MOTHER TO CHILD TRANSMISSION OF HIV:

MATERNAL AND CHILD CHARACTERISTICS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of

Doctor of Philosophy

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BROKEN TEXT AND SOME POOR QUALITY IMAGES IN ORGINAL THESIS.

TEXT BOUND INTO

THE SPINE

To Lulu, my late father and family

To children and mums in Africa

who suffer in silence from this

creeping epidemic

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PUBLICATIONS

Luo, C., Kankasa, C., Mulenga, D., Sichone, M., Mwela, C. (2000) Prevention of mother to child transmission of HIV in Africa. Specialist Doctor **XXII (1)** 14—19.

Luo, C. Strategies for prevention of mother to child transmission of HIV Plenary paper, XIIth International AIDS Conference,Durban, South Africa, 9^{th} –14^{th} July 2000 (in press Reproductive Health Matters)

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LIST OF ABBREVIATIONS

AMFAR	American Federation of AIDS Research
ACC	United Nations Administrative Committee on Coordination
AIDS	Acquired Immuno-deficiency Syndrome
ANC	Antenatal care
CBoH	Central Board of Heath
CDC	Centre for Disease Control
CI	Confidence interval
C/S	Caesarean section
DHS	Demographic Health Survey
HA	Height for age
HAZ	Height for age *z*-score
Hb	Haemoglobin
HbsAg	Hepatitis B surface antigen
HBC	Hepatitis C
HBc	Hepatitis core antigen
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus Type 1
IDU	Intravenous Drug User
I.M.	Intra-muscular
IMCI	Integrated management of childhood illnesses
IUGR	Intra-uterine growth retardation
LBW	Low birth weight
LUDCC	Lusaka Urban District City Council
MAP	Monitoring the AIDS pandemic
MCHC	Mean corpuscular haemoglobin concentration
MCH	Maternal Child Health
MCV	Mean corpuscular volume
MoH	Ministry of Health
MSPP	Maternal Syphilis Prevention Programme

MTCT	Mother to child transmission
MUAC	Mid upper arm circumference
NS	Not significant
OR	Odds Ratio
PACTG	Pediatric AIDS Clinical Trials Group
PCR	Polymerase chain reaction
PPA	Post-partum anaemia
RPR	Rapid Plasma Reagin
RR	Relative risk
SD	Standard deviation
Se	Standard error
SCN	Subcommittee on Nutrition
STDs	Sexually transmitted diseases
SVD	Spontaneous vaginal delivery
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNFPA	United Nations Fund for Population Acitivity
UNICEF	United Nations International Children Emergency Fund
UTH	University Teaching Hospital
USAID	U.S. Agency for International Development
WA	Weight for age
WAZ	Weight for age *z*-score
WB	Western Blot
WITS	Women Infant Transmission Study
WH	Weight for height
WHO	World Health Organisation
WHZ	Weight for height *z*-score

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ABSTRACT

MOTHER TO CHILD TRANSMISSION OF HIV AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA: MATERNAL AND CHILD CHARACTERISTICS

Chewe Luo

A prospective study at the University Teaching Hospital of 306 women with their infants, who were enrolled at delivery, was conducted in 1997. The primary aim was to define the magnitude and effects of maternal human immuno-deficiency virus (HIV) infection on obstetric problems and infant outcome.

Women were mainly over 19 years (87.3%), literate (73.7%) and married (91.4%), with no formal income (75.7%). 48.2% and 46.7% had antenatal or post-partum anaemia (PPA) and of these 1.8% and 6.2% were severely anaemic. Low post-partum (PP) serum retinol (<0.7μmol/L) and CD4 counts (<400 cells/mm^3) occurred in 12.8% and 16.2% of the women. The commonest obstetric problems were previous child death (32.4%), malaria treatment during pregnancy (32.6%), previous abortion (16.4%) and hypertension (13.7%).

Post-partum, 30.1% of the women were HIV infected, 14.9% rapid plasma reagin (RPR) positive and 4.5% hepatitis B surface antigen (HB_sAg) positive. Factors independently associated with HIV infection were: alcohol intake during pregnancy (RR 5.67); ante-partum haemorrhage (RR 5.85); PP HB_sAg positivity (RR 27.45); low PP CD4 cell count (RR 10.63) and PPA (RR 3.99). Primigravidae had a lower risk of HIV infection (RR O.3).

For PPA independent risk factors were: caesarean section (RR 9.95); HIV infection (RR 2.81) and low PP mean corpuscular haemoglobin concentration (MCHC) (RR 8.33); mean corpuscular volume (MCV) (RR 2.39) and serum retinol (RR 3.03). Alcohol intake during pregnancy (RR 0.22) and low PP maternal weight (RR 0.10) were associated with reduced risk of PPA.

The prevalence of low birth weight (LBW; weight <2.5kg), pre-term delivery (<37 weeks gestation) and intra-uterine growth retardation (IUGR; weight < 10^{th} centile for gestational age) were 18.9%, 23.8% and 25.9%. These showed no association with maternal HIV infection although the mean birth weight was significantly lower in children born of HIV infected mothers (P=0.006).

In HIV non-infected women, antenatal anaemia was independently associated with increased risk pre-term delivery (RR 5.12) and low birth weight (RR 5.08). Low PP serum retinol increased the risk of IUGR (RR 3.10).

In HIV infected women, lack of paternal income was associated with pre-term delivery (RR 11.7), IUGR with LBW (RR 3.59) and antibiotic treatment in pregnancy with IUGR (RR 5.85).

The cumulative rate of HIV mother to child transmission (MTCT) at 1 year of age was 31%, with 10.3%, 10.1% and 9.1% of infants DNA polymerase chain reaction (PCR) positive at birth, 1 month and 4 to 12 months respectively. On multivariate analysis, PP maternal viral load (>50,000copies /ml) was the only risk factor associated with early infant HIV acquisition (birth and 1 month) (P = 0.005) and cumulative infections at one year (P=0.001).

At a year of age, HIV infected children were severely undernourished (weight for age median Z-score -3.46) and stunted (height for age median Z-score –4.44). Stunting was the main form of malnutrition in uninfected infants regardless of maternal HIV status.

Reported morbidity in infancy was unaffected by HIV status. The infant mortality rate was 136 per 1000 live births, 85 per 1000 in HIV uninfected children of uninfected mothers, 272 per 1000 in infants of infected mothers and 424 per 1000 in infected infants. After correcting for confounders, maternal HIV infection (HR 0.28) and primigravidae (HR 0.20) were significant risk factors for infant survival. The population attributable risk percentage of infant mortality was 41.3% for maternal HIV infection and 24.9% when the infant was HIV infected as well.

CHAPTER 1

INTRODUCTION AND AIMS

1.1 BACKGROUND TO THE STUDY

Zambia is amongst the countries hardest hit by HIV in sub-Saharan Africa (UNAIDS 2000). Country-specific HIV prevalence trends have been closely monitored with epidemiological sentinel surveillance data collected from antenatal women. In the 1980s, Zambia experienced a sharp rise in antenatal HIV prevalence, which stabilised in the 1990s at very high levels (MoH/CBoH 1999).

Over a quarter of antenatal women, in urban towns in Zambia, are HIV-infected. In Lusaka, the prevalence estimates were 24.5% in 1990, 26.4% in 1992, 27.5% in 1994 and 27.3% in 1998 (MoH/CBoH 1999). It is estimated that over a third (39.5%) of these HIV infected women will transmit infection to their children (Hira *et al* 1989). The reliability of this estimate is unknown as it is based on an early study when polymerase chain reaction (PCR) testing to determine infection in the children was not available.

HIV infection in children in Zambia is already threatening the steady progress achieved with child survival programmes such as the Expanded Programme of Immunisation and Breast-feeding Promotion. The present infant mortality rate estimate in Zambia (109/1000 live births) is high and is considered to be 25% higher than it would have been without HIV (DHS 1996; Stanecki & Way 1997).

In 1994, the French-American Paediatric AIDS Clinical Trials Study Group 076 (PACTG 076), randomised trial results demonstrated that zidovudine therapy in pregnancy could reduce mother to child transmission (MTCT) of HIV by two thirds (Connor *et al* 1994). After the release of the results, many industrialised countries

adopted this regimen as a standard of care for HIV infected pregnant women and impressive reductions in MTCT were achieved in these countries (Mofenson & Fowler 1999). Subsequent studies, reported last year, have shed more light on the efficacy of cheaper and less complex regimens for wider use in women in low resource settings (Dabis *et al* 1999; Guay *et al* 1999; Shaffer *et al* 1999; Witkor *et al* 1999).

Prevention of HIV MTCT is one of the key elements of the strategic framework for the prevention and control of HIV in Zambia. An MTCT Technical Working Group was appointed by the Ministry of Health in 1998 to technically advise the government on effective approaches to intervention as well as advocacy and strategic planning. Zambia is also one of the countries supported by UNAIDS to pilot the feasibility of integrating administration of anti-retroviral therapy for the prevention of MTCT in HIV infected pregnant women in existing health systems. The pilot programme has been implemented in 6 health facilities in 3 districts with a view to scaling up as more resources become available.

One critical step to advocacy, policy development and strategic planning for prevention of MTCT in Zambia, is the evaluation of the prevailing MTCT situation, including impact on child health and mortality. The timing of acquisition of HIV in children in relation to pregnancy, delivery and breast-feeding is undefined in Zambia. Lack of resources, in previous studies, limited evaluation of the association of MTCT with biological maternal factors such as nutritional status, viral load and CD4 cell count, pregnancy morbidity and outcome as well as infant mortality.

Interventions for prevention of MTCT in Zambia have to be delivered within a comprehensive package of care to include care of both the mother and the child. There

is limited country-specific data on the association of HIV infection with other common causes of morbidity such as anaemia, low birth weight (LBW) pre-term deliveries and IUGR. The association of maternal HIV infection with post-partum and post-natal anaemia in mothers has not been evaluated in previous studies.

The contribution of HIV to malnutrition and other morbidities in children is of major concern in Zambia, given the high infant and childhood mortality. Guidelines to improve care of these children, to include counselling on feeding options, have been developed. However, issues related to preventive therapy such as antibiotic prophylaxis still require further discussion, as more information becomes available.

1.2 AIMS AND OBJECTIVES

The primary aim of this work was to define the magnitude of maternal HIV infection and its impact on pregnancy related morbidity and infant outcome, in a cohort of women, in an urban setting in Zambia, with a view to generating background data for the MTCT Prevention Programme. The research was undertaken with the following themes and specific objectives:

Theme 1

To define the characteristics of women delivering at the University Teaching Hospital (UTH) in Lusaka, Zambia in relation to HIV infection.

Specific objectives to theme 1:

a) To determine the prevalence of HIV infection, syphilis and hepatitis.

b) To describe the women's social demographic background; past and current medical and obstetric characteristics; post-partum serum retinol Hb concentration, MCV and MCHC and CD4 profiles.

c) To identify risk factors associated with maternal HIV infection.

Theme 2:

To define the magnitude and of post-partum anaemia and possible targets for control.

Specific objectives to theme 2:

To determine the prevalence of post-partum anaemia and associated risk factors.

Theme 3:

To define adverse birth outcomes.

Specific objectives for theme 3:

a) To determine the prevalence of LBW, pre-term delivery and IUGR in babies born to HIV infected and non-infected mothers.

b) To identify risk factors associated LBW, pre-term delivery and IUGR.

Theme 4:

To define the characteristics of MTCT of HIV.

Specific objectives for theme 4:

a) To determine the rate and timing of mother to child transmission MTCT.

b) To identify risk factors associated with MTCT.

Theme 5:

To define infant growth patterns, morbidity and mortality in relation to HIV.

Specific objective for theme 5:

a) To describe the infant growth patterns in relation to the National Centre for Health Statistics standards.

b) To compare infant morbidity in children born of HIV infected and non-infected mothers.

c) To determine the infant mortality rate in relation to HIV status.

d) To identify risk factors associated with infant mortality.

1.3 THESIS STRUCTURE

Chapter 2 is a review of the relevant literature with a focus on sub-Saharan Africa. **Chapter 3** summarises the study methods including laboratory methods. **Chapter 4, 5, 6, 7 and 8** describe the results according to the five themes. The structure of each result Chapter is designed to include an introduction and objectives, study methods, results, discussion and conclusion. **Chapter 9** is a summary of research findings and discussions highlighted in Chapter 4, 5, 6, 7 and 8 as well as conclusions drawn and suggested programme and research recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 The HIV/AIDS epidemic in Africa

Nearly 20 years after human immuno-deficiency virus and acquired immuno-deficiency syndrome (HIV/AIDS) was first identified, the epidemic has, by far, exceeded the worst projections in Africa. As of December 1999, the United Nations Joint Programme on HIV/AIDS (UNAIDS) and World health Organisation (WHO) estimated that world-wide about 33.6 million people were living with HIV/AIDS, of which 46% were women (table 2.1). Approximately 70% (23.3 million) of these people were living in Sub-Saharan Africa, the only region in the world where because of heterosexual transmission more women (55%) than men are affected by HIV/AIDS. It is somewhat paradoxical that even though Africa represents only 10% of the world's population, over 70% of people living with HIV/AIDS are in this region. In 1999 alone, of the estimated 5.6 million infections that occurred worldwide, 3.8 million were from Sub-Saharan Africa (table 2.1).

HIV infection in children is directly linked to the prevalence and incidence of the infection in women of the reproductive age group. Over 90% of the HIV infection in children is acquired though transmission from their mothers (UNAIDS/WHO 1999). It is estimated that 2.4 million HIV infected women deliver each year, resulting in approximately 600,000 new HIV infections in infants annually or 1,600 infections each day (UNAIDS 1998). Nearly 90% children that are born with HIV or infected through breast-feeding are from Sub-Saharan Africa (UNAIDS/WHO 1999), largely as a

consequence of high fertility rates, high HIV infection rates in women of the reproductive age group and limited resources for mother to child transmission (MTCT) of HIV interventions.

Although the HIV epidemic has spread at an alarming rate in Sub-Saharan Africa, the speed and extent of spread varies between countries and within countries. The bulk of new infections continue to be concentrated in East and Southern Africa. The reasons for this differential spread are multi-factorial.

Table 2.1 Regional HIV/AIDS 1999

Region	*Adults and children with HIV/AIDS*	*Adults and children newly infected (1999)*	*% oOf HIV infected adults who are women*
Sub-Saharan Africa	2,330,000	3,600,000	55
North Africa / Middle East	220,000	19,000	20
South & South East Asia	6,000,000	1,300,000	15
East Asia / Pacific	530,000	120,000	20
Latin America	1,300,000	150,000	35
Caribbean	360,000	57,000	20
Eastern Europe/ Central Asia	360,000	95,000	20
Western Europe	520,000	30,000	20
North America	920,000	40,000	20
Australia / New Zealand	12,000	500	10
Total	**3,360,000**	**5,600,000**	**46**

(Source:UNAIDS/WHO December1999)

2.2 Antenatal HIV prevalence rates

HIV infection in pregnancy is a major problem in Southern Africa. Up to 45% of women tested during pregnancy in this region are HIV infected, a rate 10 or more times

that in pregnant women in urban antenatal clinics in Central and West Africa (U.S. Bureau of the Census 1998). West Africa is not uniformly affected, with some countries experiencing HIV infection rates similar to Southern Africa. Estimates in 1997 indicated that the HIV infection rates in pregnant women in Abidjan, Cote d'Ivoire were 10 times higher than in Dakar, Senegal (MAP 1998). The data compiled by the Ghent International Working Group demonstrate the differences in prevalence rates in various African cities (table 2.2). The group examined data from different studies in 9 African countries as well as Thailand.

Table 2.2 HIV prevalence in pregnant women and other baseline characteristics in selected centres

Country	*Year*	*City*	*Million Population*	*Sample*	*HIV %*
Burkina Faso	1996	Bobo Dioulasso	0.4	4000	9.2
Cote d'Ivoire	1995	Abidjan	2.5	2500	14.0
Kenya	1996	Nairobi	2.0	1807	15.0
	1995	Mombassa	0.5	200	12.5
Tanzania	1994	Dar es Salaam	3.0	3000	12.0
Malawi	1997	Blantyre	0.4	814	30.0
Zambia	1994	Lusaka	1.5	595	27.5
Zimbabwe	1996	Harare	1.5	1800	28.0
South Africa	1997	Soweto	3.0	15,000	18.3
	1997	Durban	2.0	3351	27.0
Thailand	1996	Bangkok	8.0	40,000	2.3

Source: Cartoux *et al.* 1998

2.3 HIV prevalence trends

Figure 2.1 and 2.2 illustrate the antenatal HIV prevalence trends in different cities in Africa (MAP 1998). Currently the epidemic in sub-Saharan African cities can be defined under four broad trend types:

a) Cities where the infection is still steeply rising.

b) Cities that are showing stability in the epidemic though at very high infection rates.

c) Cities in which infection is on the decline.

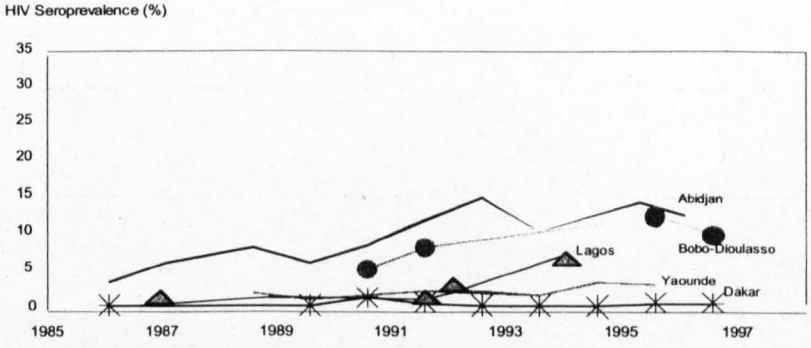
d) Cities where the infection has always remained at very low levels.

Under circumstances not fully understood, epidemics may suddenly explode; the HIV prevalence rate increasing several folds within only a few years. This type of rapid explosion has been observed in South Africa, Botswana and Zimbabwe (Torantola *et al* 1999; MAP 1998). Within 2 years, between 1993 and 1995, HIV prevalence increased about two fold from 4.3 to 11% and 9.6 to 18% in the provinces of Free State and Kwazulu/Natal, South Africa respectively (Torantola *et al* 1999). In Francistown in Botswana, the antenatal HIV prevalence increased 5 fold in 6 years between 1991 and 1997, from below 10% to above 40% (Figure 2.2). In Zimbabwe, the prevalence of HIV in antenatal women in Harare increased from 18% to 30.4% between 1994 and 1995 (Mbizvo *et al* 1996) and in Beit Bridge from 32% to 59% between 1995 and 1996 (UNAIDS/WHO 1998).

By 1992, the prevalence of HIV in pregnant women at primary antenatal clinics in Lusaka, Zambia was between 22.6 and 29.7% (Fylkesnes *et al* 1997), a rate double that observed three years earlier. At the University Teaching Hospital (UTH) in Lusaka,

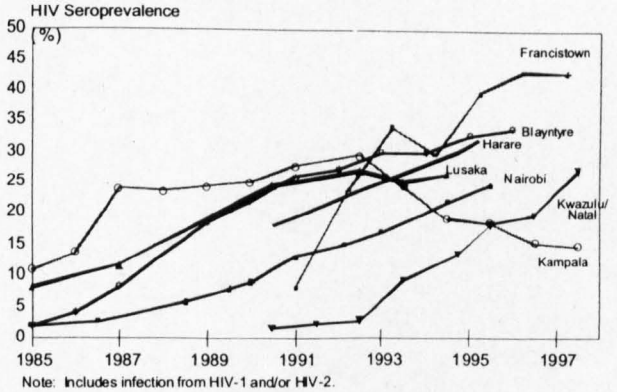
Hira *et al* (1989) in a cohort of pregnant women estimated an HIV prevalence of 12.8%. About 10 years later in 1998, the prevalence at UTH was estimated to be 27.5% (Bhat *et al* 1998).

Figure 2.1 HIV prevalence in pregnant women: Selected urban areas of West and Central Africa 1985-1997



Note: Includes infection from HIV-1 and/or HIV-2.
Source: U.S. Bureau of the Census, HIV/AIDS Surveillance Data Base, 1998.

Figure 2.2 HIV prevalence in pregnant women: selected urban areas of East and Southern Africa 1985-1997



Note: Includes infection from HIV-1 and/or HIV-2.
Source: U.S. Bureau of the Census, HIV/AIDS Surveillance Data Base, 1998.

Although the prevalence appears to have increased 2 fold over the 10 years, sentinel data indicates that between 1992 and 1998, the prevalence rates in Lusaka have remained the same (MoH/CBoH 1999).

In Dakar, Senegal, the HIV prevalence rates have been persistently low and have never gone beyond 2% (figure 2.1) whereas in Uganda, current estimates indicate that the prevalence rates have declined from very high initial levels (Stanecki & Way 1997; Asiimwe-Akiror 1997; UNAIDS/WHO 1998). Among antenatal attendees aged 15-19 at Nsambya Hospital, Kampala, HIV prevalence declined from 28% in 1991 to 10% in 1996 and in Jinja, an urban centre in Eastern Uganda, from 21% in 1990 to 4.5% in 1996. Similar trends were also found in other sentinel sites in 1995 in Uganda (Kilian *et al* 1999) and the continued in 1997 (Kigotho 1997). The differences in trends might be explained by variations in population dynamics such as mobility, patterns of sexual behaviour such as condom use, socio-economic and biological factors, fertility patterns and the commitment to prevention activities in a given country.

2.4 Differential prevalence within countries

Substantial geographical variability in HIV prevalence amongst women exists within a given country. Urban cities appear to be more affected than rural areas, especially during the early stages of the epidemic (Wawer *et al* 1991; Barongo *et al* 1992; Mnyika *et al* 1996). However, this pattern is by no means universal.

In urban Lusaka, in Zambia, the HIV prevalence amongst antenatal women in 1994 was 25-32%, whilst the rate in the rural areas was between 8 to 16% (Fylkesnes *et al* 1997). During the same period, Fylkesnes *et al* (1998) were able validate the high urban

prevalence, observed in antenatal clinics, with a community survey. The HIV prevalence in two urban community sites, Lusaka and Kapiri Mposhi was 25.7% and 33.6% in adults aged 15 to 39 years.

In the adult population in the Arusha region, in Tanzania, the HIV prevalence in urban (low and high socio-economic status), semi-urban and rural areas was 10.7, 5.2, 2.2 and 1.6%, respectively (Mnyika *et al* 1996*)*. In villages in Rakai district in Uganda, the HIV prevalence in women in 1992 ranged from 28% to 47%. Whilst in other villages outside Rakai, only up to 10% of women were HIV positive (Serwadda *et al* 1992*)*. In Zimbabwe, HIV prevalence in urban antenatal women between 1994 and 1995 in Harare, was around 30% (Mbizvo *et al,* 1994). On the other hand, 59% of the women attending a clinic at one border towns in Beit Bridge were HIV infected (Woelk 1998).

Access to HIV / AIDS information, geographic proximity to high-ways, population mobility patterns, sexual behaviour including condom use, cultural and socio-economic factors, prevalence of sexually transmitted diseases (STDs), including availability of screening and treatment facilities are possible explanations for the differences within countries (Pison *et al* 1993*;* Nunn *et al* 1996*;* Jochelson *et al* 1997*;* Whiteside 1998; Dallabetta *et al* 1999*)*.

2.5 Characteristics of HIV infection in women

1) Age distribution

Most studies involving women have observed that there are differences in the age distribution of HIV infection. In Zimbabwe between May 1994 and June 1995,

Mbizvo *et al* (1996) found that in urban Harare the prevalence rates of HIV in antenatal women in the age groups 14-19, 20-24, 25-29, 30-34 and over 34 years were 7.9%, 35.1 %, 33.1 %, 16.4% and 7.4% respectively.

Hom *et al* (1993) in Kampala, Uganda, found that although the overall antenatal prevalence was 28.1 %, the highest prevalence rate was 45.7% at age 22 years. The data also indicated a rising prevalence with increasing age in the age group 14 to 22 years. The reverse was observed in older women. A downward trend was apparent in older women between 23 and 41 years of age.

In Zambia, the highest HIV prevalence rate in urban areas was in the age group 25 to 29 years (33.9%), although this rate was not significantly higher than that in the age group 20 to 14 years (32.2%) (Fylkesnes *et al* 1997). Like in Uganda, the HIV prevalence in the 15 to 19 year age group appeared to be linear from 11% to 28% in urban areas and 4% to 11% in rural areas, an indication of rising infection with increasing age among the adolescents.

The age specific prevalence rates might be a reflection of the stage of the HIV epidemic in a particular country. Current estimates in Zambia {1999) indicate that the HIV prevalence has declined (53%) in women between 15 to 19 between 1994 and 1999 whereas the prevalence has remained stable in the older age group (MoH/CBoH 1999). A reasonable explanation for this reduced prevalence in young women is a drop in incidence in this age group and possibly a response to existing interventions and increased awareness in Zambia.

2) Marital Status

Marital status is an intriguing risk factor for HIV infection in Africa where the epidemic is generalised. In a study in Zimbabwe single marital status was associated with a two-fold increase in risk of HIV in pregnancy (Mbizvo *et al* 1996). Fylkesnes *et al* (1997) reported similar results in Zambia in the 1994 sentinel survey. HIV infection risk was higher amongst single than married women in Zambia. The observed influence of marital status on infection risk in Zambia was age specific. In women between 15 and 24 years, no difference in HIV prevalence was found between single and married women. However, the age specific analysis revealed a twofold increase in risk of HIV infection in single women between 25 and 44 years.

The results of a study in Nairobi on the effect of marital status were contradictory to the findings in Uganda and Zambia. A twofold increase in risk of HIV infection was reported for married women (Woelk 1998). These site differences in HIV infection risk with regard to marital status reflect the complexity of the HIV epidemic. HIV exposure in women can result from their own behaviour or that of their partner. Whereas single women are more likely to have multiple partners, married women are still vulnerable if husbands engage in high-risk behaviour.

3) Socio-economic status

Globally, it is the poorer and less educated who are most affected by HIV / AIDS (UNAIDS/WHO 1998). Less educated people are not likely to be informed about HIV / AIDS, including methods of prevention and control. The results of an analysis done to compare literacy and HIV prevalence in 161 countries confirmed this assumption

(UNAIDS/WHO 1998). A downward trend in prevalence was demonstrated with increasing literacy levels.

In Sub-Saharan Africa, however, the reverse prevails. HIV infection has been shown to predominantly affect the most educated of the population (UNAIDS/WHO 1998). A compilation of data from 44 countries on socio-economic status of HIV infected adults confirmed an association between HIV and being literate.

These findings are similar to the conclusions drawn by Fylkesnes *et al* (1997) from a population-based survey in Zambia. They demonstrated that higher education in women of childbearing age was associated with increased prevalence of HIV infection both in urban and rural dwellers, although in adolescents (15-19 years) the risk was the same regardless of the education level. Fylkesnes *et al* (1997) concluded that this finding might indicate differences in timing of HIV exposure and behavioural differences in the different age groups. The older educated women were exposed to HIV at a time when little information existed on HIV/AIDS. Even though educated women are more empowered in terms of their sexual choices, they may actually mix with a stratum within the community that is at high risk, such as more educated men.

