HIV-free survival rate after adjustment for baseline risk factors. The same variables were found significantly influential after adjusting for variables collected from recruitment to 36 weeks. This suggests that viral load at 3 weeks can be used as an alternative measure of average viral load between 3 weeks and 36 weeks. Likewise, as weight loss /poor weight gain of mother (during pregnancy) is found influential in both the baseline risk factors and recruitment to 36 weeks model, it can be used as an indicator of maternal health status. In our study, poor weight gain/weight loss during pregnancy also had a close correlation with mothers' average viral load (t-test result showing mean log viral load difference that is higher by 0.56 among mothers who had poor weight gain/weight loss during pregnancy; p-value=0.0009), and may be a useful proxy viral load in situations where viral load data is not obtainable.

In addition, the literature shows that CD4 count can be a proxy indicator of viral load/maternal health status[39]. The advantage of using CD4 count data is its availability from routine data, while collecting viral load data needs additional costing for blood sample collection and testing. However, although CD4 count is more easily accessible and less costly, maternal viral load is a more powerful indicator in the analysis of HIV transmission through breast feeding, as the viral load level in breast milk is directly related to plasma viral load level[40].

In both the baseline and recruitment to 36 weeks models, disclosure of status is found a significant influential factor representing the influence of psycho-social factors. Therefore, this variable can be used as a parameter for model development. In order to do so, data on disclosure of status should be collected at the 6 weeks immunization visit. The same will be true for the variable discussion of option of feeding with health professionals, which was a proxy indicator of both health-seeking behaviour and health service quality and an independent influential factor of HIV-free survival rate after adjusting for both baseline and long-term (recruitment-36 weeks) risk factors.

Among health system factors, counselling score is found to be a significant health system factor in both the baseline and recruitment-to-36 weeks model. Therefore, data should be collected on counselling score at the 6 weeks immunization visit cross-sectionally so that we can have parameter values to develop the PMTCT model that predicts the HIV-free survival rate.

In the baseline model, none of the socio-economic indicators were found to be significant. However, this could be due to the reason that HIV-free survival rate is explained more by other intermediate factors that were significant in this study. These intermediate variables are knowledge, health-seeking behaviour, getting feeding option advice and maternal and infant factors. However, most of these factors can be a direct effect of, or contributed by, the socio-economic variables. Therefore, knowing that socio-economic risk factors are the core problems of infant mortality and HIV transmission, data collection at the 6 weeks immunization visit should include socio-economic indicators such as water, electricity, and other similar indicators of socio-economic status.

In addition to the above listed baseline risk factors, other risk factor data that was not collected (or that were not statistically significant predictors) in this study, but which have strong evidence in the literature, will be identified and included in the cross-sectional data collection.

In the long-term plan of the study, the availability of parameter values from this analysis, the cross-sectional data as well as the literature will enable us to develop a PMTCT mathematical model that estimates the postnatal HIV-free survival rate of infants in each province of South Africa accounting for the significant local influential risk factors measured at 6 weeks.

However, caution has to be taken in the next step of fitting the mathematical model using parameters found influential from this study, as the effect of these parameters could be overlapping and interlinked. Most of the parameters in this study have a hierarchical cause-effect relationship to each other. Even though parameters were adjusted for confounding and contributing effects during the statistical analysis phase, the kind of strong link that exists between parameters could make it very difficult to single out the independent contribution of each risk factor to HIV-free survival rate, while using all the parameters in their current form to develop the PMTCT model could result in overlapping of effects and over-fitting of a model. Therefore, in order to properly address this problem, the subsequent step of the project (after this mini-thesis) should probe an approach that has to be followed in order to select parameters that are key predictors of HIV-free survival rate.