4) Biological factors

a) Sexually Transmitted Diseases

According to WHO estimates (1995) the annual incidence of sexually transmitted diseases (STDs) in 15 to 49 year old women in Sub-Saharan Africa is 11-35%. Sexually transmitted diseases are much more common in this region than in more developed countries but their role in propagating HIV infection remains unclear. Various authors

have reported increased prevalence of HIV infection amongst adults with an STD. Whether these individuals are engaging in behaviours that increase their chance of contracting both a STD and HIV or whether the presence of STD increases the risk of acquiring HIV is not clear.

There are compelling biological reasons for believing that untreated STDs such as herpes simplex, syphilis, gonorrhoea and chanchroid greatly increase the risk of HIV acquisition per exposure. The ulcerative lesions caused by some these diseases provide a ready portal of entry for transmission of HIV while the inflammation will result in recruitment of HIV susceptible lymphocytes and macrophages to epithelial surfaces. In a nested case control study of 431 HIV negative women followed up prospectively, women with gonorrhoea, chlamydia, or trichomoniasis, were more likely to become HIV infected than those without STDs, with an OR of 3.6 for gonorrhoea and 1.9 for chlamydia (Laga *et al* 1993).

Ulcerative STDs, like syphilis, chanchroid and herpes simplex are associated with increased risk of HIV infection (Latif *et al* 1989; Miotti *et al* 1990; Plummer *et al* 1991; European Collaborative Group of HIV in Female Prostitutes, 1993). Miotti *et al* (1990) in a study in Malawi, reported that reactive syphilis in pregnancy was associated with HIV infection. Of the HIV infected women 19% (18 out of 81) had reactive serology compared to 6% (24 out of 380) of the HIV negative women.

Women, because of their anatomical structure, are more likely than men to suffer from silent STDs without any apparent symptom. Many of them, as a result, remain untreated. The syndromic management approach without appropriate screening facility is unlikely to cover most women in antenatal clinics. Screening for STDs should be an

integral part of antenatal care in HIV prevalent settings. Syphilis rates as high as 30% have been reported in pregnant women (Mlisana *et al* 1992; Qolohle *et al* 1995). In Zambia Hira *et al* in 1990 found an overall prevalence of 8.0% in antenatal clinics in Lusaka. In 1994 to 1995, there was a notable increase in prevalence. Of 42,366 new antenatal clients screened for syphilis in antenatal clinics in Lusaka 7,419 (17.5%) were sero- positive (UNICEF 1995).

One of the most important findings in HIV prevention over the past decade is the discovery that reduction in STDs might prevent HIV transmission in populations of medium risk. A clinical trial in Tanzania demonstrated that syndromic symptomatic management of STDs could reduce HIV incidence substantially (Grosskurth *et al* 1995). The investigators randomised 12 villages into 2 defined groups with similar characteristics. The people in these villages were not expected to travel or move between villages. Community health workers were then trained in syndromic management of STDs in the intervention villages whilst referral to an STD clinic was facilitated in symptomatic persons in the control villages. Drug supplies were always adequate and regular and there were systematic supervisory visits and periodic health education in the intervention villages.

Two years later no change in behaviour was noted between the paired villages. Of the individuals who were initially HIV negative 1.2% sero-converted in the intervention villages and 1.9% in the control villages, a 42% reduction in HIV incidence in 2 years. The estimated relative risk ratio for the intervention was 0.58 (P=0.007). The incidence of serologic syphilis and symptomatic urethritis in men was also reduced in the intervention villages. There was no difference, however, in the incidence of self-

reported symptoms, in STD prevalence amongst the antenatal attendees, condom use and sexual behaviour in the control and intervention communities.

The HIV epidemic in Africa is complex and driven by multiple factors. The key determinants centre on the population dynamics. While clinical trials are the best vehicles for demonstrating the effect of an intervention, an approach that is effective in one setting may not work in another because of differences in population dynamics and the stage of the epidemic. It is then not surprising that a similar study, in Rakai district in Uganda, failed to demonstrate any impact of mass treatment of STDs on HIV incidence (Wawer *et al* 1998). This Ugandan study was designed to evaluate whether mass treatment of STDs could reduce STDs in a community and to demonstrate whether a reduction in STD prevalence and incidence would reduce HIV transmission. The study was a community based randomised trial with 5 treatment clusters and 5 control clusters. Azithromycin, ciprofloxacin and metronidazole were given as a treatment every 9 months under direct observation to adults in the intervention clusters. Treatment for syphilis was only given to those in participants found to be serology positive. Controls were given mebendazole and iron-folate tablets every 9 months. Although this study was able to demonstrate key reduction in incidence of syphilis, gonorrhoea and trichomoniasis, after 30 months there was no observed impact on HIV incidence in pregnant women, discordant couples or HIV negative concordant couples. The trial did not establish an effect of antibiotic therapy on MTCT of HIV.

b) Hepatitis B and C

Hepatitis B and C and HIV have similar routes of transmission, which include parenteral, sexual and MTCT. The prevalence HBsAg carriage reported from African studies is between 6.3 to 25% (table 2.5).

Hepatitis C prevalence in most areas in Africa, although lower than that of hepatitis A and B as well as HIV, appears to show wide variations, some countries being more affected than others (table 2.3).

Table 2.3 Prevalence of hepatitis surface antigen carriage (HBsAg) and hepatitis C positive serology (HBC)

Hepatitis B		***Hepatitis C***	
Study (reference)	***Study N (% HBsAg positive)***	***Study (reference)***	***Study N (% HCV antibody positive)***
Rural Cameroon (Kowo *et al* 1995)	166 (7.2)	Rural Cameroon (Kowo *et al* 1995)	167 (13.0)
Rural equatorial Guinea (Basaras *et al* 1999)	2042 (8.8)	Rural equatorial Guinea (Basara *et al* 1999)	2040 (1.7)
Rural Ghana (Martinson *et al* 1998)	1385 (20.9)	Rural Tanzania (Menendez *et al* 1999)	980 (5.0)
Rural Malawi (Ahmed *et al* 1998)	150 (13.0)	Rural Tanzania (Tess *et al* 2000)	517 (10.3)
Rural Tanzania (Menendez *et al* 1999)	980 (6.3)	Urban Ghana (Wansbrough Jones *et al* 1998)	936 (2.8)
Urban Tanzania (Matee *et al* 1999)	300 (11.0)	Rural Malawi (Ahmed *et al* 1998)	150 (16.5)
Urban Zambia (Oshitani *et al* 1996)	1861 (7.1)	Urban Tanzania (Matee *et al* 1999)	300 (8.0)
Urban Zimbabwe (Madzime *et al* 1999)	984 (25.0)	Urban Zambia (Oshitani *et al* 1995)	735 (0.41)

It is currently not clear whether there are real differences in the prevalence of hepatitis C or whether the differences are due to methodological differences and variability study populations (children or adults or both; urban or rural; hospital versus community individuals) and laboratory methods used to identify HCV. Initial tests seemed to have a lot of false positive results although third generation enzyme immune-assays and recombinant immunoblot assay appear to reduce false positives and indeterminate results (Damen *et al* 1995; Gretch 1997)

c) Anaemia

Anaemia in pregnancy is a major problem in Africa. Very high prevalence rates have been reported from Africa; 46.9% in Zambia, (Luo *et al* 1999), 55% in Western Kenya (Zucker *et al* 1994), 60% in Blantyre, Malawi (van den Broek *et al* 1998), 69.7% in Zanzibar, Tanzania (Mattee *et al* 1994) and 75.6% in North Mombassa, Kenya (Shulman *et al* 1996) and 78% in Liberia (Jackson 1992).

The aetiology of anaemia in Africa is multi-factorial. Some of the important factors include substrate deficiency (iron, folate and vitamin A), hookworm and malaria infestation, haemoglobinopathies and chronic infection (Beizel *et al 1976;* Fleming 1989; WHO 1993; Masawe *et al* 1999).

Anaemia is commonly seen amongst HIV infected adults (Cotton & Watts 1997). Recent studies (table 2.4) have shown an association between HIV infection and anaemia in pregnancy (van den Broek *et al* 1998; Meda *et al* 1999; Ramon *et al* 1999), although a study in Shire valley, a rural area in Malawi, did not find this association (Chimsuku 1998). Even though HIV infection was observed in 25.4% of the pregnant

women in the Shire Valley, iron and vitamin A deficiency, and malaria were probably more important causes of anaemia.

Although HIV disease is characterised by depletion of CD4 lymphocytes, depletion of other cell lines including neutrophils, thrombocytes and red blood cells have been reported (Zon *et al* 1987; Scadden *et al* 1989; Zon & Groopman 1988; Davis & Zauli 1995).

The pathogenesis of anaemia associated with HIV infection is not fully understood. Possible mechanisms include: direct effect viral infection itself on the haematopoetic system; insufficient dietary intake (vitamins, folate, iron and general malnutrition); haemolysis (infection, malignancy, splenomegaly, immune dysfunction); changes in erythropoetin synthesis; bone marrow suppression; side effects of anti-retroviral therapy and prophylactic treatments such as co-trimoxazole (Davis & Zauli 1995; Mocroft *et al* 1999).

Some reports have suggested that anaemia occurs in late HIV disease and might be an independent predictor of HIV didease progression and clinical outcome (Mocroft *et al* 1999).

Table 2.4 HIV infection and anaemia (Hb < 11g/dl) in pregnancy

Study (HIV prevalence)	*Reference*	*HIV Status N (% anaemic)*		*P value*
		HIV (+) N(%)	*HIV(-) N(%)*	
Blantyre, Malawi (33%)	Van den Broek *et al* 1998	NA(93.6)	NA(45.5)	RR.2.06
Abidjan, Coted'Ivoire (12%)	Ramon *et al*1999	197(81.7)	1442(68.)	<0.001
Bobo Dioulasso, Burkina Faso (9.7%)	Meda *et al*1999	NA(78.4)	NA(64.7)	<0.001
Shire Valley, Malawi (25.4%)	Chimsuku *et al* 1998			
Antenatal		164 (92.4)	481 (89.4)	>0.05
Delivery		164 (83.6)	481 (79.7)	>0.05

RR. Relative risk quoted as no P value available. NA not stated in report

The data in table 2.4 indicate that anaemia in pregnancy is a common problem in areas that also have high HIV prevalence. Anti-retroviral therapy is effective in reducing MTCT and its implementation currently is being piloted in some countries in Africa (Mercier 1999). For successful integration of such an intervention, the existing maternal and child-care services will need strengthening, including the control of anaemia in pregnancy. Interventions for anaemia to be addressed include malaria treatment/prophylaxis, iron, folate and multivitamin supplementation, deworming and promotion of early access to antenatal care.

c) Vitamin A

Vitamin A deficiency is a common problem in HIV infected adults (Semba *et al 1993*) but the cause of this deficiency is ill defined. Potential contributors include decreased intake, absorption and mobilisation of vitamin A, increased utilisation and abnormal

urinary losses (Semba *et al* 1993; Stephenson *et al* 1994; Jolly *et al* 1995; Sommer & West 1997; Nimmagadda *et al* 1998; Kelley *et al* 1999).

Up to 50% of HIV positive intravenous drug users (IDU's) and 29% of homosexual men have inadequate dietary intake (Smit *et al* 1996; Tang *et al* 1997). Kelley *et al* (1999) reported a lack of benefit from vitamin A supplementation in hospitalised HIV infected adults participating in a randomised controlled trial of albendazole treatment versus vitamin A, C, E, selenium and zinc. Serum concentrations of vitamin A did not increase significantly in supplemented patients compared to placebo, probably as a result of malbsorption. Another study is underway in Zambia to confirm this.

HIV infected patients with pneumonia and sepsis excrete significant amounts of retinol and retinol binding protein (RBP) in their urine compared to those without pneumonia (Stephenson *et al* 1994; Jolly *et al* 1995). A prospective study of HIV infected IDU's in Baltimore suggested that vitamin A deficiency may adversely alter the clinical course of HIV disease, with an adjusted relative risk of mortality 4.3 times higher when serum retinol concentration was less than 1.05μmol/L (Semba *et al* 1993 & 1995).

Pregnant HIV infected women in Africa are at particular risk of vitamin A deficiency, most likely because of increased nutritional demands. 58% of HIV infected mothers attending an antenatal clinic in Nairobi had serum retinol concentrations of less than 1.05 μmol/L and 17% less than 0.7μmol/L (Nduati *et al* 1995). Similarly 58-63% of HIV infected mothers in Malawi had retinol levels of less than 1.05 μmol/L. In a report from the US, 29.6% and 11% of HIV infected pregnant women low (0.7 to 1.05 μmol/L) and very low (<0.7 μmol/L) serum retinol (Burns *et al* 1999).

In Zambia, vitamin A deficiency in pregnant women should be addressed within the reproductive maternal care package. Currently WHO recommends that all women be supplemented with a 200,000 IU vitamin A capsule soon after delivery. Although these recommendations have been adopted in Zambia, vitamin A supplementation national coverage was only 13.6% in 1998 (Luo *et al* 1999).

2.6 Mother to child transmission (MTCT) of HIV

2.6.1 Rate of transmission

Mother to child transmission of HIV in the absence of intervention vary from 13-32.6% in Europe and the US to 20 to 43% in Sub-Saharan Africa (table 2.5). The higher estimates in Africa indicate differences in exposure to risk factors such as breast-feeding and methodological difficulties like paediatric HIV infection definition, following up of cohorts and allowing for high infant mortality rates.

2.6.2 Definitions of Paediatric HIV infection

In an effort to standardise the definition of Paediatric AIDS and methodologies for MTCT rates, a number of classifications have been proposed, some of which are applicable to low resource settings with limited investigative capacity.

1) WHO classification

Two clinical AIDS definitions, the Bangui classification and the modified WHO classification, applicable to settings where resources are limited were developed by WHO during the late 1980s (Table 2.6).

Table 2.5 Rate of MTCT

Country	*Rate (%)*	*Reference*
Africa	**20-43**	
Nairobi Kenya	42.8	Datta et a/ 1994
Lusaka, Zambia	39.5	Hira *et al* 1989
Kinshasa, Zaire	39.0	Ryder *et al* 1989
Butare, Rwanda	20-29	Bultereys *et al* 1993
Kigali, Rwanda	25.7	Lepage *et al*, 1993
Durban, South Africa	30.0	Bobat *et al* 1996
Blantyre, Malawi	27.0	Taha *et al* 1994
Abidjan, Cote d'Ivoire	24.7	Adjorlo-Johnson *et al* 1994
Developed Countries	**13-33**	
France	20.2	Mayaux *et al* 1995
Italy	32.6	Italian Multicentre Study, 1988
European centres	12.9	European Collaborative Study, 1991
European centres	14.4	European Collaborative Study, 1992
European centres	16.2	European Collaborative Study, 1994
New York, USA	28.0	Thomas *et al* 1994
Baltimore, USA	23.1	Nair *et al* 1993

These definitions included the commonly observed clinical features. Confirmation of maternal HIV infection with antibody testing was included as a minor sign with the understanding that not all settings would be able to perform an HIV test. AIDS diagnosis, therefore, would still be possible without the test. These definitions were evaluated in various African countries, using HIV antibody test results as the gold standard and were found to have low sensitivity and positive predictive value (Colebunders *et al* 1987; Lepage *et* al 1989; Chintu *et al* 1993). However, they were useful with country specific modifications. In Zambia, a country specific criteria was

developed and evaluated (Chintu *et al* 1993). The sensitivity, specificity and positive predictive value with the Zambian criteria were 79.3%, 91.4%, 86.8% compared to 66%, 64% and 38% with the Bangui classification.

Table 2.6 WHO case definition for paediatric AIDS

WHO clinical case definition (Bangui)	*Modified WHO clinical case definition*
Major Signs	***Major Signs***
Weight loss or failure to thrive	Weight loss and failure to thrive
Chronic diarrhoea (> 1 month)	Chronic diarrhoea (> 1 month)
Prolonged fever (> 1 month)	Prolonged fever (> 1 month)
	Severe or repeated pneumonia
Minor signs	***Minor signs***
Generalised lymphadenopathy	Generalised lymphadenopathy
Oro-pharyngeal candidiasis	Oro-pharyngeal candidiasis
Repeated common infections	Repeated common infections
Generalised dermatitis	Generalised pluritic dermatitis
Confirmed maternal HIV infection	Confirmed maternal HIV infection

With both definitions, paediatric AIDS is suspected in a child presenting with a least two major signs and two minor signs in the absence of known causes of immuno-suppression

2) Ghent classification

A working group on MTCT convened in Ghent, Belgium, in 1992 and developed guidelines to standardise estimates of transmission rates in various studies and surveillance systems (table 2.7 & 2.8) (Dabis *et al* 1993). Agreements were reached on HIV related signs and symptoms paediatric AIDS, and HIV related deaths. A direct (all

antibody positive children at 15 months combined with all with clinical AIDS) and an indirect method (all antibody positive children plus excess mortality in infants of HIV infected mothers when compared to the negative group) of estimation were developed.

Table 2.7 HIV related signs and symptoms (Ghent 1992)

Persistent diarrhoea > 15days
Oral candidiasis beyond the neonatal period
Generalised lymphadenopathy (enlarged glands in at least 2 independent anatomical sites
Failure to thrive (no weight gain for a period of 3 months or crossing two percentiles lines on the growth chart)
Chronic parotitis (> 1 month)
Herpes zoster (shingles)
Recurrent pneumonia (2 or more episodes)

The group recommended that 2 paediatricians determine clinical disease using theWHO criteria or the 'Ghent' HIV related signs and symptoms (Table 2.7) and a verbal autopsy for indeterminate children who died before 15 months for possible HIV related mortality.

Table 2.8 ***Ghent classification of children born to an HIV infected mother according to probable HIV infection status***

HIV infected
HIV WB* antibody positive at 15 months
Or
HIV related death
Or
Clinical AIDS
HIV non-infected
HIV WB negative at 15 months
Or
HIV WB negative at 9 months in a child loss to follow up without AIDS
Or
HIV WB negative in a child who died from probable non-HIV cause
Indeterminate HIV status
Death before 15 months with indeterminate relation to HIV
Or
Child died of probable non-HIV cause while WB positive or indeterminate before 15 months or WB* negative <9 months (when last seen)
Or
Child lost to follow-up while WB positive or indeterminate before 15 months or WB negative < 9 months (when last seen)
Or
Child with indeterminate WB* and alive at 15 months

WB* Western blot

The Ghent criteria was used by the group to recalculate the MTCT rates from 13 cohorts. Although lower than the estimates in the original studies, the recalculated

values were still higher (25-30%) than those from developed countries (14-25%) (Dabis *et al* 1993). The Ghent criteria, however, is complex and is only likely to work under stringent research settings.

3) Polymerase Chain Reaction (PCR) testing

Timely determination of HIV infection status with DNA or RNA (PCR) and viral culture, is possible for children born of HIV infected mothers. The advantages of PCR testing over viral culture include the availability of standardised commercial testing kits such as the NASBA and Roche and the ability to produce results within a day. The main disadvantage is the cost of the test kits, although it is possible to use cheaper in-house methods, with limited use of commercial kits for validation of the results or quality control.

Both DNA and RNA PCR methods for paediatric HIV testing have been evaluated using viral culture as the gold standard (Bremer *et al* 1996; Simonds *et al* 1998). Bremer *et al* evaluated the Roche DNA PCR kit in children enrolled in a multicentre study in the USA. The NASBA RNA kit was evaluated in a small cohort of children in the New York City Perinatal HIV Transmission Collaborative Study by Simonds *et al.*

In both studies sensitivity was low in the first few days of life, 29% for Roche DNA and 38% for NASBA RNA PCR although specificity was 100% and 99% respectively. After 1 week the sensitivity was over 90% and above and specificity over 95% for both tests. Matheson *et al* (1995) evaluated the Ghent classification with estimates obtained using PCR and viral culture. The Ghent paediatric HIV definition yielded results similar to viral culture and PCR.

2.6.3 Timing of transmission

The consensus from both clinical and laboratory evidence is that most (70-80%) of HIV infected infants acquire the virus around the time of delivery (Bertolli *et al* 1996; Rouzioux *et al* 1995; Dunn *et al,* 1995; Kuhn *et al* 1997).

A definition (table 2.9) for timing of infant infection, in non-breastfeeding populations, based on consecutive blood samples tested for tests for viral markers, like PCR, was proposed by the Paediatric AIDS Clinical Trials Group (PACTG), for defining timing of transmission (Bryson *et al* 1992).

Table 2.9 ***Paediatric AIDS Clinical Trials Group definition of timing of MTCT of HIV: PACTG 1992 (Non-breastfed infants)***

<u>In utero</u> (early):   Viral marker (polymerase chain reaction (PCR) or culture) positive within 48 hours of delivery (cord blood sample should ideally be confirmed with peripheral blood)   **Intra-partum (late):**   Viral marker negative in blood samples obtained in the first week but positive between day 7 and 90.

A child was considered to have <u>in utero</u> or early transmission if PCR positive or if HIV was isolated by viral culture within 48 hours of birth with subsequent confirmation of positive results after the neonatal period. Late or intra-partum infection was considered if the children, originally negative in the first week of life, became positive during the period from day 7 to 90 days.

Using the PACTG definition, 250 infants of HIV infected mothers, in New York City, Kuhn *et al* (1997) reported that 25% of the infections were acquired in utero and 75% intra- partum, confirming earlier observations. Further confirmation was derived from mathematical modelling, using a Markov model, on sequential results of viral markers and serology in children, to estimate the frequency of in utero and intra-partum transmission in a French cohort (Rouzioux *et al* 1995). According to the model, 65% of HIV infections in this cohort were intra-partum and 95% of the in utero infections probably occurred just before delivery (within the last 2 months of pregnancy).

In Sub-Saharan Africa, breast-feeding contributes significantly to transmission (Bertoli *et al* 1996; Coutsoudis *et al* 1999; Miotti *et al* 1999; Nduati *et al* 2000) and the risk may be higher with prolonged breast- feeding (Datta *et al* 1994; Riouzioux *et al* 1995). *A* randomised trial in Kenya (Nduati *et al* 2000) of formula feeding versus breast- feeding estimated that the cumulative probability of HIV-1 infection at 24 months was higher (36.7%) in the breast-feeding arm than in the formula arm (20.5%). The rate of breast milk transmission in this study was 16.2% with an estimation that 44% percent of HIV-1 infections were attributable to breast- feeding.

In a study in Kinshasa, the democratic Republic of Congo (D.R. Congo), 23% of the infants were thought to have been infected in utero, 65% intra-partum and 12% postpartum (Bertolli *et al* 1996). The estimated risks for in utero, intra-partum / early breast-feeding and late breast-feeding were 6%, 18%, and 4% respectively. These results indicated that the intra-partum and early breast- feeding period were important time points for transmission interventions.

An important observation by Coutsoudis *et al* (1999) from a study in Durban, South Africa, was the reduction in breastfeeding transmission risk in the early lactation period with exclusive breast-feeding. The researchers prospectively assessed feeding patterns in HIV infected women who took part in a vitamin A intervention trial in Durban, South Africa. The proportions of HIV infected children at 3 months were 18.8% for never breast-fed, 14.6% for exclusively breast fed and 24.1% for mixed feeding (breast plus supplementary feeds). After correcting for confounders, they found that exclusive breast-feeding carried a significantly lower risk of HIV transmission than mixed feeding and the rate of MTCT was similar to formula feeding. These results suggest that promotion of exclusive breast-feeding, in settings where women have few feeding options, is a potential intervention for the prevention of MTCT that requires further evaluation.

Late postnatal transmission, after 6 months of age, has been described in a number of studies (van de Perre *et al* 1991; Bertolli *et al* 1996; Ekpini *et al* 1997; Miotti *et al* 1999*)*. This rate is much higher if a lactating woman acquires infection during lactation *(*van de Perre *et al* 1991; Dunn *et al* 1994*)*.

Miotti *et al* (1999) reported that the cumulative transmission risk from breast-feeding, in children above 2 and half months old, in Blantyre Malawi, was 3.5% at 6 months, 7% at 12 months and 10.3% at 24 months. The Ghent group conducted an analysis of pooled data from 8 individual cohorts in seven different settings (Leroy *et al* 1998*)*. The group estimated the risk of late postnatal transmission of HIV-1 after 2.5 months of age as 3.2 cases per 100 breast-fed children per year.

2.6.4 Risk factors associated with MTCT

Several factors likely to contribute to MTCT of HIV have been reported and include factors relating to the mother (viral burden, immune status vitamin A deficiency, anaemia, co-infections), intra-partum period, the foetus and the child.

I) Viral burden and immune status

The stage of maternal infection is a significant predictor of MTCT. Advanced maternal immuno-suppression (low CD4 cells count or percentage), clinical disease progression and high viral load are associated with increased risk of MTCT (European Collaborative Study 1992 & 1999; St Louis *et al* 1993; Sperling *et al* 1996; Mayaux *et al* 1994; Garcia *et al* 1999; Katzenstein *et al* 1999; Mofenson *et al* 1999; O'Donovan *et al* 1999; Shaffer *et al* 1999).

a) Viral burden

Several studies, including two from Africa, identified viral load as a determinant of MTCT (table 2.10). Without zidovudine treatment, viral load is always higher in transmitting mothers. The strength of the findings differ in terms of access to zidovudine treatment and the type of viral assays used.

The current data on viral load suggest no threshold at which transmission does not occur (Sperling *et al* 1996; Mayaux *et al* 1997; European Collaborative Study 1999). Although zidovudine treatment during pregnancy reduces viral load to variable concentrations, the reduction in MTCT is not dependent on the viral concentration attained with treatment (Sperling *et al* 1996; Garcia *et al* 1999).

It appears the observed benefit with zidovudine treatment in pregnancy results from a mechanism other than the reduction of maternal viral load. The possibility of a prophylactic effect in the baby both in utero and intra-partum has been raised (Sperling *et al* 1996).

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Table 2.10 Summary of MTCT studies reporting of viral load

Study (Country Year)	*N (no. infected)*	*Median[4]/Geometric mean (anti-log calculated)[5]*		*P value*
		Transmitting	*Non-transmitting*	
European Collaborative Study 1999[1]	373 (56)	18,000	4,600[4]	<0.0001
Garcia *et al* 1999[1]	552(114)	29,2351	10,049[4]	
Katzeinstein *et al* Zimbabwe 1999[2]	251 (67)	20,893	11,492[5]	0.03
Mofenson *et al* USA 1999[3]	480 (24)			<0.001
Baseline		39,811	6,310[5]	
Third trimester		25,119	5,061[5]	
Delivery		39,811	3,981[5]	
O'Donovan *et al* Gambia[2] 1999	81 (17)	30,400	12,000[5]	0.018
Shaffer *et al* Thailand 1999[2]	280 (68)	14,400	6,500	<0.0001
Sperling *et al* USA/France 1996:				
Zidovudine group	198 (15)	19,330	4,650	0.030
Placebo group	204 (46)	8,320	5,370	0.003

[1]Combined with and without treatment [2]Without zidovudine treatment [3]With zidovudine treatment.

The risk of HIV is likely to increase with decreased maternal immune status that results from maternal disease progression. Several of the earlier studies reported this association of MTCT with decreased immune status reflected by low cell CD4 count, CD4 percentage and CD4/CD8 ratio (St Louis *et al* 1989; Ryder *et al* 1989; European Collaborative Study 1992; Lepage 1993; Thomas *et al* 1994; Landesman *et al* 1996). In the European Collaborative Study (1992), there was an increased risk of MTCT when

maternal CD4 counts were below 700 cells/mm and transmission increased linearly with decreasing CD4 cells. Decreased immune status may simply be a marker for high viral burden as opposed to a risk factor in itself. Most of the earlier studies did not control for viral load in the analysis. Multivariate analyses for in recent studies (with and without zidovudine treatment) indicate that CD4 status is not independently associated with increased risk of MTCT (Sperling *et al* 1996; European Collaborative Study 1999; Garcia *et* a1 1999; Katzeinstein et *al* 1999 Mofenson *et al* 1999; Shaffer *et al* 1999).

c) Vitamin A

An association between vitamin A deficiency and MTCT was first reported in 1994 by Semba *et al* in their MTCT cohort in Malawi. The mean vitamin A level in 74 mothers who transmitted HIV to their infants was lower than in 264 mothers who did not transmit (0.86 μmol/L versus 1.07μmol/L $P<0.0001$). In addition, the rates of transmission were inversely related to the level of Vitamin A (table 2.11). Logistic regression analysis showed that the effect of maternal vitamin A deficiency was independent of maternal age, CD4 lymphocyte percentage and body mass index. In 3 subsequent US vitamin A studies, only one demonstrated an association between serum retinol (< 0.70 μmol/L) and increased risk of MTCT, after adjusting for CD4 lymphocyte count and duration of rupture of membranes (Greenberg *et al* 1997; Burger *et al* 1997; Burns *et al* 1998).

Table 2.11 ***Mother to child transmission by maternal serum retinol concentration (Semba et al 1994)***

Serum retinol (μmol/L)	*Transmission rate (%)*
<0.70	32.4
0.70-1.05	26.2
1.05-1.40	16.0
≥1.40	7.2

Vitamin A probably contributes to transmission through several mechanisms. Vitamin A has stimulatory effects on both T-cell and B-cell function (Semba *et al* 1993). Decreased T cell activity observed with vitamin A deficiency may result in higher HIV viral titres. Vitamin A deficiency result in squamous metaplasia of mucous membranes and increased binding of pathogens to epithelial surfaces (Nimmagadda *et al* 1998). In a pregnant woman the integrity of the placenta may be impaired by these pathological changes in the foetal membranes and the uterine mucosa. The susceptibility of the birth canal to trauma may also be increased. Differences in intra-partum exposure and infant feeding patterns may also explain the observed differences in the effect of vitamin A on MTCT. Vitamin A deficiency has been shown to be associated with increased HIV virus expression in genital secretions and breast milk (Nduati *et al* 1995; John *et al* 1997).

The importance of other co-existing micronutrient deficiencies in pregnancy is suggested by the results from a randomised placebo controlled trial by Fawzi *et al* in Tanzania (1998). Receipt of prenatal multivitamins, but not vitamin A alone, was

associated with increased CD4 cell counts, decreased preterm birth and LBW, but not MTCT.

d) Anaemia

Although some recent studies have indicated an association between anaemia in pregnancy and HIV, there are limited in that most have included analysis of anaemia as a risk factor for MTCT. Recent studies in Africa have reported that HIV infection is a risk factor for anaemia in pregnancy (van den Broek *et al* 1998; Meda *et al* 1999; Ramon *et al* 1999*)*.

Three studies, which have evaluated the association between anaemia and MTCT, were reviewed, two from the US and one from DR Congo (former Zaire) (St Louis *et al* 1993; Thomas *et al* 1994; Burns *et al* 1999*)*. The two US studies demonstrated an association between anaemia and increased risk of transmission on univariate but not multivariate analysis (Thomas *et al* 1994; Bums *et al* 1999*)*. The D.R. Congo study failed to show an association (St Louis *et al* 1993*)*, possibly because malaria and nutritional deficiencies such as iron, folate and vitamin A confounded anaemia.

e) Co- infections

A number of studies have evaluated the role of co-infections (mainly chorioamnionitis and sexually transmitted diseases (STDs)) in MTCT. These infections are associated with disruption of the placenta barrier in late pregnancy, premature delivery and increased viral load in the vaginal tract (Bulterys *et al* 1993; Lallemant *et al* 1994; Ghys *et al* 1997). Ryder *et al* (1989), in a study in the D.R. Congo, reported that mothers with

severe chorioamnionitis were more likely to transmit HIV infection to their infants. These results were confirmed later, although not by all studies (Nair *et al* 1993; St Louis *et al* 1993; Simonds *et al* 1997; Mofenson *et al* 1999). Sub-clinical chorioamnionits could have been missed in studies because placentas were not examined, possibly contributing to the lack of association.

The presence of STDs in pregnancy has been correlated with increased risk of MTCT of HIV (Nair *et al* 1993; Mandelbrot *et al* 1996), although other studies have not found this association (Hira *et al* 1989; Bulterys *et al* 1993; Lepage *et al* 1993; St Louis *et al* 1993; Thomas *et al* 1994; Mofenson *et al* 1999). The reasons for the different results are not clear but could related to the influence of routine STD treatment practices in antenatal clinics and diagnostic methods used in the studies to diagnose STDs.

In the US, 30% of women with frequent unprotected sexual exposures during pregnancy transmitted HIV to their infant, compared to 9.1 % in women with no unprotected exposure (Matheson *et al* 1996). A similar association was reported in two African studies (Bulterys *et al* 1993; Lallemant *et al* 1994). Bulterys *et al* (1993) found no association of multiple sexual exposures with increased prevalence of STDs.

An increase in chorioamnionitis has previously been linked to sexual activity in pregnancy (Naeye & Ross 1983). Other possible mechanisms include increased HIV viral concentration with repeated sexual exposure, vaginal inflammation and abrasions and HIV strain diversity. These factors need further evaluation.

f) Intra-partum factors

With MTCT occurring mainly at the time of labour and delivery, obstetric factors are important determinants of transmission. Suggested mechanisms of intra-partum transmission include direct skin and mucous membrane contact between the infant and materno-cervical secretions during labour, ingestion of virus from these secretions and ascending infection (UNAIDS/WHO 1999).

i) Mode of delivery:

Mode of delivery cohort studies have yielded conflicting results. In some studies, a significant decrease in MTCT rate following caesarean section was observed (European Collaborative Study 1996; Kind *et al* 1998; Kuhn *et al* 1996) and in others there was no difference (Mandelbrot *et al* 1996; Landesman *et al* 1996; Simonds *et al 1998*). Two meta-analysis undertaken prior to 1994 reported overall decreases in MTCT rates of 25 to 30% associated with caesarean section (Villari *et* al 1993; Dunn *et al* 1994).

In March 1998, a European randomised trial was stopped after enrolling 439 women. Preliminary results indicated an MTCT rate of 1.7% among infants delivered by caesarean section compared with 10.7% among women randomised to vaginal delivery (The European Mode of Delivery Collaboration 1999).

ii) Prolonged rupture or membranes

The duration of labour does not appear to be as important as the duration of rupture of membranes for MTCT of HIV (Minkoff *et al* 1995). Prolonged rupture of membranes

has been shown to increase the risk of MTCT in several studies (Dunn *et al* 1994; Thomas 1994; European Collaborative Study 1994&1996, Landesman *et al* 1996; Kuhn *et al* 1997 & 1999; Garcia *et* a*l* 1999; Simonds *et al* 1997).

iii) Other intra-partum factors

Events during labour and delivery that expose the infant to the mother's blood may also be important for MTCT of HIV (Peckham & Gibb 1995). Such events could include antepartum haemorrhage, use of fetal scalp electrodes, episiotomy and lacerations, although it cannot be concluded from the existing data that these events increase the risk of transmission (Datta *et al* 1994; European Collaborative Study 1996; Landesman *et al* 1996; Mandelbrot *et al* 1997).

2.7 HIV and pregnancy and infant outcome

HIV infection may be a marker for poor obstetric outcome in women with previous pregnancies (Miotti *et al* 1990; Landesman *et al* 1996; Temmerman *et al* 1990; Broklehurst & French 1998).

A recent meta-analysis of 31 prospective cohorts summarised the Odds Ratios of the risk of adverse pregnancy outcomes, related to maternal HIV infection (table 2.12). Maternal HIV infection increased the risk of adverse outcomes, except for foetal abnormality and neonatal mortality. Morbidity in early infancy was closely related to care in pregnancy.

Table 2.12 Maternal HIV in pregnancy and adverse outcome: Meta-analysis results

Outcome	*Odds Ratio*	*95% CI*
Spontaneous abortion	4.05	2.75-5.96
Still birth	3.91	2.65-5.77
Foetal abnormality	1.08	0.7-1.66
Perinatal mortality	1.79	1.43-2.02
Neonatal mortality	1.10	0.63-1.93
Intrauterine-growth retardation	1.7	1.43-2.02
Low birth weight	2.09	1.86-2.35
Prematurity	1.83	1.63-2.06

Source: Brocklehurst & French 1998

Birth weight is the single most important determinant of childhood morbidity and mortality (McCormick 1985; Ashworth 1998). World Health Organisation (1992) estimates that low birth weight (LBW), as a result of prematurity or in utero growth retardation contributes significantly to the 9.1 million deaths each year. Kramer in 1987 extensively reviewed 895 studies to evaluate factors that contribute to LBW. He concluded that factors that have a direct causal effect on low birth weight included female sex, short stature, low caloric intake, inadequate weight gain in pregnancy, parity, previous history of low birth weight, general morbidity and episodic illness, malaria, cigarette smoking and alcohol consumption.

In low resource countries the major determinants were black or Indian racial origin, poor gestational nutrition, low pre-pregnancy weight, short stature, and malaria. In more industrialised countries, the most important single factor is cigarette smoking followed

by poor gestational nutrition and low pre-pregnancy weight. Data from industrialised countries suggested that maternal anaemia and iron deficiency increased LBW risk and premature delivery (Klebanoff *et al* 1991; Scholl & Hediger 1994) although the review from Kramer underplayed this effect. HIV/AIDS was not included in this analysis.

Low birth weight is associated with maternal HIV infection (Hira *et al* 1989; Taha *et al* 1995; Temmerman *et al* 1994). In Nairobi, Kenya the risk of delivering a pre-term or LBW baby was 1.9 or 2.6 (Temmerman *et al* 1994). In Zambia, Hira *et al* (1989) reported that the birth weights of babies born to HIV infected mothers were significantly lower than of those whose mothers were negative (OR 3.75).

It is projected that infant and child mortality will increase by the year 2010 due to AIDS, reversing the declines that were occurring in many countries over the past decade. The relative impact of AIDS on mortality will depend on both the level of HIV in the population and the infant and child mortality from other causes.

Today AIDS is affecting infant mortality rates (Stanecki & Way 1997). In Eastern Africa, for example, without AIDS, Kenya would have had an infant mortality rate of 46.9 per 1000 live births in 1997, but the rate was 55.3. Likewise in Southern Africa, in Zambia and Zimbabwe, the estimated infant mortality rates in 1997 were 25% higher than what they would have been without AIDS. In Zambia, the infant mortality rate was 96.1 per 1000 instead of 74.3 and in Zimbabwe it was 72.8 instead of 51.7. If the current HIV trends continue, both infant and child mortality will also continue to rise (table 2.13). By the year 2010, the infant mortality will be more than 60% higher and in Zambia, nearly half of the childhood deaths will be due to AIDS.

Table 2.13 Infant and child mortality rates with/ without AIDS by year 2010

	Infant Mortality Rate (/1000 live birth)s		*Child Mortality Rate(/1000 live birth)*	
Country	*With AIDS*	*Without AIDS*	*With AIDS*	*Without AIDS*
Kenya	55.9	32.9	110.3	45.4
Tanzania	90.9	65.2	166.1	95.8
Uganda	86.1	58.5	168.1	92.2
Botswana	66.1	26.3	147.5	38.3
Malawi	126.1	88.4	233.8	136
Zimbabwe	71.0	29.8	202.1	37.8
Zambia	97.0	58.4	152.9	96.9

Source: Stanecki and Way 1997

A number of African studies have reported increased risk of infant mortality associated with maternal HIV infection summarised in Table 2.14. There is also evidence of higher mortality among HIV infected children during the 2nd to the 5th years of life. In a cohort in Zambia, 44% of children considered HIV infected on clinical grounds had died by the 2nd year (Hira *et al* 1989).

In a three year cohort in Kinshasa, in DR Congo, mortality among HIV infected children was 44% after 3 years compared with 25% in negative children with HIV infected mothers and 6% when both the mother and child were negative (Ryder *et al* 1994).

In a 3-year cohort in Malawi, mortality in the first 2.2 years was 36% and 11% among children of HIV infected and non-infected mothers respectively (Taha *et al* 1995). Spira *et al* (1999) in their report of a 5 year natural history cohort of HIV-1 infection in children in Rwanda, Kigali, estimated that the mortality among HIV infected children at

2 and 5 years was 45% and 62% respectively, a rate 25 times higher than that of uninfected children. The median survival time after estimation of infection was 12.4 months. In a similar cohort in Kampala, Uganda amongst children with laboratory confirmed infection, 34% had died at 1 year, 66% at 3 years and 75% at 5 years (Berhane *et al* 1997; Marum *et al* 1977). Mortality at 1 year seemed lower in the Ugandan cohort. And the median survival was longer (21 months).

Table 2.14 Infant mortality by maternal HIV status in African studies

Study	***Reference***	***Number (% died)***	
		HIV Positive	***HIV Negative***
Abidjan, Cote d'Ivoire	De Cock *et al* 1994	77(13.3)	78(4.0)
Blantyre, Malawi	Taha *et al* 1995	694(22.3)	691(6.8)
Brazzavile, Congo	Lallemant *et al* 1989	64(39.0)	130(3.0)
Harare, Zimbabwe	Zijenah *et al* 1998	367(19.6)	372(25.4)
Kampala, Uganda	Marum *et al* 1997	387(16.3)	146(3.4)
Kinshasa, DR Congo	Ryder *et al* 1997	64(39.0)	130(3.0)
Kinshasa, DR Congo	Ryder *et al* 1994	333(24.9)	341(3.5)
Lusaka, Zambia	Hira *et al* 1989	109(17.4)	NA
Rakai, Uganda	Sewakambo *et al* 1994	NA(21.0)	NA(11.0)

NA - not available DR Democratic republic

2.8 Effect of pregnancy on the natural history of HIV infection

The question of whether or not pregnancy accelerates HIV replication and disease is critical to care of HIV infected women. There are no clear indications that women who have an intervening pregnancy are more likely to advance to AIDS defining illnesses or death sooner than women who do not have an intervening pregnancy in studies in Europe and the US (Cotton & Watts 1997). In pregnancy immunity is suppressed in both HIV infected and non-infected women (UNAIDS/WHO 1999). In earlier reports, these normal changes in pregnancy led to concern that pregnancy may facilitate HIV disease progression and (Jensen *et al* 1984). Follow up cohort studies, however, have failed to confirm an association (Brettle *et al* 1995; Landers *et al* 1997; Bessinger *et al* 1998; French et *al* 1998).

The data from low resource settings, on the other hand, suggests increased mortality in HIV infected pregnant women (Taha *et al* 1996; Ryder RW *et al 1994;* Ahmed *et al* 1999). Whether the increased mortality reflects pregnancy induced acceleration of disease or that more women with clinical disease are becoming pregnant is unclear. HIV infected pregnant women are more susceptible to P. falciparum malaria (Steketee *et al* 1996; Verhoeff *et al* 1998; Parise *et al* 1999)

2.9 Importance of descriptive cohorts on MTCT interventions in Zambia

The high prevalence of HIV in Zambia in women of the reproductive age group makes the prevention of MTCT a public health priority. In 1994 it became clear that MTCT could be interrupted through administration of zidovudine orally to women from 14 to 34 weeks of pregnancy, intravenously during labour and orally to the neonate in the first

6 weeks of life (Connor *et al* 1994*)*. In 1998, the efficacy of short course zidovudine (from 36 weeks) in non breast-feeding mothers in Thailand was demonstrated (Shaffer *et al* 1999*)*. This finding formed the basis for policy regarding the use of zidovudine in low resource countries adopted by WHO, UNAIDS and UNICEF in June 1998 (UNAIDS1998). Zambia is one of the UNAIDS pilot sites evaluating feasibility of implementation.

The prevention of MTCT can not be considered outside good basic understanding of the characteristics of the pregnant women and their children. Understanding the dynamics of the population to benefit from a given intervention is a critical step for control strategies. The dynamics of HIV infection are complex and there are lessons to be learnt in each country, as was shown in the Mwanza / Rakai experience with the control of STDs (Grosskurth 1995; Wawer 1999).

CHAPTER 3

SUMMARY OF METHODS

3.1 INTRODUCTION

With the recognition that paediatric HIV infection was a major problem in Zambia and paucity of data for programme advocacy and planning of interventions, this preliminary study was undertaken. The study was conducted at time when there was growing interest in developing and implementing a prevention programme for mother to child transmission (MTCT) of HIV, to be integrated within the Maternal and Child Health (MCH) Services in Zambia.

The study was divided into 5 broad themes to take account of issues related to both maternal HIV infection and maternal and child health:

1) To describe the characteristics of women delivering at the University Teaching Hospital (UTH) in Lusaka, Zambia and identify risk factors for maternal HIV infection.
2) To define post-partum anaemia. (PPA)
3) To define adverse pregnancy outcome.
4) To fine the characteristics of MTCT.
5) To define infant growth, morbidity, and mortality in relation to HIV

3.2 STUDY DESIGN

In February 1997, women delivering at UTH were enrolled into a prospective cohort study after obtaining an informed consent both for HIV testing and participation into the

study cohort. In order to meet the objectives of the study within the defined themes, 3 methodological approaches from the data collected were used:

1) Descriptive evaluation of cross-sectional and longitudinal data

2) Evaluation of probability of survival in infancy using longitudinal data, taking account of duration of follow up.

3) Unmatched case control evaluation of risk factors associated with maternal HIV infection, anaemia, LBW, preterm delivery, IUGR, MTCT and infant mortality.

Each result chapter describes the methods used in more detail.

3.3 STUDY SITE

The study was undertaken at the UTH, the only tertiary care referral hospital in Lusaka, a city with 1.3 million people. There are approximately 40,000 births in Lusaka, of which 12,000 occur at UTH. The non-UTH deliveries are conducted in 11 urban clinics, staffed exclusively by midwives. Apart from normal antenatal, intra-partum and post-natal care, all complicated cases at any stage of pregnancy, labour and puerperium are referred to UTH from these clinics, using standard referral guidelines.

3.4 STUDY SAMPLE

The sample size was calculated using the EPI INFO version 6.02 (Centers for disease Control and prevention, Atlanta, USA).

Using data from previous studies, the calculation was done with 2 main aims

1. To determine a 30% prevalence in pregnant women based on previous studies in Lusaka (Bhat et al 1998; MoH/CboH 1999).

2. To determine an infant mortality rate of 30% if the mother was HIV infected and 10% if non-infected (Spyra *et al* 1999), using a sample ratio for exposed/unexposed of 1:3 (1 HIV infected / 3 non-infected mothers).

For the first aim, it was estimated that 314 mother-child pairs would be enrolled onto the study. For second aim, the calculated sample size for infants of infected and non-infected mothers was 44 and 132. After allowing for a 30% loss to follow up, the sample sizes rose to 57 and 172.

The study sample had enough statistical power to detect a prevalence of; post-partum anaemia of 60% with a relative risk of 2.3 and LBW of 20% with a relative risk of 2.2 in infected mothers. The calculations were performed with a confidence level of 95% and power of 80%.

3.5 SELECTION OF STUDY POPULATION AND ENROLMENT

The study population included all consecutive women, presenting in the first stage of labour, between February and July 1997. Midwives, with HIV counselling skills, enrolled women each morning and evening from Sunday to Thursday. The afternoons were left for laboratory processing of blood samples collected in the morning. The samples collected in the evening were processed in the morning. Only women with a live birth, residing within a 10-kilometer radius from the University Teaching Hospital (UTH), were enrolled onto the study. The mothers were interviewed using a structured questionnaire. The weight of the mother and baby were taken using a Salter (England, West Bromwich, England 235 6S, 0-25kg) and bathroom electronic scale respectively. Measurements taken included the mother's mid-upper arm circumference (MUAC) and

the length of the baby as described in Chapter 4 and Chapter 6. Three measurements were taken and the mean calculated.

3.6 FOLLOW UP

After delivery, a follow up appointment was given to each mother, scheduled at 1 month initially and later 4, 7, 10 12 and 18 months. A study clinic was set up in the department of paediatrics and any mother who did not come for scheduled appointments was followed up actively at home by the research midwives. Pre and post-test HIV counselling was conducted by midwife counsellor, at the 1 month visit, for mothers who wished to know their status. A morbidity (mother and child) and infant feeding questionnaire was completed in at each visit (appendix 3.2). If the child or mother were unwell, a study clinical assistant attended to them in the research clinic. The mothers with positive rapid plasma reagin (RPR) and their infants were treated with benzathine penicillin (2.5 mega-units I.M. weekly for 3 weeks) and procaine penicillin (50,000 I.U./kg I.M. for 10 days) respectively.

3.7 MATERNAL INFANT FACTORS AT ENROLMENT

Information on maternal age, parity, gravidae, marital status, past and current medical and obstetric history was collected using a structured questionnaire (appendix 2.1). For the current history, information collected included malaria, antibiotic and tuberculosis treatment during pregnancy, any alcohol intake during pregnancy, ante-partum haemorrhage (ante-partum haemorrhage or APH), gestational age using the last menstrual period, duration of rupture of membranes and mode of delivery. Maternal

venous blood was collected for HIV serology, quantification of viral load, CD4 cell count and percentage, serum retinol, full blood count, RPR, HBsAg and hepatitis C serology. Baby blood was collected by heel prick for HIV PCR assessment.

3.8 MATERNAL AND INFANT FACTORS AT FOLLOW UP

The women together with their infants were followed up at 1, 4, 7, 10 and 12 months. Information on illness in the infant, requiring treatment at a health facility, and on feeding practices including breast disease was collected at each visit. The infants had their weights and heights measured at each visit. Illness in between visits in terms of clinic attendance and hospitalisation was recorded. Blood was collected from the baby for PCR estimation at 1,4,7 and 12 months and from the mother for Hb concentration. Where possible, blood was collected from the baby at 18 months for HIV serology to confirm the PCR results.

3.9 HIV COUNSELLING

Mothers wishing to know their HIV status were counselled at the one-month follow up visit by trained midwives.

3.10 LABORATORY INVESTIGATIONS

The laboratory tests were done in 3 laboratories, the University Teaching Hospital (UTH) Virology Laboratory, Lusaka, Zambia (Hb, haematocrit, MCV, CD4 cell count, RPR, HIV serology hepatitis B surface antigen and hepatitis C serology), Tropical Disease Research Institute (TDRC), Ndola, Zambia (serum retinol) and

Yamanashi University, Tokyo, Japan (validation of CD4 cell count, qualitative DNA PCR, quantitative RNA). Internal quality control was according to the systems already developed in the individual laboratories. For UTH Virology Laboratory Yamanashi University provided external quality control, on 1% of the samples and for TDRC, the serum retinol quality control was done at StellenBosch University in Cape Town, South Africa (see section on serum retinol).

3.10.1 Maternal and child (18 months) HIV Serology

The HIV antibody status in serum or plasma was determined using three types of assay kits according, in accordance with the manufacturers specifications: Capillus (Cambridge Diagnostics Iceland Ltd., Galway, Iceland) and Serodia HIV (FUJIREBIO INC., Tokyo, Japan) to detect HIV-1/2 antibodies and Wellcozyme VK56 enzyme-linked immunosorbent assay (ELISA) (Murex Diagnostics Ltd., Dartford, England) to detect HIV-1 antibodies. These tests were the tests in use in the laboratory at the time of the study. All new tests in the virology laboratory are evaluated using western blot as gold standard. The Capillus test served as the screening test (sensititivity100%) on previous laboratory evaluation. Negative results were reported as negative and all positive samples had repeat testing with Serodia (specificity 99%). If the results were discordant the Wellcozyme ELISA was used (specificity 100%).

3.10.2 HIV viral load (Amplicor monitor version 1.5, Roche Diagnostic Systems)

Maternal viral load measurements were carried out using the AMPLICOR HIV-1 MONITOR test kit (Roche Diagnostic System, INC., Branchburg, NJ, USA). This kit

permits reverse transcription and amplification of HIV-1 and quantitation standard (QS) RNA to occur simultaneously. Briefly, 200 µl of patient plasma was used for RNA extraction according to manufacturer's instructions. The processed specimens were added to the amplification mixture in reaction tubes in which both reverse transcription and PCR amplification occurred. The reaction mixture was heated to 50°C for the RT reaction and held for 2 minutes to allow specific binding of HIV-1 and QS target RNA and then at 60°C for 30 minutes for cDNA synthesis. PCR amplification using the GeneAmp PCR System 2400 thermal cyclers (Perkin-Elmer Cetus, Instrument Division, Norwalk, USA) was achieved by subjecting the reaction mixture to 4 cycles, each of 95°C, for 10 seconds (denaturation), 55°C for 10 seconds (annealing) and 72°C for 10 seconds (extension), followed by 26 cycles each at 90°C for 10 seconds, 60°C for 10 seconds and 72°C for 10 seconds (extended for 15 minutes in the last cycle) for denaturation, annealing and extension respectively. Hybridization and detection of amplified product were carried out in a micro-well-plate coated with HIV-1 and QS-specific oligonucleotide probes for patient samples and QS amplicons respectively. The micro-well-plate was then washed and 100 µl of Avid-HRP conjugate was added to each well and incubated for 15 minutes at 37°C. Optical density was measured at 450 nm.

3.10.3 In-house qualitative DNA PCR

The whole blood collected from the babies was stored at –80 and transported to Yamanashi University in Japan on dry ice for processing, using an in-house nested-PCR developed for testing Zambian whole blood samples, in collaboration with the virology

laboratory at UTH (Handema *et al* 1999). The sensitivity and specificity of this method on adult samples was 94 and 100%, using antibody serology as a gold standard. The lower limit of detection of viral DNA was 3.5 copies per ml of reaction tube.

Step 1. Lysis of red blood cells

Due to difficulties, which might be encountered in amplifying (by PCR) DNA extracted from whole blood (because of interference with haem), red blood cells (RBC) were first lysed.

a) 10X lysis buffer solution was prepared dissolving 89.9g NH_4Cl, 10.0g $KHCO_3$ and 0.37 g EDTA.4Na in one litre of distilled water.

b) 1X of the lysis buffer was used to lyse the cells.

c) 1.5 ml of the 1X lysis buffer was added to 100µl of whole blood.

d) The mixture was vortexed for 2 minutes, incubated at room temperature for 10 minutes and then centrifuged at 1000 rpm (Eppendorf Bench top centrifuge Model 5417R, Germany) for 5 minutes.

e) The supernatant was poured off, the pellet washed for with 1.5ml of PBS and then vortexed for 1 minute.

f) The mixture was centrifuged at 1000 rpm for 5 minutes

g) Step e and f were repeated and the liquid poured off leaving about 100 µl with the pellet.