5.6. CONCLUSION

In general, this study has given us an idea that postnatal HIV-free survival is determined by the inter-related effect of multi-level co-factors such as feeding patterns, infant and maternal factors, health system factors, socio-economic factors and psycho-social factors. Although our sample size limited us from engaging with measurement of the effect of different types and durations of feeding patterns, our study has shown that, at five weeks, the majority of mothers had already started mixed feeding and this resulted in an increased risk of transmission and mortality. Poor counselling and health service provision have been shown as a major influential factor in feeding choices that are risky and inappropriate. The majority of socio-economically poor mothers have been shown to engage in inappropriate feeding choice and mixed feeding. In this regard, a lot has to be done at health facility level to increase the quality of counselling and support services with the aim to ensure informed choice and compliance to exclusive feeding in the early months of an infant's life. In this study, disclosure of status has been found to be one of the important protective elements of HIV transmission and mortality. Therefore, disclosure of status (at least to a partner) has to be promoted by designing interventions that prevent discrimination and stigma.

On the other hand, the prevention of mother-to-child-transmission and/or mortality among children needs the attention of sectors other than the health system. This includes government in ensuring adequate support (grants) to HIV-positive mothers, to help raise their babies with adequate and nutritious complementary feeding, especially after the sixth month of infant age. In addition, long-term plans should be designed to alleviate the problem of poverty and unemployment, which are the core problems driving the HIV transmission and mortality among children.

This study is different from other previous analyses made using Good Start data in that we have analysed multi-level factors resulting in a comprehensive list of risk factors of postnatal HIV-free survival. None of the previous Good Start analyses focused on postnatal HIV-free survival or looked at the risk factors of HIV-free survival rate in a broader way, as examined in this analysis. Knowledge and health- seeking behaviour and anthropometric measurements at 3, 24 and 36 weeks are risk factors that have never been analysed before in any of the Good Start analyses.

In the literature, there are several studies that analyse the effect of feeding pattern and maternal and child indicators of HIV-free survival rate. Also, few studies elaborate on the effects of broader risk factors such as counselling, disclosure and socio-economic status using a qualitative approach. However, our study could be the first quantitative study to measure the magnitude of the independent influence of broader risk factors such as knowledge and health-seeking behaviour, anthropometric measurement, disclosure of status, socio-economic status and health system factors.

An additional contribution of this analysis is that the baseline risk factors from this analysis potentially could be used for developing a risk assessment tool at the 6 weeks immunization visit. These baseline risk factors are important predictor factors of HIV-free survival rate. In addition to this, these same risk factors can easily be collected during the immunization visit at 6 weeks, without any additional cost, as part of the routine programme. Therefore, these baseline risk factors could be examined in future research for utility as a good indicator for development of a risk assessment tool at the 6 weeks immunization visit to identify high risk infants born from HIV-positive mothers.

5.7. RECOMMENDATIONS

- Disclosure of status should be promoted using initiatives such as continued integration of partners into PMTCT programmes in order to increase compliance with appropriate feeding patterns and utilization of health services (pilot programmes in Lusaka, Zambia and other places[73,74] are successful examples that can be adopted).
- Urgent action has to be taken to improve the quality of counselling and health services given in health facilities (the WHO infant feeding counselling training guideline can be used to train health professionals[24]).
- Counselling services should give strong emphasis to socio-economic circumstances and other essential conditions that should be in place for mothers to make decisions to formula feed (essential conditions for formula feeding are listed in Doherty, 2007[72]).
- Interventions to reduce postnatal transmission and mortality should be started from the early pregnancy period in order to reduce transmission and mortality as a result of premature/low birth-weight and poor maternal weight gain.
- Government should prevent food insecurity problems among HIV-positive mothers by providing grants to HIV-positive mothers and designing long-term plans for poverty alleviation and unemployment reduction programmes [75].
- Further research should assess the impact of timing the start of mixed breastfeeding and the impact of cessation of breastfeeding at 6 months in the South African context using a larger sample size and longer follow-up period.

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ANNEX I- Brief explanation and definition on Counselling score:

For *HIV-positive women:* was ever discussed antenatally? (+4 if yes, -4 if no and 0 if don't know), number of times discussed (0 -none, 1 - once only, 2 – twice, 3 -3 times and 4 if >3 times) and whether the following topics were mentioned: risks of MTCT and breastfeeding (+4 if yes), different formula feeding and breastfeeding options (+4 if yes), risk of giving formula feeds (+4 if yes),, how to make best feeding choice (+4 if yes), if the mother intended to breastfeed, then avoiding mixed feeding and stopping breastfeeding early (+4 for each), how women were helped to make a choice - if women were helped to make an appropriate choice (score = +12); if health staff recommended a suitable option (score=+8); if little / no help or guidance provided with choice (score = +4). If health staff simply told women to breastfeed, score =-4. Thus maximum score was +44 and minimum was -8.