Step 2 Extraction of DNA

Proteinase K (Tritirachium album limber, Worthington catalogue) was used for DNA extraction. Proteinase K is serine protease with broad specificity towards aliphatic, aromatic and hydrophobic amino acid. It has applications in molecular cloning and DNA sequencing , nucleic acid research and protein and peptide structural analysis.

a) Proteinase digestion buffer was prepared by adding 2ml of 0.5M EDTA pH 8.0 (final concentration 20mM), 1ml 1M Tris-Cl pH 8.0 (final concentration 20mM) 2.5 ml 10% SDS (final concentration 0.5%) and 44.5 ml distilled water. The buffer was stored at room temperature.

b) The required volume of proteinase digestion buffer was prepared with proteinase K (199 µl of proteinase digestion buffer and 1µl of 20 mg/ml proteinase K per tube).

c) 200 µl was of this mixture was added to the cell suspension in the tube. The sample was incubated overnight at 60°C

d) The mixture was briefly spun down and 300 µl of TE-saturated phenol and 300 µl of 24:1 chloroform /isoamyl alcohol added to the digestion mixture.

e) The mixture was mixed gently by inverting the tube several times and then micro-centrifuged at 12000 rpm for 10 minutes at room temperature. The aqueous base containing the DNA was transferred to a new micro centrifuge tube.

f) The aqueous phase was extracted twice 300 µl of 24:1 chloroform /isomyl alcohol and centrifuging at 1000 rpm for 10 minutes at room temperature, each time to separate the phases.

g) 10 M ammonium acetate was added to 2.5 M (final), in the last aqueous solution, in the aqueous phase and mixed gently. For example, if the volume of aqueous phase was 200 µl , the volume of 10M ammonium acetate added was 66.7 µl.

h) 5 volume of cold 100% ethanol was added and tube inverted gently several times to mix. The tubes were placed at -20°C overnight.

i) The pellet mixture was micro-centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was then poured out.

j) The pellet was washed with 1000 µl of 70% ethanol by inverting the tube several times, micro-centrifuged at 12,000 rpm for 15 minutes for 15 minutes.

k) The supernatant was then poured off and the pellet air dried at room temperature for 40 minutes.

l) The pellet was dissolved in 25 µl of distilled water by placing the micro-centrifuge tube at 60°C (water bath) for 15 minutes.

m) The DNA was stored at 4°C

Step 3 Amplification

Amplification of the *pol* region was 2 staged involving two pairs of primers, HPOL4235-2 (5'-CCCTACAATCCCCAAAGTCA-3') nt 4235 to 4255 and HPOL4538 (5'-TACTGCCCCTTCACCTTTCCA-3') nt 4538 to 4559 was used in the first amplification and HPOL4327 (5'-TAAGACAGCAGTACAAATGGCAG-3') nt 4327 to 4350 and HPOL4481 (5'-GCTGTCCCTGTAATAAACCCG-3') nt 4481 TO 4502 was used for the second amplification.

The PCR were performed in a total volume of 100 µl and 50 µl of reaction mixture in the first and second amplification, respectively. The reaction mixture contained 500 µM KCL, 100 µM TRIS-HCL (pH 8.0), 1.5 mM $MgCl_2$, 0.5 µM EDTA, 0.0025% Tween 20, 5 µM DTT, 0.25% Glycerol, 200 µM each dNTPs (dATP, dTTP, dGTP, dCTP), 0.025 units/µl of *Taq* DNA polymerase (NIPPON GENE, Toyama, Japan), 0.5 pmol/µl of each primer and DNA sample in a total volume of 100ml. Round one amplification was done under the following cycling conditions: temperature for the first denaturation was held at 94°C for 2 minutes followed by 35 cycles each at 94°C for 1 minute (denaturation), 50°C for 1 minute (annealing) and 72°C for 1 minute (extension), with an extra extension period of 7 minutes in the last cycle in a DNA thermal cycler machine (DNA Thermal Cycler 480, Perkin Elmer Cetus, Instrument Division, Norwalk, USA). One microlitre of the amplified DNA was used in the second round amplification of 25 cycles under the same cycling conditions as described.

Eight microlitres of the amplicons and a marker (TAKARA SHUZO LTD., Otsu, Japan) were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized by transillumination at 254 nm. A PCR reaction was considered as positive when a band of the right size from the second round amplification was observed.

Specimen processing for DNA extraction and preparation of PCR reaction mixture were performed in different rooms to avoid contamination.

3.10.4 CD4 T-lymphocyte counting

CD4 measurement was done within 12 hours of blood collection

Adherent CD3

The typing of lymphocyte subsets was performed by flow cytometry (FACScan, Becton Dickinson, San Jose, California) and the total lymphocyte count was performed by an automated blood analyzer (Coulter Counter; Coultronics, Margancy). The following monoclonal antibodies were used for staining: phycoerythrin labelled CD4+ (Becton Dickinson, San Jose, California) and FITC labelled CD3+ (Becton Dickinson, San Jose, California). EDTA-treated blood was stained with combinations of CD3+/CD4+ monoclonal antibodies following the manufacturer's instruction. The blood was incubated at room temperature in the dark. FACS lysing solution (Becton Dickinson, San Jose, California) was used to lyse the red blood cells and the labelled cells were washed with FACS Cell Wash and fixed with FACS Cell Fix solution (Becton Dickinson, San Jose, California). The labelled cells were then counted and analysed by FACScan.

3.10.5 Haemoglobin, MCV and MCHC

Haemoglobin, packed cell volume (PCV) and mean corpuscular volume (MCV) were obtained using an automated cell counter (Coulter Counter; Coultronics, Margancy) calibrated regularly according to the manufacturers specifications using appropriate controls. The MCHC was calculated as: Hb(g/dl)x100/PCV%.

3.10.6 Rapid plasma reagin

Papid plasma reagin (RPR) was carried out using a standard RPR kit according to the manufacturers specifications. The sample was withdrawn with pipstirrer, taking care not to transfer any cellular elements. One drop of the sample was placed carefully onto the

test card. The sample was spread over the entire area of the test circle. One drop of the antigen was the added to the test sample and at 8 minutes of the test card was examined macroscopically for agglutination particles.

3.10.7 Hepatitis B Surface antigen

A sub-sample of serum was tested, according to the manufacturer's specifications, using the ***wellcozyme HBsAg*** test, a rapid sensitive radio-immunoassay.

a) 50µl of conjugate containing freeze dried mouse monoclonal antibody to HBsAg labelled alkaline phosphatase, was reconstituted 20 minutes prior to use and added to each mouse monoclonal antibody coated microwell.

b) 150 µl of negative control (normal human serum non-reactive for HBsAg) was added to 2 wells.

c) 150 µl of positive control (heat inactivated human serum reactive for HBsAg) were added to 2 wells.

d) 150 µl of the sample was added to the other wells.

e) The wells were then incubated at 20-25°C for 20 hours.

f) The prepared wells were washed at the end of the incubation period.

g) 50 µl of the substrate solution (freeze dried NADP) was reconstituted and added to each well. The wells were then incubated for at 20-25°C for 40 minutes.

h) 100µl amplifier solution (alcohol dehydrogenase and diaphorase freeze dried in a protein base) was reconstituted and added to each well and incubated at 20-25°C for 10 minutes.

i) 50µl of stop solution was added to each well.

j) The specimens were read at 429nm within 15 minutes using a microwell reader (Dyna tech).

k) The results were obtained as absorbency at 492 nm. The cut-off value was arrived at by adding 0.10 absorbancy units to the negative control mean.

3.10.8 Hepatitis C serology

A sub-sample of serum was tested for hepatitis C, according to the manufacturer's specifications, using Serodia (Fugirebio, Tokyo, Japan) HCV gelatin particle (sensitized with recombinant antigens).

Agglutination test serum diluent was placed into 3 wells of a microplate in equal volume (25μl). Plasma or serum of the specimen (25μl) was added to diluent. The mixture was mixed well. 25μl of the mixture was transferred to well 2 and mixed. The procedure was repeated for well 3. The control was added to well 2 and the sensitised particle to well 3. The contents were mixed with a tray mixer for 8 to 10 minutes. The micro-plate was allowed to stand for at room temperature for 2 hours. The results were read according to the interpretation table supplied with the kit.

3.10.9 Serum retinol

A sub-sample of blood (2ml) was placed in an opaque container for estimation of serum retinol. Blood was centrifuged on the day of collection, serum separated and stored at 4°C at UTH. It transported to Ndola (4 hours away by car) in a cooler box with ice packs for processing at the Tropical Disease Research Centre. 50 μl of 50 μg/ml ethanolic retinyl acetate was added to 50μl of serum sample in a screw glass top tube

and vortexed machine for 15 seconds to precipitate serum proteins. 750 µl of n-hexane was added and the contents vortex mixed for 45 seconds. The mixture was then centrifuged at 300 rpm for 2 minutes before the top hexane layer was was separated to a separated tube. A second extraction with n-hexane was done using the same procedure. The double extracted n-hexane was then separated evaporated to dryness under a stream of nitrogen gas and the residue redissolved in dichloromethane: propanol (4:1 v/v) solution. Using a hamilton syringe 20µl of this solvent was applied onto the column for separation.

HPLC conditions:

Column: Supelcosil LC 18 5um 25cm x 4.6 mm i.d.

Slovent: methanol/water (98:2)

Flow Rate: 2.0 ml/min

Temperature: Ambient

Standardization

For internal standardization retinyl acetate was used. The concentration of the standard was calculated using an absorbency read at 325nm using E^1 % of 1560 in ethanol. Known concentrations of retinol and retinyl acetate were entered into the integrator that was programmed to calculate retinol levels based on chromatogram areas. A E^1 % of 1835 was used to calculate the value of retinol at 325 nm.

Quality Control

Stellenbosch University, Department of Human Nutrition, Cape Town, South Africa, provided external quality control of the laboratory analysis. This was done in three phases as follows:

1. Analysis by TDRC of a 'high level' control sample sent by Stellenbosch laboratory. This sample was analysed 45 times on different days.
2. Analysis by TDRC of a 'low level' control sample sent by Stellenbosch Laboratory. This sample was analysed twenty two times on different days.
3. Analysis of randomly selected study field samples by both TDRC and Stellenbosch

The data obtained indicated that, apart from one or two examples in the low retinol level samples, TDRC was able to identify samples from deficient subjects compared to those from normal subjects. However, the upward deviation in results from the external "low retinol" control and those from comparison data from sample analysed by TDRC and those by DOHN (differences versus means) suggested a possible bias. Application of a correction factor (0.82, based on comparison of TDRC and DOHN "low level" controls) to TDRC's results so as to counter this bias was recommended.

3.11 RELEVANCE OF STUDY METHODS

Women who were in the first stage of labour were invited to participate in study after an informed consent in order to cut down the cost of the study. Enrolling women ante-natally would have meant losing over 30% of the sample of women who deliver outside a health facility or closer to their home.

It is often possible to investigate a particular question using either a descriptive cross-sectional, case control or cohort design. To define the prevalence of a disease or an event cross-sectional data is ideal. It was possible to determine the prevalence rates such as maternal HIV infection, LBW, pre-term delivery, IUGR, antenatal and post-partum anaemia with the cross-sectional data collected at enrolment of the women and the infants.

One major source of bias is the selection of the study site. The study population was a select group of women from an urban tertiary referral hospital, catering for all high-risk pregnancies. Other potential biases in the study include recall and data bias. The women were asked about events such as the last menstrual period and antibiotic and malaria treatment during pregnancy, without any methods of validating the information received. The nurses underwent 3 days training to standardise data information collection and measurements. Observer error was checked using the World Health Organisation standard method (1983)

Missing values between cases were a major concern during analysis. A comparative analysis of nutritional and biological factors shown in table 4.2 was done to establish whether there were major differences in proportions determined when all cases were included in the analysis compared to when cases with missing values were excluded. Antenatal haemoglobin was not included in the data because only 56 women had haemoglobin screening during routine antenatal services. The results indicated no significant differences in proportions, suggesting that the missing values were probably randomly distributed amongst cases (table 3). Because of the small sample size (306), subsquent analysis did not exclude cases with missing values.

Table 3 ***Comparison of occurrence nutritional and biological factors; an analysis including all cases compaed to when cases with missing values are excluded***

Study Variable	*All cases included n/N (%)*	*Cases with missing values excluded n/N (%)*	*P Value*
Poor post-partum maternal weight (<50kg)	46/282 (16.3)	30/191 (15.7)	0.86
Poor post-partum maternal weight (<45kg)	13/282 (4.6)	10/191 (4.2)	0.76
Low post-partum MUAC (<23cm)	24/298 (8.1)	14/191 (7.3)	0.90
Post-partum anaemia (Hb< 11g/dl)	152/306 (49.7)	92/191 (48.2)	0.74
Post-partum severe anaemia (Hb< 7g/dl)	19/306 (6.2)	13/191 (6.8)	0.79
Low post-partum serum retinol (<1.05μmol/L)	108/304 (35.5)	69/191 (36.1)	0.89
Low post-partum serum retinol (<0.7μmol/L)	39/304 (12.8)	24/191 (13.1))	0.93
Post-partum CD4 cell count (<700 cells/mm^3)	152/297 (51.2)	94/191 (49.2)	0.67
Post-partum CD4 count cell (<400 cells /mm^3)	48/297 (16.2)	31/191 (16.2)	0.98

MUAC mid-upper arm circumference, Hb haemoglobin

To answer the question of risk factors associated with particular events, such as low birth weight, post-partum anaemia, a population based case control study approach was adopted from the cross-sectional data. Out the women or children enrolled, cases (eg HIV infected women) were defined and those unaffected were classified as controls. This methodology allowed the estimation of the relative risk associated with the specific variables.

A longitudinal cohort was conducted to address growth patterns, morbidity and mortality of the children. Sixty of the 306 women did not come back for the one month follow up visit probably because they did not want to discuss their HIV results, despite having reassured them that the dissemination of results was going to be voluntary. One

hundred and fifty six of the 302 women and their infants (51.7%) were seen at the 12-month visit. The limitations of the laboratory investigations are discussed in the individual chapters.

CHAPTER 4

CHARACTERISTICS OF WOMEN DELIVERING AT THE UNIVERSITY TEACHING HOSPITAL AND HIV-1 INFECTION

4.1 INTRODUCTION AND OBJECTIVES

Heterosexual transmission is the major cause of HIV infection amongst adults in Zambia and women in the reproductive age group are at high risk of acquiring infection. HIV infection has become one of the leading causes of morbidity amongst pregnant women and their children in Zambia. The reproductive health care package in Zambia should be modified to include HIV/AIDS. However, a thorough understanding of the characteristics of maternal HIV infection is crucial for effective and targeted planning of interventions, not only for the prevention of maternal HIV infection, but also safe motherhood strategies for HIV infected women.

The objectives of this chapter were:

a) To describe the socio-demographic and biological characteristics of women delivering at UTH.

b) To determine the prevalence of maternal HIV infection.

c) To identify risk factors associated maternal HIV infection.

4.1.1 Background

In 1998, the estimated national HIV prevalence, using antenatal women (between 15 to 49 years of age) sentinel surveillance data, was 19.7%, 28% in urban and 13.6% in rural areas (MoH/CBoH 1999). In Lusaka, the HIV prevalence rate amongst antenatal

women was estimated as 27.3%. At the University Teaching Hospital (UTH), a referral tertiary hospital in Lusaka, there has been an increasing HIV prevalence antenatal women, 12% in 1989 to 29.5% in 1998 (Hira *et al*, 1989; Bhat *et al* 1998).

Despite this knowledge, until recently, very little has been done to address the issue of HIV in pregnant women attending antenatal clinics in Zambia. Research on HIV infection both in pregnancy and in children has been minimal. At UTH, maternal HIV infection has not received much attention. At the time of the study, voluntary counselling and testing services for pregnant women were not available and HIV was not routinely discussed in the antenatal clinic.

4.2 METHODS

4.2.1 Enrolment

Between February and July 1997, a systematic sample of women presenting at the UTH in established early labour (cervical dilation of 3 to 5 centimetres) were asked for consent to enrol into the study cohort and to have blood investigations done on their babies and themselves. All consenting women, with a live delivery, were interviewed within 24 hours of delivery using a standard structured questionnaire (appendix 3.1).

4.2.2 Questionnaire and subject management

The women were asked about their background characteristics. These characteristics included; age, marital status and duration of marriage, literacy status, occupation including that of their partner, number of pregnancies and children, the economic background, obstetric and medical history, pregnancy morbidity and social habits such as smoking and alcohol intake. Asking the woman to read a simple script in English or

in her local language, which ever was appropriate, as well as to write her name was used to assess literacy. Information collected from the women's antenatal card included recorded treatment for malaria and antibiotics used for infection, antenatal Hb and the syphilis test results. These services are routinely provided in the antenatal clinics.

After delivery, the women were weighed and neonatal head circumference and length measured. Since enrolment of the women was after delivery and pre-pregnancy weight was not known, the mid-upper arm circumference (MUAC; left arm and hanging loosely) was used to assess maternal nutritional status during pregnancy (Krosovec 1990).

4.2.3 Blood Investigations

All maternal samples were tested for HIV type-1. HIV infection was defined as two positive antibody tests. The HIV antibody status in serum or plasma was determined using three types of assay kits according, in accordance with the manufacturers specifications: Capillus (Cambridge Diagnostics Iceland Ltd., Galway, Iceland) and Serodia HIV (FUJIREBIO INC., Tokyo, Japan) to detect HIV-1/2 antibodies and Wellcozyme VK56 enzyme-linked immunosorbent assay (ELISA) (Murex Diagnostics Ltd., Dartford, England) to detect HIV-1 antibodies. The Capillus test served as the screening test. Negative results were reported as negative and all positive samples had repeat testing with Serodia. If the results were discordant the Wellcozyme ELISA was used. (Wellcozyme Recombinant HIV-1 VK 57/57, Murex Diagnostics, Dartford, UK) with confirmation of positives by a particle agglutination test (Serodia HIV, Fujirebio,

Tokyo, Japan). This algorithm has previously been pre-evaluated in the laboratory. Rapid plasma reagin (RPR) was done to screen for maternal syphilis.

The serum was screened for hepatitis B surface antigen (HBsAg) and hepatitis C antibody by ELISA (Wellcozyme, UK) and passive haemaglutination test (Serodia Fugirebio, Tokyo, Japan), respectively.

A full blood count (FBC) including Hb and red cell indices was done by Coulter Counter (Coulter Counter; Coultronics, Margancy) calibrated regularly according to the manufacturer's specifications using appropriate controls. Absolute T lymphocyte subsets (CD4 and CD8) were measured, within 24 hours of blood collection, using a Facscan (Becton Dickenson, San Jose, USA). Validation of the results was carried out at Yamanashi University, Japan. Serum retinol was measured by high performance liquid chromatography (HPLC). Stellenbosch University in Cape Town, South Africa undertook quality control of the HPLC results.

Maternal HIV RNA viral load was measured using plasma samples frozen at -70 and assayed by quantitative reverse transcriptase polymerase chain reaction (Roche Molecular Systems, Branchburg, New Jersey, USA). The babies had heel prick samples taken for HIV DNA PCR determined using an in-house methodology (Chapter 3 and 7)

4.2.4 Follow Up

A postnatal follow appointment a month after delivery was given when HIV counselling was available for women wishing to know their HIV results. Other results were also discussed at this visit. Women found to be RPR positive were treated with benzathine penicillin 2.4 mega-units weekly for 3 weeks. The partners of the RPR positive women

were also contacted to receive similar treatment. Blood samples were drawn from the women for Hb including red cell indices estimation and CD4 cell count (Chapter 7 and 8)

4.2.5 Statistical analysis

The statistical programme SPSS (version 10) and EPI-INFO 6.02 (Centers for Disease Control and Prevention, Atlanta, GA) were used for analysis.

Background characteristics were analysed using means and standard deviation as well as proportions and 95% confidence limits. Range was provided for continuous variables and geometric mean or median was calculated for data that was not normally distributed. The standard deviation (SD) of the geometric mean was calculated using the formula: gemetric SD = antilog (log mean + log SD) – geometric mean.

Women were stratified as HIV infected or non-infected. Analytical tests for discrete variables associated with HIV infection were Pearson's X^2 or *Fisher* exact test. The independent *t*-test was used to compare continuous variables. Relative risk estimates with 95% confidence intervals were estimated. Risk factors with a $P<0.05$ on univariate analysis as well as those considered to be of public health significance were fitted in a multiple logistic regression model to determine independent factors associated with HIV infection after adjusting for the effect of the other variables. Modelling was accomplished by sequential removal of each variable found least significant on each analysis. All tests were two tailed at $P < 0.05$.

4.2.6 Definitions

Poor nutritional status using anthropometric indices was defined as post-partum maternal weight of less than 45 or 50 kilograms and a maternal MUAC of less than 23 centimetres (Krosovec 1990). Anaemia and severe anaemia were defined as Hb concentration of less than 11g/dl and 7 g/dl (WHO 1992). Antenatal anaemia was determined with antenatal booking Hb and post-partum anaemia referred to anaemia detected within 24 hours of delivery. 80fl and 31g/dl were cut points for low MCV and MCHC (Tietz 1990) and low CD4 count was defined as cell count of less than 400 and 700 cells/mm^3 (European Collaborative Study 1996). 20μg/dl and 30μg/dl were the cut-off points for low serum retinol (WHO 1996). The viral load cut-off points were 1,000 and 10,000 copies /ml (Mayaux *et al* 1995). The medical histories such hypertension and tuberculosis were extracted from the patients record.

4.3 RESULTS

4.3.1 Socio-demographic background

Of the 382 women that delivered during the study period, 306 (80%) were enrolled in the study after informed consent was obtained. Most women who refused to be enrolled did not wish to have an HIV test. The socio-demographic characteristics of the women enrolled are shown in table 4.1. The majority were aged 20 years and above (87.3%), could read and write (73.7%) and were married (91.4%). The geometric mean (±SD) duration of marriage was 3.9 (2.9) years (range 0.1 to 38 years).

Most of the women did not have a source of income (75.7%) whereas 90.8% of their spouses were wage earners. The partners' were engaged in professional and technical work (27.1%), business (22.5%) and non-professional jobs (17%).

Table 4.1: Socio-demographic characteristics

Characteristic	*n/N*	*% (95% CI)*
Age (20 years and above)	261/299	87.3 (83.5-91.1)
Marital status (married)	275/301	91.4 (88.2-94.6)
Literate (read and write)	216/293	73.7 (68.7-78.7)
Partner earns income	268/295	90.8 (87.5-94.1)
Mother with no income	206/272	75.7 (70.6-80.8)
Maternal alcohol intake	29/285	10.2 (6.7-13.7)
Maternal cigarette smoking	4/291	1.4 (0-2.7)

The 66 working women were either secretaries or clerks (12.2%) or engaged in business, mainly selling goods at a market (7.4%). Of the social habits, 10.2% of women admitted to drinking alcohol but cigarette smoking was rare (1.4%).

1. Anthropometric measurements

Out of the 306 women, 298 and 282 had post-partum MUAC and weight recorded. The mean (± SD) post-partum maternal weight was 57.6 (10.1) kilograms (range 38 to 110 kilograms) and MUAC was 25.9 (2.9) centimetres (range 18 to 40 centimetres). A few women (8.1%) had a post-partum MUAC of less than 23cm. The proportion of women with a post-partum weight less than 45 kilograms was 4.6% (table 4.2).

2. Anaemia prevalence

Anaemia prevalence is described in table 4.2. Of the 306 women, 56 (18.3%) had antenatal Hb entered on their antenatal record. The mean antenatal Hb (± SD) was 10.9 (2.0) g/dl (range 6.3 to 14.1g/dl). Almost half (48.2%) of the 56 women were anaemic (Hb <11g/dl). The proportion with severe anaemia (Hb < 7mg/dl) was 1.8%.

4.3.2 Nutritional status and biological factors

Table 4.2: Nutritional and biological characteristics

Characteristic	*n/N*	*% (95% CI)*
Poor post-partum maternal weight (<50kg)	46/282	16.3 (12-20.6)
Poor post-partum maternal weight (<45kg)	13/282	4.6 (2.1-7)
Low post-partum MUAC (<23cm)	24/298	8.1 (5-11.2)
Antenatal anaemia (Hb<11g/dl)	27/56	48.2 (31.1-61.3)
Antenatal severe anaemia (Hb<7g/dl)	2/56	1.8 (0-5.7)
Post-partum anaemia (Hb< 11g/dl)	152/306	49.7 (44.1-55.3)
Post-partum severe anaemia (Hb< 7g/dl)	19/306	6.2 (3.5-8.9)
Antenatal and post-partum anaemia[1]	17/27	63 (44.8-81.2)
Antenatal and post-partum severe anaemia[2]	5/27	18.5 (3.8-33.1)
Normal antenatal Hb but post-partum anaemia	17/29	58.6 (40.7-76.5)
Normal antenatal Hb but post-partum severe anaemia	1/29	3.2 (0-9.6)
Low post-partum serum retinol (<1.05µmol/L)	108/304	35.5 (30.1-40.9)
Low post-partum serum retinol (<0.7µmol/L)	39/304	12.8 (9-16.6)
Post-partum CD4 cell count (<700 cells/mm^3)	152/297	51.2 (45.5-56.8)
Post-partum CD4 count cell (<400 cells /mm^3)	48/297	16.2 (12.0-20.4)

[1]Antenatal anaemia (Hb<11g/dl) and postnatal anaemia (Hb <11g/dl)

[2] Antenatal anaemia (Hb<11g/dl) and postnatal severe anaemia (Hb <7g/dl)

All 306 women were screened for anaemia post-partum at enrolment. The mean (± SD) post-partum Hb was 10.7 (2.3) g/dl (range: 2 to16g/dl). Post-partum anaemia prevalence was similar (46.7%) to that of the antenatal period (48.2%). Although severe anaemia was slightly higher in the post-partum period (6.2%) than in the antenatal period (1.8%), this difference was not statistically significant.

In the 56 women who had an antenatal Hb record, the prevalence of anaemia (<11g/dl l) significantly increased post-partum (P value < 0.001) (figure 4.1) although the increase in severe anaemia was not statistically significant. Seventeen (63%) of the 27 women with antenatal anaemia were still anaemic post-partum and in 5 (18.5%) the anaemia was severe. Of the 29 women with no antenatal anaemia, 58.6% had post-partum anaemia and severe anaemia was found in 3.4%

3. Vitamin A status

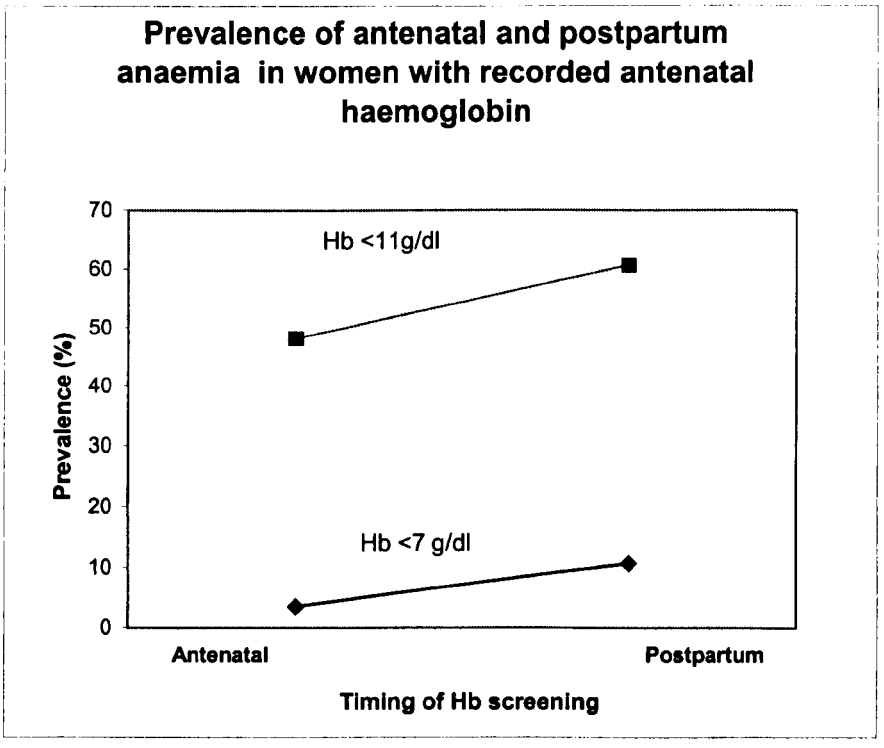
Three hundred and four of the 306 specimens collected had sufficient blood for serum retinol analysis. The mean (± SD) serum retinol was 1.24(0.47) μmol/L (range 0.06 to 2.71μmol/L). Low serum retinol of less than 0.7μmol/L and 1.05μmol/L was found in 12.8% and 35.5% of the women respectively (table 4.2).

4. CD4 cell counts

CD4 cell counts were estimated in 297 of the 306 samples collected. The geometric mean (± SD) of the CD4 cell count was 627.9(540) cells/mm^3 (range 78-3589 cells/mm^3). One hundred and fifty two (51.2%) of the women had CD4 cell counts less

than 700 cells/mm^3. The proportion of women with CD4 cell counts below 400 cells per mm^3 was 16.2% (table 4.2).

Figure 4.1:



4.3.3 Obstetric characteristics

Table 4.3 describes obstetric characteristics. Almost one third (30.4%) of the women were primiparous. The median number of children per woman was 2 children (range 1 to 12). About a third (32.4%) reported having lost a child and 16.4% had had a previous abortion. The commonest cause of morbidity during the pregnancy was suspected malaria (32.6%).

Table 4.3: Obstetric characteristics

Characteristic	*n/N*	*% (95% CI)*
Primiparous	91/299	30.4 (25.2-35.6)
Previous history of:		
Prematurity	23/299	7.7 (4.7-10.7)
Abortion	49/299	16.4 (12.2-20.6)
Child death	97/299	32.4 (27.1-37.7)
Preceding pregnancy:		
Spontaneous vaginal delivery	245/297	82.5 (78.2-86.8)
Hypertension	35/255	13.7 (9.5-17.9)
Malaria treatment	86/264	32.6 (26.9-38.3)
Infection antibiotic treatment	26/180	14.4 (9.3-19.5)
Antepartum haemorrhage	12/251	4.8 (2.6-7.4)
Rupture of membranes < 4 hours	175/258	67.8 (62.1-73.5)

Treatment for malaria at the local or UTH antenatal clinic was mainly on clinical grounds and the drug of choice was chloroquine. Antibiotic treatment was commonly given for suspected chest infection.

The modes of delivery, recorded for 297 women, were spontaneous vaginal (82.5%) emergency caesarean section (10.2%), breech extraction (4%), elective caesarean section (2.0%) and instrumental extractions (1.3%). Most of the women (67.8%) delivered within 4 hours of rupture of membranes. Only sixteen (6.2%) delivered after 24 hours.

4.3.4 Prevalence of HIV infection and other sexually transmitted diseases

1. HIV infection

Ninety-two (30.1%) of the 306 women were infected with HIV. Infection rates were high for all age groups, with a peak at 20 to 35 years of age (figure 4.2). Viral load was measured in 70 of 92 post-partum samples from HIV infected women. The geometric mean (± SD) viral load was 21325.5(95964.5) viral copies/ml (193.6 to 479,826 viral copies/ml). Most women (68.6%) had HIV RNA viral loads of 10,000 viral copies/ml or more. 14.3% of the women had more 100,000 viral copies/ml (table 4.3)

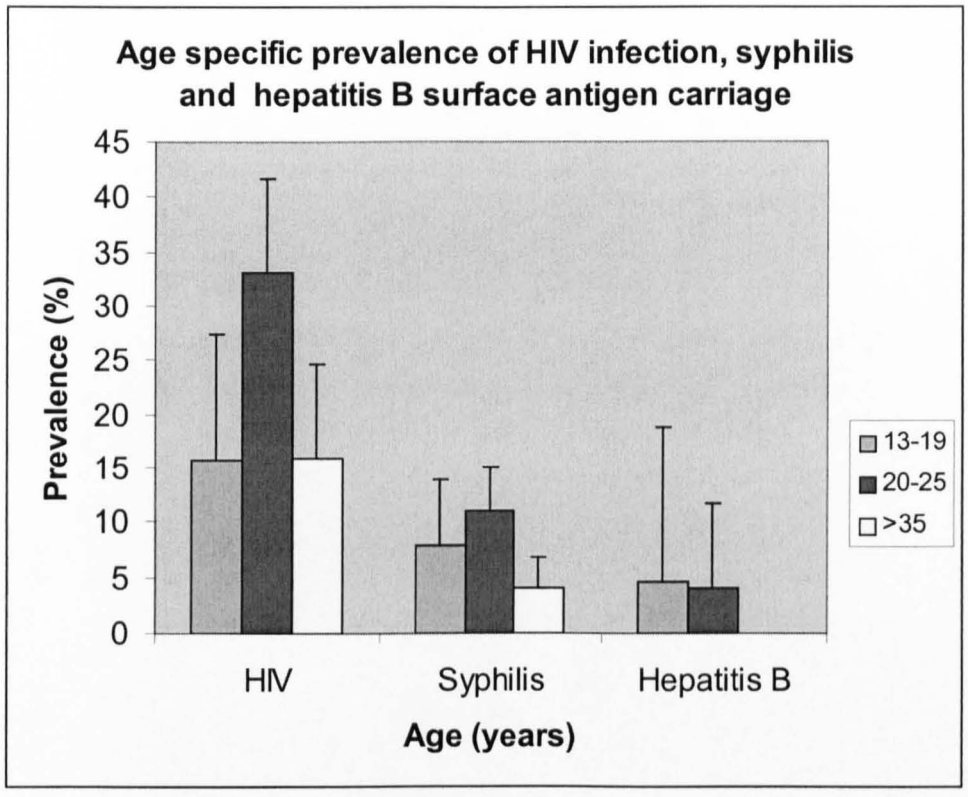
2. Syphilis infection

Two hundred and one (65.7%) of the 306 women had a syphilis (RPR) result record on their antenatal card and 30 (14.9%) were positive. Post-partum, all 306 women were tested for syphilis and 31 (10.1%) were RPR positive. The age specific prevalence of syphilis exposure for the 31 RPR positive women is illustrated in figure 4.2.

Of the women RPR positive (30) in the antenatal period, all reported having been treated with benzathine penicillin, 2.4 mega units weekly for 3 weeks. The RPR remained positive in 17 (56.7%) of these women (30) post-partum.

New infection with syphilis was noted in 8 (4.7%) of 275 women who were RPR negative in the antenatal period but positive on the post-partum sample. Six of the 8 women were between 20 and 35 years of age and 2 less than 20 years. HIV infection was present in 4 (50%) of the 8 women with new syphilis infection.

Figure 4.2:



Bar charts represent 95% confidence limits

3. Hepatitis infection

Hepatitis B surface (HBsAg) antigen was measured in 224 post-partum samples and 10 (4.5%) were positive. The age specific prevalence of HBsAg carriage for the 10 positive women is shown in figure 4.2. Serology for hepatitis C was negative in all samples.

4.3.5 Factors associated with HIV infection (univariate analyses)

1. Socio-demographic factors:

Women less than 20 years of age were marginally at lower risk of maternal HIV infection whereas any alcohol consumption during pregnancy was associated with increased risk of infection. (table 4.4). Geometric mean (± SD) duration of marriage

was similar in HIV infected and non-infected women; 3.4 (2.4) years versus 4.2(3.0) years respectively ($P>0.05$)

Table 4.4: Socio-demographic factors associated with maternal HIV infection

Variable	*HIV positive n/N (%)*	*HIV negative n/N (%)*	*Relative Risk*	*95% CI*	*p value*
Maternal age (less than 20 years)	6/88 (6.8)	32/211 (15.2)	0.50	0.24-1.07	**0.048**
Marital status (married)	84/89 (94.4)	191/212 (90.1)	1.59	0.71-3.56	NS
Maternal education (literate)	67/86 (77.9)	149/207 (72.0)	1.26	0.81-1.95	NS
Mother earns income	17/83 (20.5)	49/189 (25.9)	0.80	0.51-1.27	NS
Spouse earns income	81/87 (93.1)	187/208 (89.9)	1.36	0.66-2.82	NS
Maternal smoking	2/84 (2.4)	2/207 (1.0)	1.75	0.65-4.74	NS
Maternal alcohol intake	16/81(19.8)	13/204 (6.4)	2.17	**1.47-3.21**	**0.001**

NS not significant

2. Obstetric and medical factors associated with maternal HIV infection

Analysis of medical and obstetric factors associated with maternal HIV infection is shown in table 4.5. Antepartum haemorrhage, HBsAg carriage and CD4 cell counts (< 400 or 700 cells/mm^3) were associated with maternal HIV infection.

Table 4.5: Obstetric/ medical factors associated with maternal HIV infection

Variable	*HIV positive n/N (%)*	*HIV negative n/N (%)*	*Relative Risk*	*95% C.I.*	*P value*
1st pregnancy	18/88 (20.5)	63/211 (29.9)	0.69	0.44-1.09	NS
1st Child	26/88 (29.5)	65/211 (30.8)	0.96	0.65-1.41	NS
Past history of:					
Prematurity	7/88 (8.0)	16/211 (7.6)	1.04	0.54-1.98	NS
Abortion	19/88 (21.6)	30/211(14.2)	1.40	0.94-2.11	NS
Child death	34/88 (38.6)	63/211(29.9)	1.31	0.92-1.87	NS
Preceding pregnancy					
Antenatal haemorrhage	8/74 (10.8)	4/177 (2.3)	**2.41**	**1.54-3.78**	**0.007***
Tuberculosis	4/76 (5.3)	7/177 (4.0)	1.22	0.55-2.73	NS
Hypertension	11/76 (14.5)	24/179 (13.4)	1.06	0.63-1.81	NS
Malaria treatment	26/79 (32.9)	60/185 (32.4)	1.02	0.69-1.50	NS
Infection treatment	10/55 (18.2)	16/125 (12.8)	1.32	0.76-2.27	NS
HBsAg *positive*	7/70 (10.0)	3/154 (1.9)	**2.38**	**1.51-3.75**	**0.021***
RPR positive	13/92 (14.1)	18/214 (8.4)	1.46	0.93-2.30	NS
ROM > 4 hours	29/77 (37.7)	54/181 (29.8)	1.27	0.87-1.86	NS
Elective C/S	1/85 (1.2)	5/212 (2.4)	0.58	0.10-3.49	NS
CD4 count <400	35/90 (38.9)	13/207 (6.3)	**3.30**	**2.47-4.41**	**0.000**
CD4 count <700	72/90 (80.0)	80/207 (38.6)	**3.82**	**2.40-6.07**	**0.000**

*Fisher's exact test used to estimate P value (RR estimate unreliable), NS not significant

None of the factors in the past obstetric history (prematurity, abortion and childhood deaths) were shown to increase the risk of HIV infection. The geometric mean (± SD) CD4 count in HIV infected was lower than in negative women, 397.6(365.8) cells/mm^3 versus 765.8 (482.5) cells/mm^3 (P<0.001) (figure 4.3).

Positive RPR and current treatment for tuberculosis were not associated with maternal HIV infection. Mean parity and gravidae and mean number of pre-term deliveries, abortions and child deaths were calculated for HIV infected and negative women after

excluding primigravidae (table 4.6). The results indicated that mean parity and gravidae were lower in HIV infected than non-infected women.

Figure 4.3

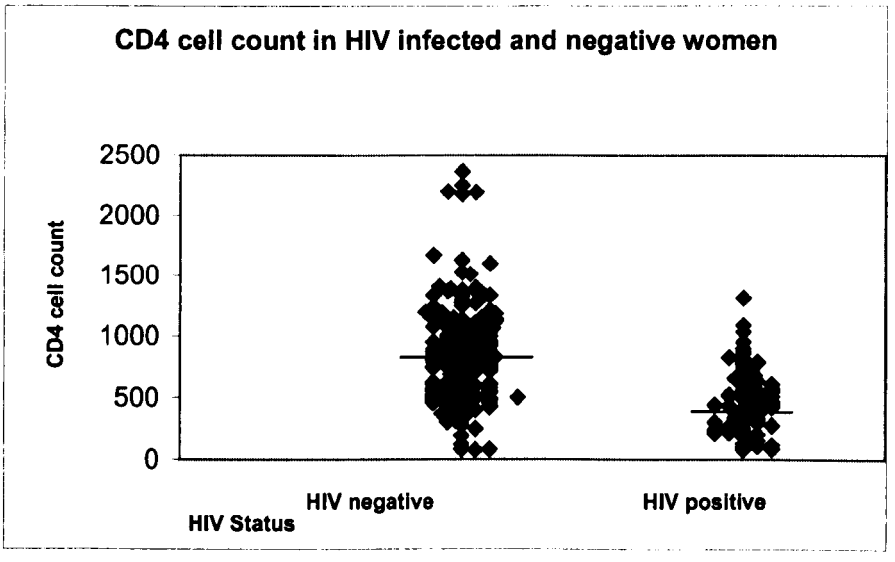


Table 4.6: ***Past obstetric factors associated with HIV infection (first pregnancies excluded)***

Factor	*HIV Infected*	*HIV Negative*	*P value*
	Mean±SD[1]	*Mean±SD*	
Parity	3.19 ± 1.56	4.14 ± 2.32	0.002
Gravidae	3.53 ± 1.56	4.33 ± 2.18	0.006
Premature delivery	0.19 ± 0.71	0.11± 0.37	NS
Abortion	0.31 ± 0.66	0.27± 0.65	NS
Children dead	0.62 ± 0.50	0.43 ± 0.50	NS

NS not significant

3. Nutritional factors associated with HIV infection

Mean post-partum maternal weight and MUAC were lower in HIV infected women (table 4.7) but there was no association between low post-partum maternal weight (<45 or 50 kilograms) and low MUAC (<23 centimetres) with risk of maternal HIV infection (table 4.8).

Table 4.7: Nutritional factors associated with maternal HIV infection (continuous variables)

Factor (total number screened)	*HIV Infected*	*HIV Negative*	*P value*
	Mean±SD[1]	*Mean±SD*	
Maternal weight (kg)	54.8±7.8	58.7±10.7	0.004
Mid upper arm circumference (cm)	25.1±2.2	26.2±3.1	0.002
Antenatal Hb (g/dl)	10.6±1.6	11.0±2.1	NS
Post-partum Hb (g/dl)	10.0±2.3	11.0±2.2	0.001
MCHC at delivery (Hb/cell)	31.8±3.1	32.2±2.5	NS
MCV at delivery (fl)	85.2±9.1	85.5±8.9	NS
Serum retinol (μmol/L)	1.18±0.55	1.29±0.47	NS

NS not significant

Table 4.8: Nutritional factors associated with HIV infection

Variable	*HIV (+) n/N (%)*	*HIV (-) n/N (%)*	*Relative Risk*	*95% CI*	*P value*
Post-partum weight < 45kg	5/80 (6.3)	8/202 (4)	1.38	0.68-2.82	NS
Post-partum weight < 50kg	15/80 (18.8)	31/202 (15.3)	1.18	0.74-1.88	NS
Post-partum MUAC < 23cm	8/88 (9.1)	16/210 (7.6)	1.14	0.63-2.07	NS
Antenatal Hb <11g/dl	13/20 (65.0)	14/36 (38.9)	2.00	0.94-4.24	NS
Antenatal Hb< 7g/dl	0/20 (0)	2/36 (65.6)	ND	ND	ND
Post-partum Hb < 11g/dl	56/92 (60.9)	87/214 (40.7)	1.77	1.24-2.53	0.001
Post-partum Hb< 7g/dl	9/92 (9.8)	10/214 (4.7)	1.69	0.99-2.72	NS
Post-partum PCV <33%	49/92 (53.3)	74/214 (34.6)	1.70	1.21-2.38	0.002
Post-partum MCHC <31 Hb/cell	28/92 (30.4)	46/214 (21.5)	1.37	0.96-1.96	NS
Post-partum MCV <80 fl	24/61 (39.3)	24/79 (30.4)	1.23	0.77-1.65	NS
Post-partum serum retinol < 0.7μmol/L	21/91 (23.1)	18/ 213 (8.5)	2.04	1.43-2.90	0.000
Post-partum serum retinol <1.05 μmol/L	44/91 (48.4)	64/213 (30.0)	1.70	1.21-2.38	0.002

MUAC mid upper arm circumference, MCV mean corpuscular volume, MCHC mean corpuscular haemoglobin concentration, PCV packed cell volume, ND not done due to some empty cells, NS not significant

Post-partum maternal anaemia (<11g/dl), PCV (<33%) and low post-partum serum retinol (< 0.7 or 1.05 μmol/L) was associated with increased HIV infection risk. On the other hand, antenatal maternal anaemia did not increase the risk (table 4.7 and 4.8). Low serum retinol (< 0.7μmol/L) was associated with a CD4 count of less than 400 cells /mm^3 (*P<0.001*) and Hb less than 11g/dl (*P<0.001*).

Table 4.9: Variables selected for inclusion in the logistic regression model

Variable	*Unadjusted RR (95% CI)*	*P value*
Maternal age (<20 years)	0.50 (0.24-1.07)	0.048
Alcohol intake	2.17 (1.47-3.21)	0.001
Antepartum haemorrhage	2.41 (1.54-3.78)	0.007
HBsAg carriage	2.38 (1.51-3.75)	0.021
Post-partum CD4 cell count (<400 cells/mm^3)	2.82 (2.40-6.07)	<0.001
Post-partum anaemia (Hb <11g/dl)	1.77 (1.24-2.53)	0.001
Post-partum serum retinol (< 0.7μmol/L)	1.70 (1.21-2.38)	<0.001
First pregnancy,	0.69 (0.44-1.09)	NS
Mother earns income	0.80 (0.51-1.27)	NS
Spouse earns income	1.36 (0.66-2.82)	NS
Literate	1.26 (0.81-1.95)	NS
Married.	1.59 (0.71-3.56)	NS

HbsAg hepatitis B surface antigen, NS not significant

4.3.6 Multivariate analysis of factors associated with HIV infection

A multiple logistic regression model was used to describe variables significantly associated with HIV infection on univariate analysis with P *<0.05* as well as factors considered of public health or clinical significance (table 4.9)

Table 4.10 shows that alcohol intake, antepartum haemorrhage, hepatitis B surface antigen carriage, low CD4 cell and post-partum anaemia were strongly associated with

increased maternal HIV infection risk. Although first pregnancy was not associated with maternal HIV infection on univariate analysis, reduced risk was observed after adjusting for other variables.

Table 4.10: Adjusted odds ratio of variables associated with maternal HIV infection

Variable	*Adjusted odds ratio (95% CI)*	*P value*
First pregnancy	0.30 (0.10-0.92)	0.034
Alcohol intake	5.67 (1.69-19.07)	0.005
Antepartum haemorrhage	5.85 (1.16-29.45)	0.032
HBsAg carriage	27.45 (2.90-259.39)	<0.001
Post-partum CD4 cell count (<400 cells/mm^3)	10.63 (3.41-33.14)	<0.001
Post-partum anaemia (Hb <11g/dl)	3.99 (1.65-9.61)	0.002

HbsAg hepatitis B surface antigen, Hb haemoglobin

4.4 DISCUSSION

4.4.1 Study sample

Approximately 40,000 women deliveries occur in Lusaka annually and 12,000 of these are at UTH. The women who deliver at UTH are mainly referred from the clinics in Lusaka for suspected high-risk pregnancy. The sudy sample, therefore may not be representative of pregnant women in Lusaka urban. Another potential source of bias, was the enrolment process. Only women who agreed to HIV testing were included in the study.

4.4.2 Socio-demographic characteristics and programme implication

Most women were married (91.5%) and literate (73.8%). Similar findings were reported in the 1998 Zambia situation analysis survey. Of the antenatal women surveyed, 88% were married and 71% had some form of education.

Despite the high literacy level in the current study, 73.7% of the women had no formal income whilst 90.8% of their partners were either employed or engaged in some business. This gender inequality puts women in a less favourable negotiating position regarding their own sexuality and reproductive choices. For HIV prevention, there are no methods available for women to use to prevent HIV infection independent of the male partner other than the female condom (Gibney et al 1999). Provision of voluntary testing and counselling to married women without participation of their husband did not result in a reduction in HIV incidence rates in Rwanda, despite counselling, education and knowledge of HIV test results (VanderStraten *et al* 1995). It was evident in Rwanda that men dominated sexual decision making and it was also clear that involvement of men in counselling and testing contributed significantly to reduced rates of gonorrhoea amongst women. (Allen *et al* 1992)

HIV infected women for the first time in developing countries are being requested to make decisions on feeding choices for their babies (WHO/UNICEF 1998) and yet antenatal clinics do not routinely offer partner counselling. Avenues to make the clinics partner friendly will need exploring.

4.4.3 Maternal anaemia and implications for care and control

1. Prevalence

Anaemia, defined as Hb of less than 11g/dl in pregnant women (WHO, 1993), was common both in the antenatal and post-partum period (46.7 and 48.2%). These rates were similar to the national estimate (46.9%) in Zambia (Luo *et al* 1999).

Research in other African countries using defined populations of pregnant women has shown variable results ranging from 41.5% to 95% (Msolla & Kanabo 1997; van den Broek *et al* 1998; Meda *et al* 1998 & 1999; Ramon *et al* 1999). This variability relates to timing of Hb measurements during pregnancy, variable exposure related to infection, including malaria, helminthic infection and HIV and differences in diet and underlying nutritional deficiencies.

2 Anaemia screening

Very few women had antenatal screening for anaemia (18.3%) during their routine antenatal attendance. This could be explained either by poor recording (the midwife simply did not record the result onto the antenatal card), lack of screening facilities or irregularities in the screening services such as delays in delivery of supplies or actual shortages. This finding requires further evaluation as it has major implications for control of anaemia in pregnancy, an important component of maternal and child health.

Zambia is one of the countries piloting the feasibility of implementing interventions aimed at reducing MTCT of HIV. The maternal care package for this programme includes screening of women for anaemia and it is recommended that women with Hb less than 8g/dl at 36 weeks gestation should not be given zidovudine short course

preventive therapy (WHO, 1998). This is because one of the major side effects observed with zidovudine therapy is bone marrow suppression manifesting as anaemia, neutropaenia and thrombocytopaenia (AMFAR treatment directory).

Without Hb routine screening facilities, all midwives will require training in clinical detection of tongue, conjunctiva or palmar pallor for the programme to be successful. High clinical screening sensitivity and positive predictive values are possible for Hb less than 7g/dl but not higher (van den Broek *et al* 1998; Meda *et al* 1999; Stoltsfus *et al* 1999).

An important observation of programmatic importance with regard to post-partum anaemia, was the high proportion of women who developed anaemia in the post-partum period (58.6%), after a previously normal antenatal recording, and the proportion who had anaemia both ante-natally and post-partum (63%). One of the limitations of this finding is the different Hb testing methods used (Coulter counter estimation at UTH and haematocrit at most local clinics) but the findings are interesting. They reflect some of the practical elements related to anaemia control. Iron sulphate supplements are routinely given to all pregnant women in Zambia. The supplies are usually irregular in antenatal clinics and even when regular, compliance amongst women is low (Ekstrom *et al* 1996; Luo *et al* 1999). Some of the reasons for low compliance in Zambian pregnant women include fear of delivering a big baby resulting in a difficult labour, nausea and vomiting and influence on younger pregnant women by older women in the community (Luo *et al* 1999).

4.4.4 Post-partum maternal vitamin A status

Serum retinol has been used to widely measure vitamin A status although levels can fluctuate with concurrent infections, poor protein status, inadequate liver function, other micronutrient deficiencies such as zinc, and concurrent malaria. No single measure provides adequate estimation of status (Sommer and West 1996). In this study, serum retinol was used to estimate vitamin A status in the study population in order to be able to compare with prevalence estimates proposed by WHO and in other studies.

Whilst serum retinol above 0.7μmol/Ll is generally considered normal, there are important caveats (Sommer and West 1996). Impaired dark adaptation in otherwise healthy adults is observed at serum vitamin A between 0.7 and 1.05 μmol/L or higher. Non-xerophthalmic children with serum retinol above 0.7μmol/L have evidence of sub-clinical dysfunction such as conjunctiva metaplasia, which has been directly linked to infectious morbidity. A significant proportion of children with xerophthalmia have retinol levels above 0.7μmol/dl.

To compare different populations and define at risk groups, specific serum cut-off points have been developed (Sommer & West 1996). Maternal vitamin A deficiency (serum retinol <0.7μmol/L) in the current study was 12.8%, a level slightly higher than recommended by WHO (1996) for indicating prevalence of public health significance for lactating mothers (10%). It has been estimated that, in Zambia, vitamin A deficiency affects 21.5% of women in the reproductive age group (Luo *et al* 1999). Other estimates in the region include 58% in antenatal women in Nairobi and 58-63% in HIV infected pregnant women in Blantyre (Nduati *et al* 1995; Semba *et al* 1994).

4.4.5 Obstetric morbidity.

The majority of the women were multiparous although the median number of children was 2. Child mortality and abortions were high, 32.4% and 16.4% respectively. The study did not establish, however, whether these abortions were induced or spontaneous but induced abortions are among the common causes of maternal mortality in Zambia (Mhango *et al* 1986).

The commonest cause of morbidity during pregnancy was suspected malaria (32.6%), determined mainly on clinical grounds. A previous retrospective study on maternal mortality at UTH concluded that malaria was the commonest cause of mortality (Ahmed *et al* 1999). The authors of this study did not indicate whether these malaria cases were confirmed by microscopy. Preliminary data in an ongoing cohort study of women delivering at UTH, however, indicates that the prevalence of malaria among pregnant women in Lusaka might be as low as 1.8% (ongoing Human Herpes Virus 8 study)

4.4.6 Maternal HIV prevalence

HIV infection was the commonest STD to which women were exposed. The prevalence of HIV infection amongst women delivering at UTH in the current study of 30.1% is not significantly higher than that observed in 1998 (29.5%), indicating that the infection may be stabilising in Lusaka (Bhat *et al* 1998). A recent sentinel surveillance survey in Zambia (MoH/CBoH, 1999) revealed similar results in four primary antenatal clinics in Lusaka (25.3-31.6%).