ANNEX 2 - Variables selected for analysis

Category	Indicator variables
Socio-demographic factors	Age of mother
	Mother's last standard passed
	Marital status
	Mother alive/dead
	Number of children alive and dead
	Source of drinking water, How they store drinking water, type of toilet, type of fuel, use of fridge or freezer, Availability of working/cooking items
	Any one employed in the house, main income provider, source of income, Mothers main activity during the day, employment status of mother, total monthly income, any one on grant/pension (type of grant/pension)
Mothers knowledge and Health seeking behavior	Number of mothers who have knowledge on MTCT (mentioned all, particularly BF, as a route of transmission, and know the chances of transmission) No of mothers who used this knowledge to change their plans for feeding to the most AFASS method Number of mothers who tried to discuss with health professionals during pregnancy about the best way of feeding Mothers first choice to turn to for advice when they face difficulty in feeding their child No of mothers who have seen advert or heard about formula milk No of mothers who share the formula from clinic/hosp with other children in the house Mothers practice with left over formula milk No of mothers who correctly demonstrated how formula milk is prepared No of mothers who stopped breastfeeding applying the correct

	method No of mothers who seek treatment for themselves/ their child illness No of mothers who are planning to have their child tested for HIV
Infant feeding patterns	Number of infants exclusively breastfed based on the definition Number of infants predominantly breastfed based on the definition Number of infants mixed breastfed based on the definition Number of infants exclusively formula fed based on the definition Number of infants fed by wet nurse No of mothers who stopped breastfeeding applying the correct method (rapid weaning < 4 weeks) Infant feeding duration
Maternal factors	Number of mothers whose water broken > 4 hours before delivery Proportion of mothers that had illness since the last visit – Number admitted to Hospital (how long) Number of children that received fluid/substance as mother was unwell/separated from the child Number of mothers with engorged breasts painful breasts when tried to stop BF Number of mothers given multivitamin HGB result of mother, Viral load
Infant factors	Sex of infant Number of children completed vaccination for a given age Proportion of children who had illness (since the last visit) that caused to change feeding type Proportion of children with illness/hospitalization Proportion of children who were given fluid/ substance due to that they were unwell

	Number of infants received Multivitamin /bacterium Number of children with malnutrition/underweight
Psychosocial factors	Number of mothers discussed feeding decision with some one else apart from the health staff Number of mothers who anticipate one of the family might disagree with their feeding decision (Who) Number of mothers who Disclosed their status (when, to whom, how did they react) # of mothers who are planning to disclose (to whom) # of mothers who provided with fluid and substance at the 1st week due to family/traditional recommendation # of mothers who purchased formula instead of receiving from the clinic due to fear of stigma # of mothers who were criticized/helped when trying to stop BF (by whom)
Health care system Factors	Number of mothers that were counseled by Nurse/Doctor/Social worker/Midwife on Infant feeding/formula milk/weaning/complementary food # of mothers claimed that health workers recommended them to give fluid and substance in the 1st week of child birth No of mothers who heard (seen advert at clinics) about formula milk # of mothers who ever have received/ still receiving free formula milk from the clinic/hospital Quantity of formula milk received when discharged? How long it last? Number of mothers who purchased formula milk in addition to what they received from the clinic/hop Number of times formula milk was out of the clinic/hosp stock # of mothers who has something they don't like about the

	PMTCT programme given in their clinic Number of times formula milk run out before the scheduled date to return to the clinic Number of mothers informed by health staff to test their child for HIV
Socioeconomic status indicators	# of mothers who run out of food Number of food items not available during the food inventory list / in the last month Source of water – no of mothers who use rain water, river, borehole, Toilet type – # of mothers who use pit latrine Fuel source – # of mothers who use wood as a fuel source Access to service (distance to the nearest shop, distance to clinic/hospital, clinic too far for collection of formula milk) No of children died from the total number of children born by a mother Socioeconomic scores