On average 12, 000 deliveries at UTH every year. At the current estimate of 30.1% maternal HIV prevalence, then 3,612 of these women will have HIV infection. Without

HIV screening facilities at UTH, the majority of the infected women will go through pregnancy undetected and will pass on infection to about a third of their babies (Hira *et al* 1989), resulting in about 1200 paediatric HIV infections annually. HIV screening in this study was done at the time delivery in early labour. It was not the intention of this study to evaluate the acceptability of screening during labour but it has been suggested as a feasible option for intra-partum and post-partum interventions to reduce mother to child transmission of HIV in the USA (CDC 1999). A sensitivity of 99.4% and testing acceptability of over 80% has been previously achieved in Zambia with rapid testing (Mckenna *et al* 1997; Bhat 1998; Plourde *et al* 1998).

4.4.7 Maternal syphilis prevalence

RPR was used to test for syphilis exposure but this test is non-specific. False positives are common with other infections such as hepatitis, pneumonia, early HIV infection (Mandell *et al* 2000) and false negatives can occur in about 2% of pregnant women (Mandell *et al* 2000). The test, however, is still useful as a screening tool. More specific treponema tests, the *Treponema pallidum* haemaglutination test, *T. pallidum* immobilisation test and fluorescent treponemal antibody, are more specific and are used as confirmatory tests. They were not available for this study or for most syphilis screening programmes in Africa due to limited resources.

In 1994, the Maternal Syphilis Prevention Programme (MSPP) was initiated in Lusaka by the Urban District City Council (LUDCC) with technical and financial support from UNICEF and WHO. This programme has integrated routine antenatal screening using the RPR test with treatment of women, their partners and babies.

In the current study, two hundred and one women (65.7%) were screened for syphilis during routine antenatal attendance at the local clinic and at UTH. This estimate of the screening rate is lower than that reported (91%) during the evaluation of the syphilis programme in Lusaka in March, 1995. This difference reflect either a deterioration in the delivery of service or that since UTH is a referral hospital, some of the women delivering there will come from outside Lusaka district where syphilis screening facilities are not available.

During the antenatal and post-partum periods 14.9% and 10.1% respectively were RPR positive. These estimates are similar to an earlier observation (12.5%) (Hira *et al* 1986). During evaluation of the syphilis programme, however, a prevalence of 17.5% was reported for Lusaka (UNICEF 1995).

New infection with syphilis was noted in 8 (4.7%) of the study women previously negative during the antenatal period. This estimate could be used as a measure of disease incidence although a specific treponemal test was not done to confirm infection. Moreover, RPR sensitivity is between 70-100% during primary and secondary infection (Mandell 2000). Despite these limitations in the interpretation of results, it is possible that these women are being exposed to new infection during the later part of pregnancy and a single test during pregnancy may not be adequate.

4.4.6 Maternal Hepatitis B and C prevalence

1. Hepatitis B

Hepatitis B surface antigen (HBsAg) is the most important marker of active hepatitis infection because its presence in serum indicates active infection in almost all cases but

for detection of recent infection, hepatitis core antibody (HBc) should be measured (Mandell 2000). In this study HBsAg was done to determine hepatitis B carriage in the women but anti-HBc was not done due to limitations in funding. A prevalence of HBsAg antigen carriage of 4.7% was found, a value similar to that reported earlier by Oshitani *et al* (5.7%) in 1996. Results from other endemic countries have wide variations; Gabon (19%), Central African Republic (14%), rural Malawi (13%), Cameroon (7.2%) and Tunisia (6.5%) (Traore *et al* 1995; Pawlotsky *et al* 1995; Ahmed *et al* 1998; Triki *et al* 1997). One major reason for variability in results is the type of HBsAg assay used, with the newer generation assays being more sensitive. The test used in this study was a second generation assay.

2. Hepatitis C

No Hepatitis C antibodies were detected in the current study. These results were not surprising and are in agreement with an earlier report from Zambia by Oshitani and colleagues in 1995. Of the 735 samples they tested, only 3 (0.4%) were positive and all from the samples were from hospital inpatients with clinical signs. There seems to be marked variability in the prevalence amongst adults of hepatitis C in various African settings, 16.5% in pregnant women in Malawi, 5.0% in rural Tanzania, 3.2% amongst pigmies in Cameroon and 0.4% and 2.8% in urban Ghana (Kowo *et al* 1995; Ahmed *et al* 1998; Wansbrough-Jones *et al* 1998; Menendez *et al* 1999). In the studies second generation assays and the results of these assays tend to be more specific than the first generation ones (Tibbs, 1997). The current study used a second generation test.

4.4.7 Factors associated with maternal HIV infection:

1. Maternal age:

Although the prevalence of HIV, in Zambia, seems to be showing a downward trend in adolescence, infection prevalence has remained stable or has increased in older women (MoH/CBoH, 1999). When maternal age was evaluated on univariate analysis, in the current study, women less than 20 years were at reduced risk of HIV infection. After controlling for other factors in the logistic regression model, first pregnancy had an inverse relationship with maternal HIV infection but not young age. This finding has important implications current preventive efforts. If women in their first pregnancy are less likely to be HIV infected, reinforcing preventive efforts such as education, condom promotion and couple counselling might help them remain negative.

2. Literacy and income:

In the current study, maternal education or having a source of income were not associated with maternal HIV infection. The data, however, suggested that the relative risk of HIV infection was lower when the woman had her own source income, although the estimate was not statistically significant. Globally, it is the poorer and less educated that are most affected by HIV/AIDS (UNAIDS, 1998) probably because they are less likely to be informed about HIV/AIDS. Educated women are less vulnerable because they are able choices about their sexual practices. This analogy, however, may not be applicable to women in Africa, where multiple factors are more likely to be involved.

3. Maternal alcohol consumption:

10.1% of the study women reported drinking alcohol during pregnancy. The data suggested that alcohol consumption was strongly associated with increased risk of maternal HIV infection after correction for confounding variables. These findings were similar to previous reports in Zambia and other studies (Boerma *et al.*1999; Morrison *et al.*1997; Kapiga *et al*; 1998, Mnyika *et al* 1996), although Demisse *et al* (1996) in Ethiopia failed to demonstrate a similar association in Ethiopian sailors. The relationship with alcohol consumption may be related change in behaviour with the effect of alcohol. On the other hand it is possible that women who engage in high-risk sexual practices are more likely to drink alcohol. The situation in Zambia will need further evaluation.

4. Maternal syphilis:

Positive RPR in the current study was not associated with increased maternal HIV infection risk. Many studies have shown an increased risk of HIV infection amongst persons who have other sexually transmitted diseases (STDs) including genital ulcer disease (Mosha *et al.*1993; Latif *et al.* 1989; Johnson *et al.* 1989; Plourde *et al.* 1994). The study women were not clinically examined for genital ulcer disease. The RPR test is limited, as it can remain positive for up to a year after exposure (Mandell *et al* 2000). New infections can be detected on RPR repeat testing. In the current study, 4 of the 8 women who became RPR positive post-partum were also HIV infected, suggesting that HIV infection risk might be increased in women who newly acquire syphilis infection. Further analysis to confirm this association was not done due to limited numbers.

5. Maternal Hepatitis B carriage

Hepatitis B surface antigen carriage was strongly associated with maternal HIV infection, although this association was not found in previous studies in Zambia and Malawi no association was found (Ahmed *et al* 1999; Oshitani *et al* 1996). However, there was an observed increase in risk of hepatitis e antigen (HbeAg) positivity in pregnancy with HIV infection, a finding that is unusual in Africa (Oshitani *et al* 1996). These findings might be related to immunological profiles of the women.

6. Obstetric factors:

Maternal HIV infection was not associated with previous history of prematurity, abortion, child mortality, tuberculosis, hypertension and treatment although the factors were common in HIV infected than non-infected women (table 4.6).

The data suggested that ante-partum haemorrhage was associated with increased risk of of maternal HIV infection, similar to what has been previously reported (Braddick *et al* 1990). The mechanism or reason for this association is unknown and requires further study.

7. CD4 cell count

CD4 cell counts less 400 cells /mm^3 and 700 cells /mm^3 were associated with increased of maternal HIV infection in the current study. HIV/AIDS has become a common cause of maternal mortality (Ryder *et al* 1994; Brettle *et al* 1995; Temmerman *et al* 1995; Taha *et al* 1996; Landers *et al* 1997; Bessinger *et al* 1998; Burns *et al* 1998). It could be argued that more women with advanced clinical HIV disease were becoming pregnant

rather than HIV infection influencing CD4 cell count. In this study, however, women with an AIDS defining illness, such as tuberculosis or wasting, with post-partum maternal weight less than 45 kilograms, were not were not at increased risk of maternal HIV infection.

8. Vitamin A deficiency:

The results in this study indicated that low post-partum serum retinol of less than 0.7 μmol/L was associated with increased risk of maternal HIV infection in the univariate analysis. The relationship between vitamin A deficiency and HIV infection has received significant attention in the last few years (Semba *et al* 1994&1997; Fawzi.*et al* 1999). Several observations have shown that serum retinol levels are significantly depressed in HIV infected individuals (Semba *et al* 1993; Karter *et al* 1995; Nimmagadda *et al* 1998). The effect low maternal serum retinol, however, was lost after adjusting for other variables, suggesting that low serum retinol might be marker of advanced HIV disease.

4.4.8 Post-partum maternal anaemia

Low antenatal Hb was not associated with maternal HIV infection, although only a few of women (18.3%) had Hb measured during the antenatal period. However, post-partum maternal anaemia was associated with increased risk of maternal HIV infection. Various reports in the current literature indicate that anaemia in pregnancy is associated with maternal HIV infection (van den Broek *et al* 1998; Meda *et al* 1998&1999; Ramon *et al* 1999), although the significance of this association may not be high (Chimsuku 1998).

The finding that severe anaemia (Hb < 7 g/dl) was not associated with increased risk maternal HIV infection is in agreement with the results from Burkina Faso (Meda *et al* 1999), although Ramon and colleagues in Ivory Coast (1999) did find an association.

The association of anaemia with maternal HIV infection is of programmatic significance. Women should be monitored for anaemia as it may be a marker of HIV disease progression and a predictor of maternal mortality.

4.5 CONCLUSION

Maternal HIV is a common problem at UTH. Of the women delivering at UTH in 1997, 30.1% were HIV infected. Voluntary counselling and testing services should, therefore, be an integral part of antenatal care in order to identify these women and reinforce preventive efforts.

The association maternal HIV infection with alcohol consumption, ante-partum haemorrhage, HBsAg antigen carriage, low CD4 cell count and post-partum anaemia raises important prevention and care issues. The reduced risk of HIV infection observed with primigravidae, observed in this study, suggests that this group of women might be an important target for intervention efforts.

With such high levels of antenatal (46.7%) and post-partum (48.2%) anaemia, control and treatment efforts for anaemia in pregnancy require strengthening. These efforts should be extended to include the post-partum and post-natal period. RPR testing should be repeated toward the end of pregnancy in women previously found to be negative and positive women afforded treatment together with their spouses and babies.

There is need for further research to evaluate the interaction between ante-partum haemorrhage and maternal HIV infection.

CHAPTER 5

PREVALENCE AND RISK FACTORS FOR POST-PARTUM ANAEMIA IN AN URBAN SETTING WITH HIGH HIV PREVALENCE

5.1 INTRODUCTION AND OBJECTIVES

Human immuno-deficiency virus (HIV) infection and pregnancy related anaemia, both common problems in Zambia, are major contributors to maternal and child morbidity (Hira *et al* 1989; WHO 1992; Luo *et al* 1999; MoH/CBoH 1999). There are no previous studies in Zambia on the interaction of these two causes of morbidity. Current literature suggests that the risk of developing anaemia in pregnancy is increased with HIV infection (van den Broek *et al* 1998; Meda *et al* 1999; Ramon *et al* 1999). These studies, however, did not examine the effect on post-partum anaemia (PPA). Uncorrected maternal anaemia would diminish work capacity and when associated with iron deficiency could increase the risk of bacterial infections (Viteri *et al* 1994; Allen *et al* 1997). For a lactating mother, therefore, PPA could reduce her ability to feed and care for the baby adequately. The objective of this chapter was to determine the prevalence of PPA anaemia and associated risk factors, including maternal HIV infection.

5.1.1 Definitions

The definition of pregnancy related anaemia is not clear-cut. In most published studies the minimum cut-off for normal haemoglobin (Hb) in healthy pregnant women living at sea level is between 11 and 12 g/dl (van den Broek *et al* 1998). The cut–off concentration considered to be of public health importance, recommended by WHO, is

11g/dl (WHO 1972). With normal physiological changes in pregnancy, plasma volume expands by 46 to 55% and red cell volume by 18 to 25%, resulting in haemodilution in the latter part of pregnancy (van den Broek *et al* 1998). It is postulated that the drop in osmolarity results in reduced blood viscosity, enhancing blood flow in the intervillous space, improving foetal growth (Steer 2000).

This haemodilution makes the assessment of anaemia in pregnancy using haemoglobin (Hb) concentration difficult when Hb measurements are done at different time points. The Hb concentration declines throughout the first and second trimester, reaches a nadir at about 30-32 weeks and then rises again nearer to term (Kitay & Harbot 1975; Scholl & Hediger 1994). For this reason, anaemia can only be optimally assessed early in pregnancy or in the post-natal period.

5.1.2 Prevalence, associated risk factors and effects of pregnancy related anaemia

Pregnancy related anaemia, is a common problem, affecting 35 to 75% of women in Africa (WHO 1992). In Zambia, 46.9% of pregnant women are anaemic (Luo *et al* 1999). In contrast, in industrialised countries, anaemia prevalence is reported in less than 20% of pregnant women, although poorer populations may still have higher rates (WHO 1992). In industrialised countries, the prevalence of anaemia in pregnancy has declined over the past few decades, including anaemia attributable to iron deficiency. Scholl & Hediger (1994) reported that the prevalence of anaemia and iron deficiency, in low income pregnant women in the United States, was 3.5% in whites and 12.7% in blacks in the first trimester, and correspondingly 6.4% and 17.8% in the second and 18.8% and 38.1% in the third trimester (CDC 1988).

In poor resource settings in Africa, anaemia in pregnancy is caused by a number of factors including iron, folate and vitamin A deficiency, hookworm infestation, malaria and haemoglobinopathies (Fleming 1989; WHO 1993; Masawe *et al* 1999). In Zambia, prior treatment for malaria, passage of worms and pregnancy were associated with anaemia in women (Luo *et al* 1999).

Recent studies in Africa suggest that maternal HIV infection is a risk factor for anaemia in pregnancy. In urban Malawi, the estimated antenatal risk of anaemia in 150 HIV non-infected and infected women was 45.5% and 93.6%, with a relative risk of 2.06 (van den Broek 1998). Similar findings were reported from much larger studies in Burkina Faso and Cote d'Ivoire (Meda *et al*, 1999; Ramon *et al*, 1999). This association has important implications for HIV management strategies in pregnant women since zidovudine has well know haematological toxicity.

Anaemia is an independent predictor of clinical outcome in HIV infected patients in Europe (Mocroft *et al*, 1999). In a prospective cohort of 6,725 adults with HIV infection from 52 centres in Europe, Mocroft *et al* found that 40.4% had normal Hb, 58.2% mild anaemia (defined as Hb 8-14 in men and 8-12 g/dl in women) and 1.4% severe anaemia (<8g/dl). At 12 months post–enrolment 3.1% non-anaemic and 15.9% mild and 40.8% severe anaemics had died.

Measurement of maternal haemoglobin is relatively inexpensive and if done post-partum might be useful in predicting maternal morbidity and performance during lactation. It has been suggested that poor lactation or inability to feed adequately might increase the risk of breast milk transmission of HIV secondary to sub-clinical mastitis and increased HIV viral load in breast milk because of milk stasis (Semba *et al* 1999).

The maternal mortality ratio in Zambia has been estimated at around 640/100,000 live births (DHS 1997) but the contribution of PPA to this mortality is not clear. A recent study indicated that post-partum haemorrhage was the commonest contributing factor to maternal mortality in Zambia (UNFPA 1999). The risk of death in anaemic women with post-partum haemorrhage is likely to be increased.

5.2 METHODS

5.2.1 Study population

Women enrolled during labour for the longitudinal cohort had peripheral blood collected within 24 hours of delivery for HIV serology, Hb, MCV and MCHC, CD4, RPR and serum retinol as described in Chapter 3. Maternal haemoglobin was also measured at one-month after delivery. At enrolment, a standard enrolment questionnaire, described in Chapter 4 was administered after obtaining an informed consent.

5.2.2 Definitions

Anaemia was defined as Hb <11 g/dl and severe anaemia as Hb was <7g/dl (WHO 1992). The cut-offs of <7, 7-10.9 and ≥11 g/dl defined severe and mild anaemia and normal values. Post-partum and post-natal anaemia were defined as Hb concentration <11 g/dl detected within 24 hours of delivery and within 4-6 weeks of delivery respectively. The cut-offs for microcytosis were MCV <80 fL and for macrocytosis MCV >100 fL and hypochromasia was defined as MCHC <31 Hb/cell and hyperchromasia as >37 Hb/cell (Tietz 1990).

Immuno-deficiency was classified as severe if the CD4 cell count was <200 cells /mm^3 and mild if the count was between 200-500 cells /mm^3 (CDC 1992). Viral load cut off points were ≥1000, ≥10,000 and ≥100,000 copies per ml.

Severe, moderate and mild vitamin A deficiency were defined as serum retinol <0.35, 0.35-0.7 and 0.7-1.05 μmol/L (Sommer & West 1996). For maternal weight and mid upper arm circumference (MUAC), the cut-offs were 45 kg and 23 cm.

Antepartum haemorrhage was defined as clinically diagnosed ante-partum haemorrhage (with ultrasonography when possible) by the attending physician. The remaining definitions were described in Chapter 4.

5.2.3 Statistical analysis

SPSS version 10 and Epi-Info 6.04 were used for analysis. Relative risks and 95% confidence limits were calculated to determine factors associated with PPA. P values were estimated using Pearson's X^2 or Fishers exact tests for small numbers. Mean values for continuous variables were compared using the Student's t-test. For skewed data, the mean of the log of the values was calculated and geometric means were compared. All tests were two tailed at P <0.05.

Variables found to be associated with PPA by univariate analysis and those considered of public health or clinical relevance were included in a logistic regression model. This was followed by a backward step selection with a significance level of P<0.05, to arrive at the final model to identify independent risk factors for anaemia.

5.3 RESULTS

5.3.1 Prevalence of post-partum anaemia

All women (306) enrolled had post-partum haemoglobin measurements and 143 had PPA (46.7%; 95% CI 41.1-52.3%). Severe PPA occurred in 19 women (6.2%; 95% 3.5-8.9%). 13.3% of anaemic women had severe PPA.

5.3.1 Univariate analysis of factors associated with post-partum anaemia

1) Background maternal characteristics (table 5.1)

Caesarean section (elective and emergency), significantly increased the risk of PPA (table 5.1). Thirty-six (12.1%) women had a caesarean section, of which 6 were elective. Of the 6 women who had elective caesarean section and of the 30 who had emergency caesarean section, 4 (66.7%) and 25 (75%) had PPA. Two hundred and forty five (82.5%) of the women had a spontaneous vaginal delivery (SVD), 12 (4%) breech extraction, and 4 (1.3%) instrumental delivery. The proportion of anaemic women for these other modes of delivery was 43.3% for SVD, 41.7% for breech extraction and 25% for instrumental delivery.

None of the women who delivered by breech extraction or instrumental delivery had severe PPA. An increase in proportion of PPA was observed with SVD (4.9%), elective caesarean section (16.7%), and emergency caesarean sections (20%) (χ^2.for trend 9.94; P=0.002).

Alcohol intake was independently associated with reduced risk of PPA. Reported malaria treatment during pregnancy was not associated with PPA even after adjusting for gravidae and maternal HIV infection.

Table 5.1 Maternal factors associated with post-partum anaemia

Variable	*Hb <11g/dl* *n/N (%)*	*Hb≥11g/dl* *n/N (%)*	*RR*	*95% C.I.*	*P value*
Maternal age <20	19/141 (13.5)	19/158 (12.0)	1.07	0.76-1.51	0.707
Marital status (married)	129/142 (90.8)	146/159 (91.8)	0.94	0.62-1.41	0.763
Maternal education (illiterate)	30/139 (21.6)	47/154 (30.5)	0.77	0.57-1.05	0.083
Maternal employment (none)	98/131 (74.8)	108/141 (76.6)	0.95	0.72-1.26	0.731
Paternal employment (none)	14/138 (10.1)	13/157 (8.3)	1.12	0.76-1.65	0.579
Maternal alcohol intake	8/136 (5.9)	21/149 (14.1)	0.55	0.30-1.01	**0.022**
Primigravida	34/141 (24.1)	47/158 (29.7)	0.86	0.64-1.14	0.274
Primipara	44/141 (31.2)	47/158 (29.7)	1.04	0.80-1.34	0.784
Ante-partum haemorrhage	8/125 (6.4)	4/126 (3.2)	1.36	0.89-1.07	0.231
Malaria treatment in pregnancy	84/128 (34.4)	94/136 (30.9)	1.08	0.84-1.40	0.545
Caesarean section	29/141 (20.6)	7/156 (4.5)	1.18	1.52-2.32	**0.000**

2) HIV infection and immuno-deficiency

Maternal HIV infection but not viral load was significantly associated with PPA but not HIV viral load (table 5.2).

Table 5.2: Maternal HIV infection, immuno-deficiency and post-partum anaemia

Variable	*Hb <11g/dl*	*Hb≥11g/dl*	*RR*	*95% C.I.*	*P*
	n/N (%)	*n/N (%)*			*value*
Maternal HIV infection	56/143 (39.2)	36/163 (22.1)	**1.50**	**1.19-1.89**	**0.001**
Viral load (copies/ ml)					
≥1000	42/43 (97.7)	25/27 (92.6)	1.88	0.38-9.42	0.55
≥10,000	28/43 (65.1)	20/27 (74.1)	0.86	0.59-1.2	0.43
≥100,000	8/43 (18.6)	2/27 (7.4)	1.37	0.94-2.0	0.30
CD4 count (cells/mm^3)					
<200	14/143 (9.8)	9/163 (5.5)	1.34	0.94-1.90	0.157
<500	49/143 (34.6)	50/163 (30.7)	1.09	0.85-1.40	0.502
CD4 percentage					
<15%	17/140 (12.1)	10/163 (6.4)	1.38	1.01-1.90	0.084
<25%	37/140 (26.4)	30/157 (19.1)	1.25	0.90-1.62	0.115
CD4 count (cells/mm^3)					
<200 (HIV positive)	10/56 (17.9)	3/36 (8.3)	0.93	0.93-1.88	0.33
<500 (HIV positive)	29/56 (51.8)	24/36 (69.4)	0.79	0.57-1.09	0.16
CD4 percentage					
<15% (HIV positive)	13/55 (23.6)	9/35 (25.7)	0.96	0.64-1.42	0.82
<25% (HIV positive)	30/55 (54.5)	27/35 (77.1)	**0.69**	**0.51-0.95**	**0.03**

Geometric mean (± SD) viral load was not associated with PPA 22,594 (98,058) in anaemic women compared to 19,485 (95,866) in non-anaemic women. Similarly, HIV viral burden, at different cut of points (≥1000, ≥10,000 and ≥100,000 viral copies per ml), was not associated with PPA (table 5.2).

The geometric mean (± SD) CD4 cell count was lower in anaemic than non-anaemic women, 583.0 (606.32) versus 670.7 (449.4), although this difference was not statistically significant. Low CD4 cell count (< 200 & 500) was not associated with

anaemia but CD4 percentage < 25% was significantly associated with reduced risk of PPA in HIV infected women (table 5.2).

3) Nutritional factors associated with post-partum anaemia (table 5.3)

There was a significant reduction in risk of PPA with low maternal weight (< 45 kg) and low MUAC < 23cm (table5.3). Antenatal anaemia was not associated with PPA. PPA, however, was significantly associated with post-natal anaemia (one month after delivery). Mean (± SD) post-partum MCHC, MCV and serum retinol were significantly lower in anaemic than non-anaemic women; 31 (3.2) Hb/cell versus 33.1 (1.5) Hb/cell, 83.6 (10.1) fl versus 87.0 (7.5) fl and 1.15 (0.51) μmol/L versus 1.35 (0.45) μmol/L respectively (P < 0.002 for all comparisons). This association was explored using cut-off values. There was a significantly increased risk of PPA with MCHC (< 31 Hb/cell) and MCV values (< 80 fL) (table 5.3). Of the 143 anaemic women, none had MCHC >37 Hb/cell and only 7 (4.9%) had MCV >100fl.

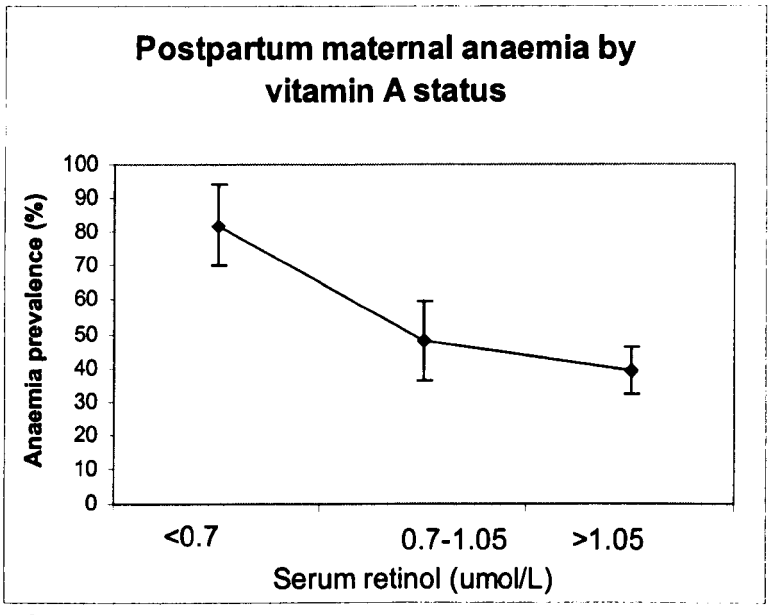
PPA was more prevalent in women with post-partum serum retinol < 0.7 and < 1.05μmol/L (table 5.3) and the association was significantly negative and linear (figure 5.1). Of 18 severely anaemic women 10 (55.6%) had serum retinol less than 0.7 μmol/L compared to 29 (10.1%) of women with haemoglobin ≥ 7g/dl. This difference in proportion was statistically significant (P<0.001). Only 2 women were vitamin A deficient (serum retinol<0.35μmol/L) and both were anaemic.

Table 5.3: Nutritional factors associated with maternal post-partum anaemia

Variable	*Hb<11g/dl n/N (%))*	*Hb≥11g/dl n/N (%))*	*RR*	*95% C.I.*	*P value*
PP maternal:					
Weight < 45kg	2/131 (1.5)	11/151 (7.3)	**0.32**	0.09-1.16	**0.021**
Weight < 50kg	19/131 (14.5)	27/151 (17.9)	0.87	0.60-1.26	0.444
MUAC < 23cm	6/140 (4.3)	18/158 (11.4)	**0.51**	0.25-1.03	**0.025**
Hb <11g/dl:					
Antenatal at booking	17/34 (50.0)	10/22 (45.5)	1.07	0.71-1.64	0.74
Post-natal (1 month)	39/73 (53.4)	12/54 (18.2)	**1.98**	**1.46-2.68**	**<0.01**
PP maternal:					
MCHC<31 Hb/cell	58/143 (40.6)	16/163 (9.8)	**2.14**	**1.74-2.63**	**<0.01**
MCV <80 fL	47/143 (32.9)	26/163 (16.0)	**1.57**	**1.24-1.97**	**0.001**
MCHC<31 & MCV <80	19/39 (32.8)	4/16 (25)	1.08	0.48-1.38	0.762
Serum retinol < 0.7μmol/L	32/142 (22.5)	7/162 (4.3)	**1.98**	**1.61-2.43**	**<0.01**
Serum retinol <1.05 μmol/L	65/142 (45.8)	43/162 (26.5)	**1.53**	**1.22-1.93**	**<0.01**

Hb haemoglobin, PP post-partum, MUAC mid upper arm circumference, MCV mean corpuscular volume, MCHC mean corpuscular haemoglobin concentration

Figure 5.1



χ^2_{trend} **20.89 P<0.001**

5.2.3 Multivariate analysis of factors associated with post-partum anaemia

Adjusted odds ratios were calculated for factors associated with PPA, using logistic regression. Included in the model were factors that were significant on univariate analysis (table 5.5). Postpartum MUAC < 23 cm and CD4 % <15% were included in the model because they were close to significant.

Maternal HIV infection, delivery by caesarean section and low post-partum MCHC, MCV and serum retinol were independently associated with increased risk of PPA (table 5.5). The risk of PPA was inversely associated with alcohol intake during pregnancy and with a low post-partum maternal weight < 45 kilograms. These results are shown in table 5.6.

Table 5.4: Variables selected for inclusion in multivariate analysis

Variable	*Unadjusted RR (95% CI)*	*P value*
Any Alcohol intake during pregnancy	0.55 (0.30-1.01).	0.022
Caesarean section	1.15 (1.23-1.97)	0.002
HIV infection	1.50 (1.19-1.89)	0.001
Post-partum weight <45kg	0.32 (0.09-1.16)	0.021
Post-partum MUAC<23cm	0.51 (0.25-1.03)	0.025
Post-partum MCHC <31Hb/cell	2.14 (1.74-2.63)	<0.001
Post-partum MCV <80fl	1.57 (1.24-1.97)	0.001
Post-partum serum retinol < 0.7μmol/L	1.98 (1.61-2.43)	<0.001
CD4% less than 15%	1.38 (1.01-1.90)	0.084

MUAC mid-upper arm circumference, MCHC mean corpuscular haemoglobin concentration, MCV mean corpuscular volume

Table 5.5: Adjusted odds ratio of variables associated with post-partum anaemia

Variable	*Adjusted OR (95% CI)*	*P value*
Alcohol intake	0.22 (0.07-0.70)	0.011
Caesarean section	9.95 (2.83-34.96)	<0.001
HIV infection	2.81 (1.34-5.90)	0.006
Post-partum weight <45kg	0.10 (0.01-0.74)	0.024
Post-partum MCHC <31Hb/cell	8.33 (3.87-17.95)	<0.001
Post-partum MCV <80fl	2.39 (1.18-4.82)	0.015
Post-partum serum retinol < 0.7μmol/L	3.03 (1.09-8.42)	0.033

MCHC mean corpuscular haemoglobin concentration, MCV mean corpuscular volume

5.3 DISCUSSION

5.4.1 Prevalence of post-partum maternal anaemia

This study provides some of the first data on PPA in an area of high HIV prevalence. The results demonstrate that PPA is a common problem in women delivering at UTH. The prevalence of 46.7% was similar to that reported for pregnancy related anaemia by WHO (1992). Higher antenatal prevalence values have been reported in recent surveys from rural and urban areas in Africa. For rural areas: 95% of women in Morogoro, Tanzania; >90% in Chikwawa, Malawi; 84.4% in Mbarara, Uganda; 75.6% in Kilifi, Kenya (Shulman 1996; Msolla *et al* 1997; Verhoeff *et al* 1999). For urban areas: 70.1% in Abidjan, Cote d'Ivoire; 60% in Blantyre, Malawi; 66% in Bobo Dioullasso, Burkina Faso (Msolla *et al* 1997; Meda *et al* 1998; Ramon *et al* 1999).

This variation in antenatal anaemia prevalence relates to timing of Hb measurements during pregnancy, variable exposure related to infection, including malaria, helminthic infection and HIV and differences in diet and underlying nutritional deficiencies.

5.4.1 Risk factors for pregnancy related anaemia

1) Post-partum anaemia and caesarean section

The results showed an association between PPA and caesarean section. Alterations in total circulating plasma volume and haemoglobin mass determine whether or not anaemia occurs (Hoffbrand & Pettit 1984). The data in the current study suggests that there is excess loss of haemoglobin mass with caesarean section deliveries. This outcome has serious implications for recommending caesarean section as an intervention option for reducing MTCT of HIV. The initial results of the European

Mode of Delivery Collaborative trial (1999) showed a transmission rate of 1.7% among infants delivered by caesarean section compared with 10.7% among women randomised to vaginal delivery. The association of caesarean section with PPA in our study requires further evaluation. It is likely to be influenced by medical practices, such as blood transfusion for anaemic women before or following caesarean section. Ideally in low resource settings with high HIV prevalence, blood transfusions should be avoided.

2) HIV infection and immuno-deficiency

Human immuno-deficiency virus infection is a major cause of morbidity in pregnant women in Zambia (MoH/ CBoH 1999). In this study, maternal HIV infection was an independent risk factor for PPA. The association of maternal HIV infection with anaemia has been previously described in antenatal women in African centres with different HIV and anaemia prevalence (Chimsuku 1996; van den Broek *et al* 1998; Meda *et al* 1998&1999; Ramon *et al* 1999). The current study, is the first to describe the association of HIV with PPA in Africa.

In Bobo Dioullasso, Burkina Faso, the prevalence of anaemia was 78.4% in HIV infected antenatal women, versus 64.7% in HIV non-infected women and the prevalence of maternal HIV infection was 9.7% (Meda *et al* 1998). Similar anaemia prevalence was found in Abidjan, Cote d'Ivoire amongst a population of antenatal women with slightly higher (12%) prevalence of HIV infection (Ramon *et al* 1999). The prevalence of anaemia in Abidjan in HIV infected women was 81.7% compared to 68.9% in non-infected women. In Blantyre, Malawi, 93.6% of HIV infected antenatal women were anaemic, compared to 45.5% in non-infected women (van den Broek

1998). The prevalence of HIV infection in Blantyre (30.2%) was similar to that found in the current study in Lusaka (30.1%). The estimated relative risk for anaemia associated with maternal HIV infection in Blantyre (2.06) was similar to that found in Abidjan (2.05) (van den Broek *et al* 1998; Ramon *et al* 1999). The estimated relative risk for the Zambian data on univariate analysis was lower (1.5), although the Odds ratio on multivariate analysis, was 2.8.

The pathogenesis of HIV related anaemia is unclear but is likely to be multifatorial. Possible causes are bleeding (gastrointestinal malignancy/severe infection) insufficient dietary intake, (vitamins, folate, iron and general malnutrition), effect of chronic HIV infection on micro-nutrient metabolism (acute phase reactants), haemolytic anaemia (infection, malignancy, splenomegaly, immune dysfunction), changes in erythropoetin synthesis, bone marrow suppression, anti-retroviral therapy and prophylactic treatments such as cotrimoxazole (Thurnham 1997; Mocroft *et al* 1999).

HIV viral load as well as CD4 count and percentage were not associated with increased risk of PPA in the current study. Low CD4 count was reported to increase the risk of anaemia in adults from 53 European centres (Mocroft *et al* 1999). These investigators also reported the risk of death was increased by 57% for each 1g/dl drop in Hb, after controlling for CD4 cell count, viral load and use of ARV drugs (Mocroft *et al* 1999). This risk was greater than that observed with a 50% reduction in CD4 cell count, or a log increase in HIV viral load. The authors concluded that Hb concentration measurement could be used as a tool to monitor disease progression where resources were too limited for CD4 estimation. Post-partum Hb estimation could be an important

indicator related to disease progression. This would complement its use as a screening tool for PPA.

Anaemia intervention studies have suggested that reversing anaemia could slow down HIV disease progression (Moore *et al* 1998). Moore *et al* observed that treatment of anaemia with erythropoeitin was associated with improved prognosis, possibly by allowing higher doses or prolonged use of drugs such as zidovudine. Studies on the effect of cheaper interventions in low resource settings, such as micro-nutrient supplementation, are urgently required to test this hypothesis.

3) Nutritional status

Iron deficiency anaemia is considered the most commonly recognised nutritional deficit both in industrialised and low resource settings (Scholl & Hediger 2000). In the present study MCV and MCHC values were used as indicators of microcytosis and hypochromasia. The results should be interpreted with caution, as confirmatory tests for deficient iron status were not available in this study. The microcytosis and hypochromasia observed could result from other causes, such as lead poisoning, α and β thalassemia and sideroblastic anaemias. The prevalence of these conditions in Zambia is unknown.

With increased erythropoeisis in pregnancy, there is a relative increase in circulating larger young erythrocytes which reduces the sensitivity of MCV as an indicator of iron deficiency (Chanarin *et al* 1977; Celada 1982; Thomson 1988; van den Broek 1998). A recent study in Malawi estimated the sensitivity of MCV in pregnancy at cut-off values of 85, 80 and 70 fL. The sensitivities were 58.1, 41.9, 41.9% respectively, indicating a

high level of false negatives with MCV as a screening tool for iron deficiency (van den Broek *et al* 1998).

Despite these limitations, the low values of MCV and MCHC in this study would indicate a significant component of iron deficiency anaemia in these women. Although women received ferrous sulphate supplementation during the antenatal period, either the amount given (200mg once daily), or compliance was unsatisfactory on the basis of these haematological results. A further study is required to ascertain the prevalence of iron deficiency anaemia in the post-natal period in order to justify haematemic supplementation post-natally.

Anaemia in the antenatal period was not associated with PPA. This lack of association could be confounded by blood loss at delivery. One month after delivery, post-natal anaemia was associated with PPA. The loss of Hb mass at delivery in women with marginal nutritional status could explain this finding. The analysis did not include evaluation of risk factors of post-natal anaemia because the number of women with haemoglobin results at one month was limited. These results require validation as haemoglobin measured post-partum could be useful as screening tool for identifying women at risk of post-natal anaemia in Safe Motherhood Programmes.

Post-partum serum retinol < 0.7 and < 1.05 μmol/L was significantly associated with PPA. Anaemia has long been recognised to be associated with vitamin A deficiency. Bloch (1994) noted that Danish orphans with xeropthalmia were not only weak and thin, they were also markedly anaemic. Wolbach and Howe (1925) noted loss of stem cells in the marrow of vitamin A deficient rats. With advanced deficiency this haematopoetic deficiency was more profound with loss of lymphoid cells and was

associated with heavy haemosiderin deposits in the spleen. Blackfan and Wolbach (1933) observed sequestered iron deposits (as haemosiderin) in the liver or spleen in malnourished infants who died with histopathologic evidence of vitamin A deficiency.

Vitamin A supplementation increases Hb concentration and improves iron status in children and pregnant women (Mejia *et al* 1988; Thurnham 1993; Panth *et al* 1990; Suharno *et al*; 1993). In a randomised clinical trial involving pregnant women in West Java, Indonesia, after 2 months of vitamin A supplementation (2400μg RE/day), Hb levels increased by 4gm/l over that seen in the placebo group (Suharno *et al* 1993). Although iron supplementation increased Hb by 8g/dl over controls, the greatest response (13g/dl increase) was observed with taking both vitamin A and iron. The prevalence of anaemia was reduced by 23% with vitamin A alone, 62% with iron and 98% with both vitamin A and iron. Similar observations were reported in pregnant women from India (Panth *et al* 1990).

These results suggest that vitamin A might be involved in the metabolism or mobilisation or utilisation of iron for haemoglobin synthesis. A possible mechanism suggested by Thurnham (1989) involves suppression of vitamin A and iron transport associated with underlying infection commonly found in poor populations. Synthesis of transport proteins, transferrin and retinol binding protein, are suppressed by infection. Following vitamin A supplementation, with improved immunity and infection clearance, there is a stimulus to synthesise transport proteins, which releases the trapped iron in the liver for haemoglobin synthesis.

The explanation for the negative association of low maternal weight (<45 kilogram) with PPA is unclear. Malnutrition is frequently associated with nutritional anaemia but

the association may be confounded by several factors including poor socio-economic status, access and use of health care and underlying nutritional deficiencies.

A history of alcohol consumption was significantly associated with lower risk of PPA. This unexpected finding might reflect the better socio-economic status of women who consume alcohol in pregnancy. The type of beverage consumed could be important. Locally prepared beers in Zambia are often prepared from fermented maize flour. In a study in China, feeding children with fermented soya for 6 months significantly reduced the incidence of iron deficiency anaemia from 21.7% to 1.25% (Qin 1989). The author attributed this improvement to better iron absorption as result of fermentation. Consumption of fermented beers could have a similar effect. Several reports have associated iron overload with heavy consumption of fermented beer even when cooked in aluminium pots (Gordeuk *et al* 1992).

4) Malaria

Pregnancy is an important predisposing factor for malaria and studies conducted in holo-endemic areas in Malawi and Kenya have indicated that maternal HIV infection is associated with increased risk of *P. falciparum* parasitaemia in pregnancy (Steketee *et al* 1996; Parise *et al* 1999;Verhoeff *et al* 1999). The data also showed an increased risk of parasitaemia in multiparous HIV infected women who are more likely to have been infected with HIV for a longer period.

The present study was conducted in an urban area with a low prevalence of malaria. Watts *et al* (1990) examined 423 children during the malaria in 1984 season and only 2.4% had scanty parasitaemia. The spleen rate in children under 15 years old was 3%,

indicating hypoendemic malaria. Although Lusaka is an area of low malaria prevalence, over 30% of women in our study reported taking treatment for malaria during pregnancy. There was no increased risk of PPA observed with malaria treatment in primigravidae and HIV infected women. This treatment was mainly based on presumptive diagnosis without blood-slide confirmation. This limitation could explain the finding.

5.4 CONCLUSION

Anaemia during pregnancy and after delivery is common and its reduction should be treated as priority in Safe Motherhood Programmes in Zambia. HIV infected women, especially those who undergo caesarean section are at increased risk of post-partum and possibly post-natal anaemia. Haemoglobin estimation should be part of the routine care during pregnancy and post-partum and HIV infected anaemic women should be closely monitored for HIV disease progression and post-natal anaemia. Micro-nutrient supplementation in pregnancy and post-partum should be part of the minimum package of care of these women.

CHAPTER 6

PREVALENCE AND RISK FACTORS FOR PRE-TERM DELIVERY, LOW BIRTH WEIGHT AND INTRAUTERINE GROWTH RETARDATION IN HIV INFECTED AND UNINFECTED WOMEN

6.1 INTRODUCTION AND OBJECTIVES

Low birth weight is an important indicator of pregnancy outcome and is associated with poor child survival. Improving child survival is a priority component of Safe Motherhood Programmes and interventions aimed at reducing mother to child HIV transmission (MTCT). Low birth weight (LBW) babies have a reduced chance of surviving through the critical neonatal and postnatal periods, and if they survive, they are at higher risk of linear growth retardation and impaired mental development (Lechtig 1985). The objectives of this chapter were, to estimate the prevalence and risk factors of pre-term delivery, LBW and intra-uterine growth retardation (IUGR) in children born to HIV infected and uninfected mothers.

6.1.1 Prevalence of Low Birth Weight

Prevention of low birth weight, an important determinant of infant and childhood morbidity and mortality (Lechtig 1988; McCormick 1985; Forsas *et al* 1999), remains a major challenge in Sub-Saharan Africa. Globally, almost 25 million LBW babies are born, accounting for 17% of total births (WHO 1992; UNICEF 2000). The State of the World Children 2000 report (UNICEF) estimated the prevalence of LBW in Sub-

Saharan Africa between 1990 to 1997 to have been 15%, a rate over twice that observed in industrialised countries (6%).

6.1.2 Risk factors for pre-term delivery, low birth weight and intra-uterine growth retardation

In industrialised countries, LBW predominantly results from pre-term delivery, whereas in low resource settings, intrauterine growth retardation (IUGR) is the main contributor (Kramer 1987; Lechtig 1988; Verhoeff *et al* 1998). In a study at Baragwanath Hospital in Johannesburg, South Africa, Stein and Ellis (1974) observed that 73% of LBW babies had IUGR. Further, Villar and Belizan in 1982 reported that when the incidence of LBW is higher than 10%, it is most exclusively due to increase in IUGR while prematurity remains almost unchanged. Two clinical types of growth retardation, type I and II, have been defined (Rosso & Winick 1974). In type I the insult begins before 30 weeks gestation causing symmetrical growth retardation and in type II the retardation is asymmetrical as the insult begins after 30 weeks, with a greater effect on weight reduction than linear growth.

In Zambia, studies on LBW are limited and there are no previous studies examining risk factors for LBW, pre-term delivery and IUGR. The prevalence of LBW in two previous Zambian studies reported in 1977 and 1990 were 11.4% and 11% respectively (Davis 1977; Watts *et al* 1990). Both studies did not define the relative contribution of pre-term delivery or IUGR to LBW. In another study involving 2,353 normal singleton consecutive births at the University Teaching Hospital (UTH) in Lusaka, 208 (17%) of the babies were born pre-term (Bhat *et al* 1989). Although the mean birth weight in that

study was reported as 3.13 kilograms, the proportion of LBW babies was not determined.

In Kramer's (1987) extensive systematic review of 895 studies on LBW, several consistent factors were related to increased risk of pre-term delivery and IUGR. For IUGR, these factors included infant sex, racial/ethnic origin, low maternal height and pre-pregnancy weight, paternal weight and height, maternal birth weight, parity, a previous history of a low birth weight infant, poor caloric intake and gestational weight gain, general maternal morbidity or episodic illness, malaria, cigarette smoking, alcohol consumption and tobacco smoking.

In developing countries, Kramer concluded that the major determinants of IUGR were black or Indian racial origin, poor gestational nutrition, low pre-pregnancy weight, short maternal stature and malaria. For pre-term delivery, the major determinants were low pre-pregnancy weight, a previous history of pre-term delivery or spontaneous abortion, exposure during pregnancy to diethylstilbesterol and cigarette smoking. In the majority of children, however, the aetiology of prematurity remains unknown.

In the last 20 years HIV has become a major cause of morbidity in sub-Saharan Africa. However, Kramer did not evaluate the contribution of HIV to LBW. More recent data in the 1990s suggests that maternal HIV infection does reduce birth weight (Taha *et al* 1995; Leroy *et al* 1998; Verhoeff *et al* 1997; Brocklehurst & French 1998; Castetbon *et al* 1999). Similarly the risk of pre-term delivery is also increased with maternal HIV infection (Broklehurst & French 1998).

Data from industrialised countries has suggested that maternal anaemia increases the risk of LBW and pre-term delivery (Klebanoff 1991; Scholl & Hediger 1994). As

anaemia is also associated with HIV infection in pregnancy (van den Broek *et al* 1998; Meda *et al*, 1999; Ramon *et al*, 1999), an additive effect on birth outcome could result in settings with high maternal HIV prevalence.

The World Health Organisation (1972) and INAG (1999) have defined haemoglobin (Hb) cut-off points for anaemia in pregnancy and lactating women considered of public health significance. However, Hb concentrations that impact pregnancy outcome are not clearly defined and will vary according to the population studied (Garn *et al* 1981; Steer *et al* 1995). It has been suggested that the impact of Hb concentration on the incidence of LBW and pre-term delivery is U-shaped, with the proportion of LBW babies rising and mean birth weight falling with maternal haemoglobin values that are either at the low or high end of the range. The demonstration of this association was reported from three large studies in industrialised countries; the National Collaborative Perinatal Project from the United States, the Cardiff Birth study and the North West Thames Regional surveys in the United Kingdom (Garn *et al* 1981; Murphy *et al* 1986; Steer *et al* 1995). The importance of these findings in African mothers, with nutritional deficiency and malaria, is unclear. For comparative purposes, most researchers in Africa use the WHO cut-off points.

Low birth weight, whether due to pre-term delivery or IUGR, is a major cause of morbidity and mortality in young children with or without HIV infection. Modifiable factors with large effects on reducing IUGR and pre-term delivery should form targets for Safe Motherhood Programmes. In settings highly affected by HIV, modifying these factors could be crucial for improving child survival and targeted interventions should

be integrated, where possible, into prevention programmes aimed at reducing MTCT of HIV.

6.2 METHODS

6.2.1 Subject management

The research midwives undertook a three-day training workshop in order to standardise anthropometric measurement procedures accordin to the World Health Organisation methodology (1983). Measurements were done on both mothers and babies within 24 hours of delivery. The weight was measured with the infant nude using a Salter scale to the nearest 0.1 kilogram (Salter England, West Bromwich, England, model 235 6S, 0-25kg). The scale was calibrated daily before starting each clinic. Recumbent length of the children was measured to the nearest 1 cm.

The upper mid-arm circumference (MUAC) of the mother was measured at the mid-point in the left arm, with the elbow at a right angle, to the nearest 0.1 cm. Care was taken to ensure that the tape did not compress the arm.

All anthropometric measurements were done in triplicate and the mean value calculated. The reported date of the last menstrual period (LMP) was used to determine gestational age of the infant. Socio-demographic, obstetric, medical and biological factors collected at enrolment are described in Chapter 4.

6.2.2 Laboratory methods

Methods for haemoglobin (Hb), vitamin A, RPR test, HIV ELISA and CD4 cell count were described in chapter 4.

6.2.3 Definitions

Low birth weight was defined as weight of less than 2500 grams, pre-term delivery as gestational age of less than 37 weeks and IUGR as birth weight below the 10^{th} percentile for gestational age as defined by Williams *et al* (1982) and recommended by WHO (Kelly *et al* 1996). Rohrers ponderal index was calculated as birth weight (gm) x 100, divided by $(length)^3$ (cm) to define growth symmetry (Rosso 1989). The total study population was used to define the 10^{th}, 50^{th}, and 90^{th} percentiles. Asymmetrical growth retardation was defined as a ponderal index below the 10^{th} centile in IUGR babies (Lubchenco *et al* 1966). The rest of the parameters were defined as described in previous chapters.

6.2.3 Statistical analysis

Analysis included only children who had information on gestational age and birth weight. Growth curves were drawn and all outliers outside the 99^{th} centile of the British standards were excluded from analysis. Most of these, had unacceptably high birth weights for gestational age and it was assumed that this occurred because of large error in the LMP estimation. Data was analysed using SPSS version 9.0 for Windows and Epi Info version 6.04. Pearson X^2 and relative risk (with 95% confidence limits) were used for comparison of discrete variables and X^2 for trend for measuring linear associations. Means were compared using the student t test. Multiple logistic regression analyses was applied to identify independent factors associated with pre-term delivery, LBW and IUGR. Variables at a significant level of $P < 0.1$ in the univariate analysis were included in the multivariate regression analysis. A P value <0.05 was considered significant and

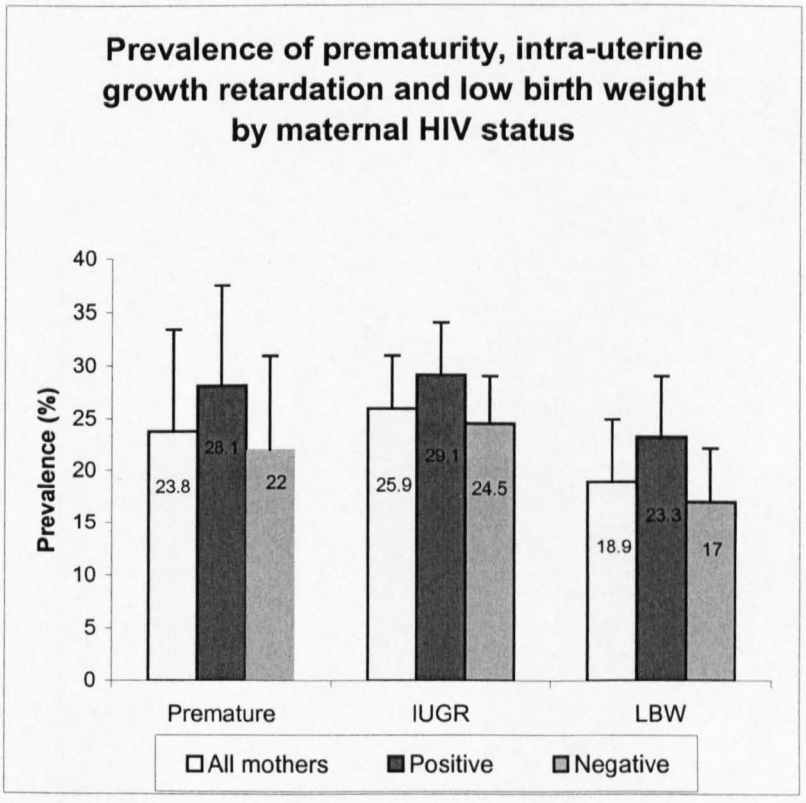
all tests were 2 tailed. Separate analyses were done for the whole study population (all mothers) and HIV infected and non-infected mothers.

6.3 RESULTS

6.3.1 Prevalence of prematurity, low birth weight and intra-uterine growth retardation

Two hundred and eighty six children were included in the analysis. The prevalence of pre-term delivery, IUGR and LBW was higher in babies born to HIV infected mothers, although the differences were not statistically significant (figure 6.2).

Figure 6.1



Bars represent 95% confidence limits

1) Pre-term delivery

Of the 286 babies, 68 were premature (23.8%; 95% CI 18.8-28.7%). Stratified analysis by maternal HIV status showed that of babies born to HIV infected mothers, 24 out of 86 were premature (27.9%; 95% CI 18.4-37.4%) compared to 44 babies of 200 non-infected mothers (22.0%; 95% CI 16.2-27.7%).

2) Low birth weight

Low birth weight was present in 54 of the 286 babies (18.9%; 95% CI 14.3-23.4%). Twenty of these babies were born to the 86 HIV infected mothers (23.3%; 95% CI 14.3-32.2%) and 34 to 200 non-infected mothers (17.0%; 95% CI 11.8-22.2%). Of the 54 LBW babies 26 (48.1%) had IUGR and 33 (61.1%) were born prematurely.

3) Intra-uterine growth retardation

Intra-uterine growth retardation was the commonest birth outcome. Of the 286 children, 74 had IUGR (25.9%; 95% CI 20.8-30.9%). Stratified analysis of IUGR babies by maternal HIV status (86 HIV infected and 200 HIV non-infected mothers) showed that 25 were born to infected (29.1%; 95% CI 19.5-38.7%) and 49 to non-infected mothers (24.5%; 95% CI 18.5-30.5%). The ponderal index was calculated for 279 babies, including 69 with IUGR. Seven of the IUGR babies (10.1%) had asymmetrical growth retardation with an index below the 10^{th} centile. Of the babies without IUGR, 10.5% had asymmetrical growth.

6.3.2 Univariate analysis of factors associated with pre-term delivery

The univariate analyses of risk factors associated with pre-term delivery are shown in table 6.1. Maternal HIV infection had no effect on the risk of pre-term delivery or mean gestational age at delivery. Stratified analysis by maternal HIV status indicated that lack of paternal income, low post-partum maternal weight (< 45 kg), previous pre-term delivery and malaria treatment during pregnancy were associated with significantly increased risk of pre-term delivery in HIV infected mothers (table 6.1). CD4 cell count < 500cells/mm^3 or percentage <25% were not associated with prematurity in HIV infected mothers.

In non-HIV infected mothers pre-term delivery was statistically more prevalent when mothers were young (< 20 years), primigravidae, primiparous and anaemic (Hb< 11g/dl) or with low post-partum maternal weight (< 45 kg) and CD4 percentage (< 25%). Stratified analysis showed that the relative risk of prematurity associated with low CD4 percentage in mothers with low post-partum maternal weight (<45kg) was 4.86 (95% CI 1.25-18.91) and 4.24 (95% CI 1.18-15.16) in those with antenatal maternal anaemia (Hb< 11g/dl). IUGR was significantly less frequent in pre-term than term births of HIV non-infected mothers.

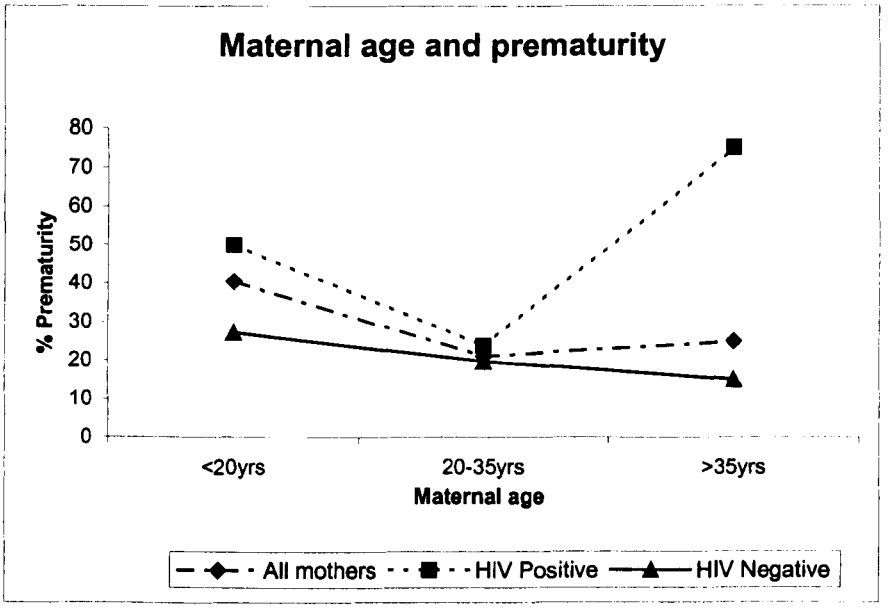
For all deliveries, there was a negative linear relationship between the proportion of pre-term births gravidae, parity and post-partum maternal weight (figure 6.2, 6.3, 6.4 and 6.5). In children born to HIV non-infected mothers, a significant downward trend was observed increasing maternal age, gravidae and parity, whereas in children born to HIV infected mothers, an association was observed only with increasing post-partum maternal weight.

Table 6.1 Risk factors for pre-term delivery

Factor	*Unadjusted Relative Risk (95% confidence interval)*		
	All mothers	*HIV non-infected*	*HIV positive*
Maternal HIV infection	0.79 (0.51-1.21)		
Father has no income	**2.05 (1.24-3.38)**	1.51 (0.73-3.11)	**3.66 (2.13-6.28)**
Maternal age < 20years	**1.89 (1.20-3.0)**	**2.03 (1.82-3.49)**	1.90 (0.79-4.59)
Primigravida (first pregnancy)	**1.66 (1.09-2.51)**	**2.73 (1.65-4.52)**	0.37 (0.10-1.42)
Primiparity (first baby)	**1.78 (1.18-2.68)**	**2.60 (1.57-4.32)**	0.81 (0.37-1.81))
Previous h/o of pre-term delivery	**1.96 (1.17–3.30)**	1.81 (0.90-3.61)	**2.26 (1.0-4.75)**
H/o malaria treatment during pregnancy	1.45 (0.94-2.24)	1.16 (0.64-2.08)	**2.0 (1.05-3.82)**
Positive RPR during pregnancy	1.45 (0.78-2.68)	0.87 (0.30-2.56)	2.17 (0.97-4.85)
Positive post-partum RPR	1.06 (0.84-1.34)	1.02 (0.78-1.35)	1.10 (0.71-1.67)
H/o alcohol intake during pregnancy	0.3 (0.08-1.17)	ND	0.45 (0.12-1.72)
Maternal weight < 45kg	**2.86 (1.76-4.67)**	**3.16 (1.72-5.82)**	**2.34 (1.04-5.27)**
MUAC (cm)	1.44 (0.79-2.65)	o.89 (0.25-3.10)	1.80 (0.90-3.59
Antepartum haemorrhage	1.79 (0.88-3.65)	2.40 (0.86-6.68)	1.28 (0.49-3.39)
Antenatal maternal haemoglobin <11g/dl	**3.46 (1.07-11.18)**	**7.69 (1.01-58.59)**	1.35 (0.35-5.24)
Post-partum maternal haemoglobin (<11g/dl)	1.31 (0.87-2.00)	1.09 (0.65-1.85)	1.78 (0.8-4.01)
Post-partum serum retinol < 1.05μmol/L	1.35 (0.89-2.04)	1.29 (0.76-2.21)	1.33 (0.67-2.63)
CD4 cell count <500 cells/mm^3	0.99 (0.63-1.55)	0.53 (0.18-1.52)	1.27 (0.52-3.07)
CD4% < 25	1.38 (0.88-2.15)	**2.17 (1.07-4.39)**	0.87 (0.44-1.71)
Intra-uterine growth retardation[1]	**0.28 (0.13-0.61)**	**0.72 (0.01-0.51)**	0.64 (0.27-1.53)

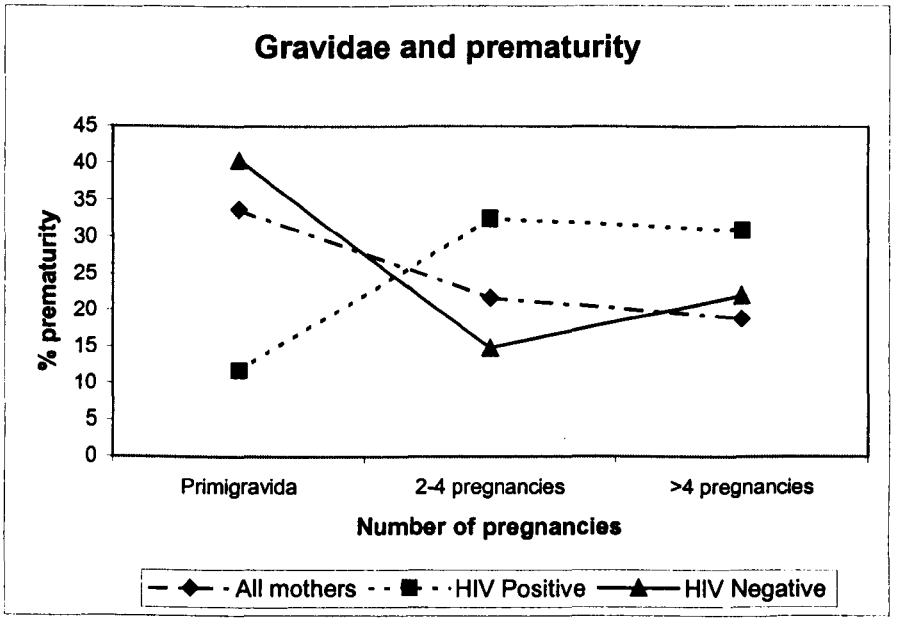
[1]Below 10th centile for gestation, ND not done as one of the cells empty, RPR rapid plasma reagin test for syphilis MUAC mid –upper arm circumference
Significant values in bold

Figure 6.2



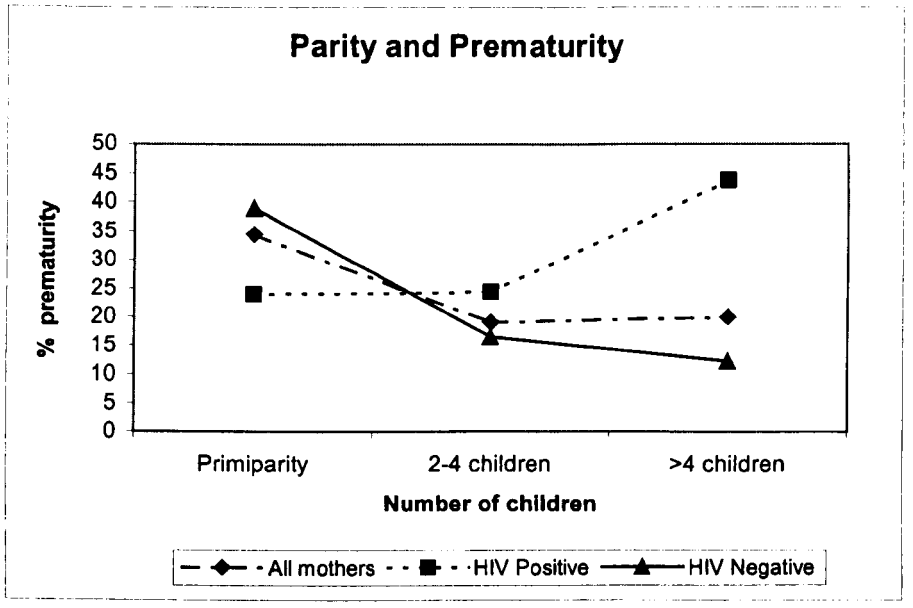
χ^2_{trend} All mothers 6.7 P=0.076; HIV positive 6.5 P= 0.70; HIV non-infected 6.1 P= 0.03

Figure 6.3



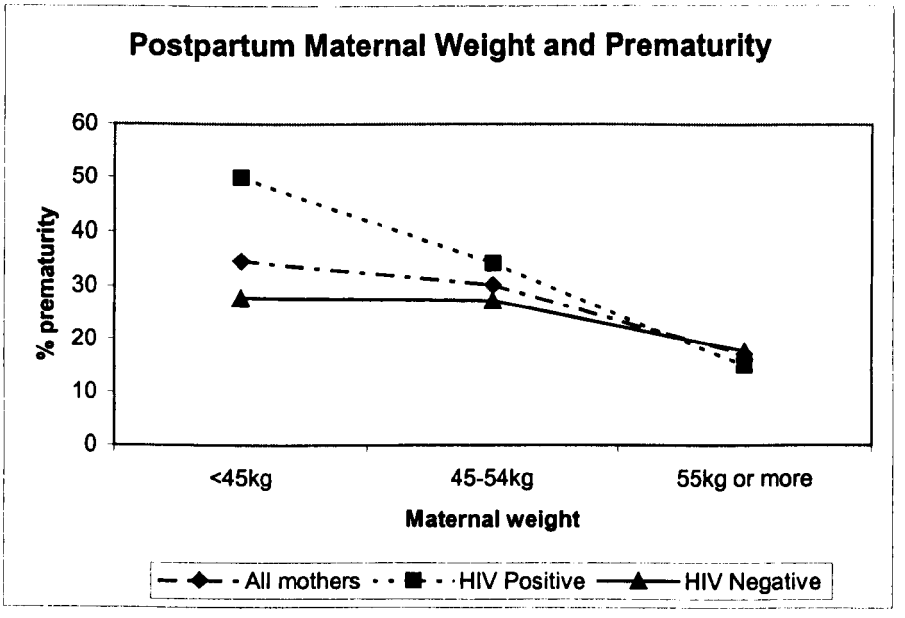
χ^2_{trend} All mothers 5.63 P= 0.028; HIV positive 2.76 P= 0.25; HIV non-infected 15.43 P< 0.001

Figure 6.4



χ^2_{trend} **All mothers 7.47 P=0.027; HIV positive 2.45 P=0.29; HIV non-infected 14.20 P=0.001**

Figure 6.5



χ^2_{trend} **All mothers 7.23 P= 0.009; HIV positive 5.37 P= 0.022; HIV non-infected 2.37 P=0.16**

6.3.2 Univariate analysis of factors with low birth weight (table 6.2)

Maternal HIV infection was not associated with increased risk of low birth weight, although mean birth weight (±SD) was significantly lower in babies of HIV infected compared to non-infected mothers 2.7 (± 0.6) versus 2.9 (± 0.5) kilograms respectively P = 0.006). For all deliveries, young maternal age, (<20 years), previous history of pre-term delivery, low post-partum maternal weight (<45 kg), serum retinol (< 1.05μmol/L), low CD4 percentage (<25%) and IUGR were associated with increased risk of LBW.

In HIV infected mothers, a significant increase in risk of LBW was observed with a previous history of pre-term delivery, low post partum-maternal weight (<45 kg), IUGR and positive post-partum maternal RPR. The association with post-partum maternal weight was negative and linear. There was no significant association with increasing parity (figure 6.7). There was a non-significant U-shaped relationship with increasing maternal age (figure 6.8), although the highest prevalence of LBW was in babies of mothers between <20 and >35 years.

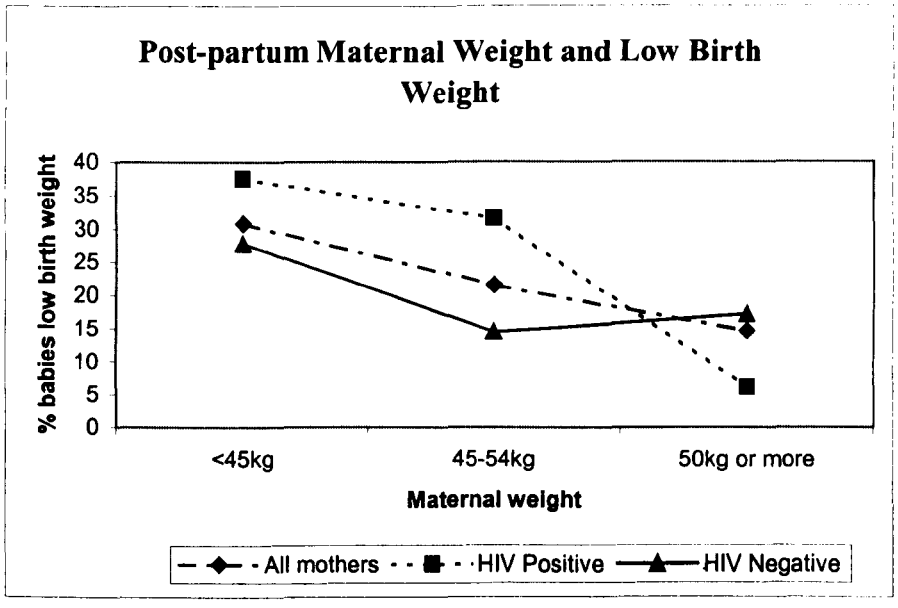
In HIV non-infected mothers, young maternal age (<20 years), primigravidae, primiparity, previous history of pre-term delivery, antepartum haemorrhage, low post-partum maternal serum retinol (< 0.7μmol/L) and IUGR were associated with increased risk of LBW. The relationship was negative and linear with increasing parity (figure 6.7), maternal serum retinol (figure 6.9) and CD4 cell count (6.10). Stratified analysis with CD4 cell count cut-off at < 200 cells/mm^3, gave a relative risk for LBW of 3.96 (95% CI 1.67-9.36). There was no association between antenatal Hb, post-partum and MUAC with LBW in HIV infected or non-infected mothers.

Figure 6.2 Risk factors for Low Birth Weight

Factor	*Unadjusted Relative Risk (95% confidence interval)*		
	All mothers	*HIV non-infected*	*HIV positive*
Maternal HIV infection	1.37 (0.84-2.24))		
Father has no income	1.07 (0.47-2.43)	0.58 (0.51-2.24)	2.47 (0.99-6.15)
Maternal age < 20years	**2.13 (1.26-3.57)**	**2.26 (1.20-4.24)**	2.35 (0.95-5.81)
Primigravida	1.43 (0.82-3.0)	**2.21 (1.22-4.03)**	0.45 (.12-1.76)
Primiparity	1.52 (0.93-2.47)	**2.11 (1.16-3.85)**	0.81 (0.33-2.0)
Previous h/o of pre-term delivery	**3.25 (2.01-5.26)**	**2.97 (1.54-5.71)**	**3.76 (1.96-7.23)**
H/o malaria treatment during pregnancy	0.98 (0.56-1.69)	0.85 (0.40-1.79))	1.17 (0.52-2.61)
Positive RPR during pregnancy	1.27 (0.62-2.59)	1.03 (0.35-3.06)	1.45 (0.54-3.87)
Positive post-partum RPR	1.77 (0.97-3.24)	1.04 (0.36-3.05)	**2.64 (1.27-5.52)**
H/o alcohol intake during pregnancy	0.79 (0.31-2.0)	0.50 (0.08-3.31)	0.85 (0.28-2.58)
Ante-partum haemorrhage	1.81 (0.78-4.28)	**4.89 (2.50-9.57)**	0.40 (0.02-3.75)
Post-partum weight < 45kg	**2.19 (1.05-4.57)**	1.47 (0.42-5.08)	**3.17 (1.35-7.47)**
Antenatal haemoglobin <11g/dl	2.77 (0.82-9.31)	6.15 (0.77-49.12)	1.08 (0.26-4.49)
Postnatal haemoglobin <11g/dl	1.02 (0.63-1.66)	1.01 (0.54-1.88)	0.89 (0.41-1.94)
Post-partum serum retinol < 1.05 μmol/L	**2.03 (1.25-3.28)**	**2.40 (1.30-4.44)**	1.38 (0.64-2.97)
CD4 cell count (cells/mm^3) <500	1.31 (0.80-2.13)	0.68 (0.22-2.10)	1.95 (0.77-4.87)
CD4 % < 25	**1.69 (1.02-2.80)**	1.65 (0.59-4.56)	1.74 (0.69-4.35)
Intra-uterine growth retardation	**2.66 (1.67-4.23)**	**2.74 (1.52-4.95)**	**2.44 (1.16-5.13)**

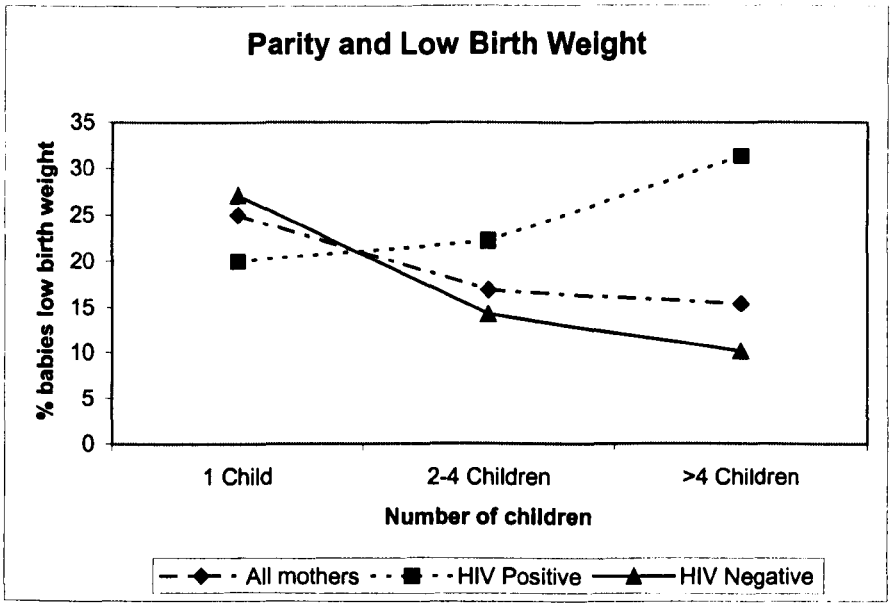
Significant values in bold

Figure 6.6



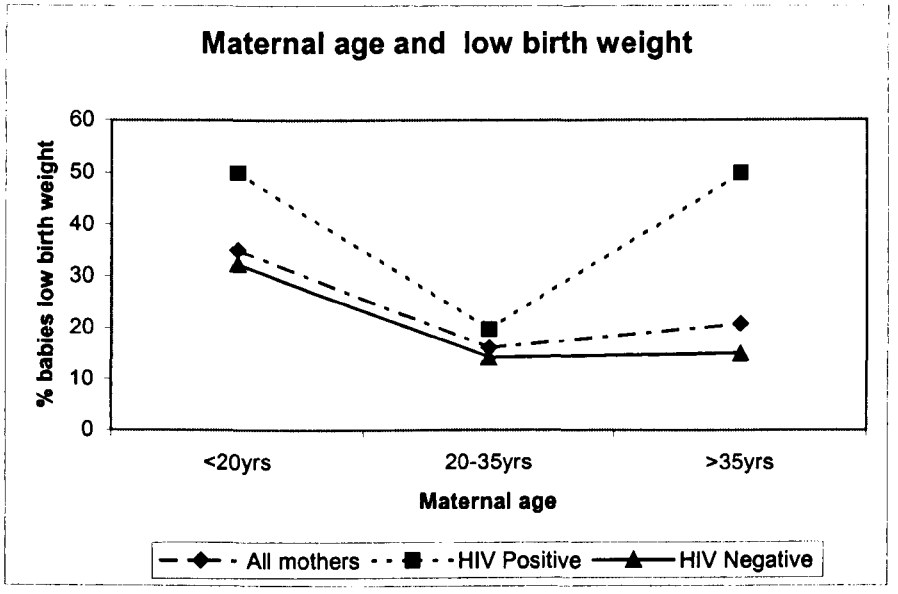
χ^2_{trend} **All mothers 4.60 P= 0.033; HIV positive 8.16 P=0.007; HIV non-infected 1.67 P=0.527**

Figure 6.7



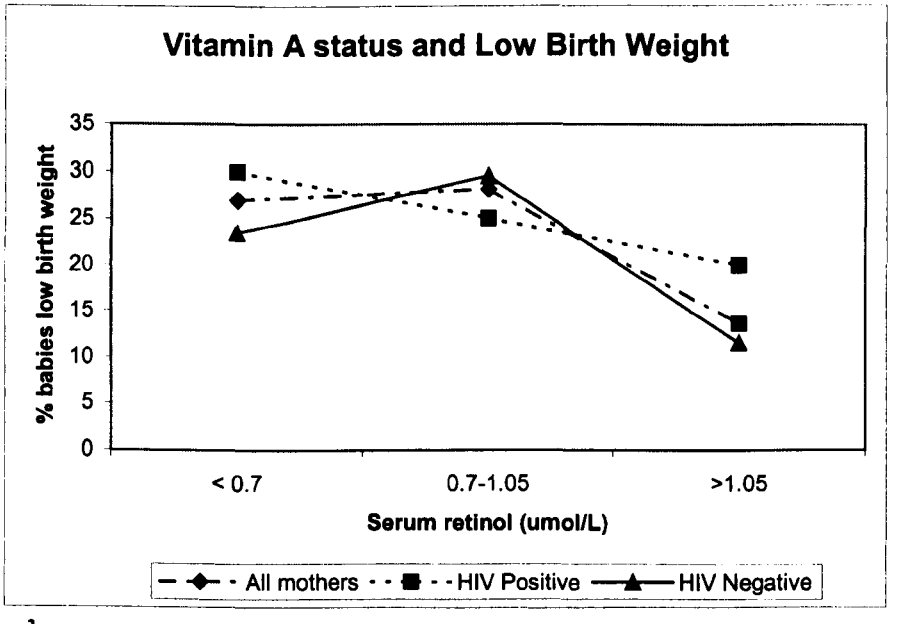
χ^2_{trend} All mothers 2.908 P=0.121; HIV positive 0.075 P= 0.436; HIV non-infected 6.33 P=0.018

Figure 6.8



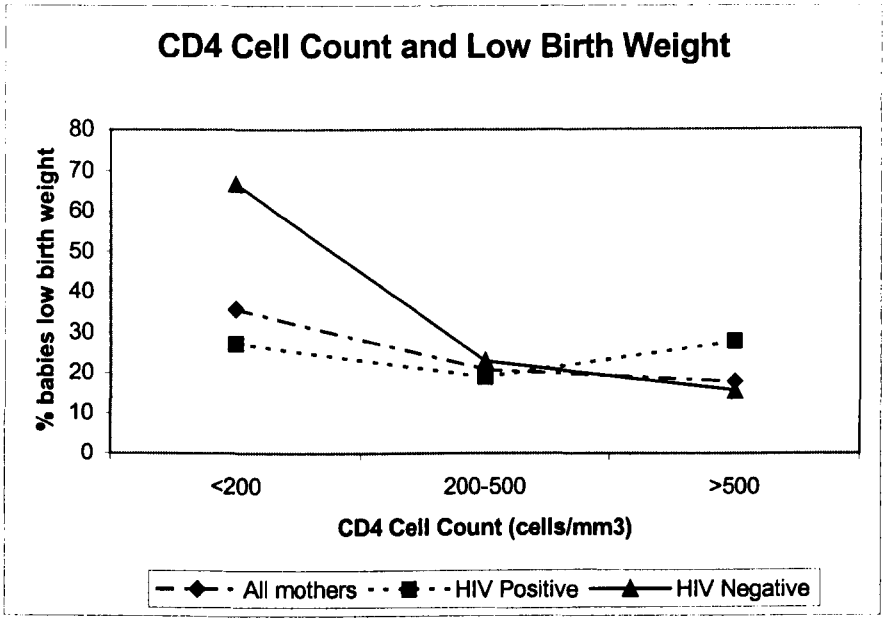
χ^2_{trend} All mothers 7.56 P= 0.070; HIV positive 4.53 P= 0.690; HIV non-infected 5.98 P=0.06

Figure 6.9



χ^2_{trend} All mothers 8.50 P= 0.009; HIV positive 0.80 P=0.374; HIV non-infected 8.42 P=0.017

Figure 6.10



χ^2_{trend} **All mothers 2.77 P= 0.148; HIV positive 0.87 P= 0.697; HIV non-infected 6.12 P=0.043**

6.3.3 Univariate analysis of factors associated with intra-uterine growth retardation

Table 6.3 summarises the analysis for determinants of IUGR. Maternal HIV infection was not associated with risk of IUGR. For all deliveries maternal antibiotic treatment during pregnancy, low post-partum maternal serum retinol (<0.7μmol/L) and LBW (<2.5 kg) were associated with increased risk of IUGR. Premature babies were significantly less likely to be growth retarded.

In babies born to HIV non-infected mothers, tuberculosis treatment during pregnancy, low maternal serum retinol (< 0.7μmol/L) and LBW (< 2.5 kg) increased the risk of IUGR. Pre-term delivery, was associated with significantly reduced reduced risk. The relationship with increasing serum retinol concentration and IUGR was negative and linear (figure 6.11).

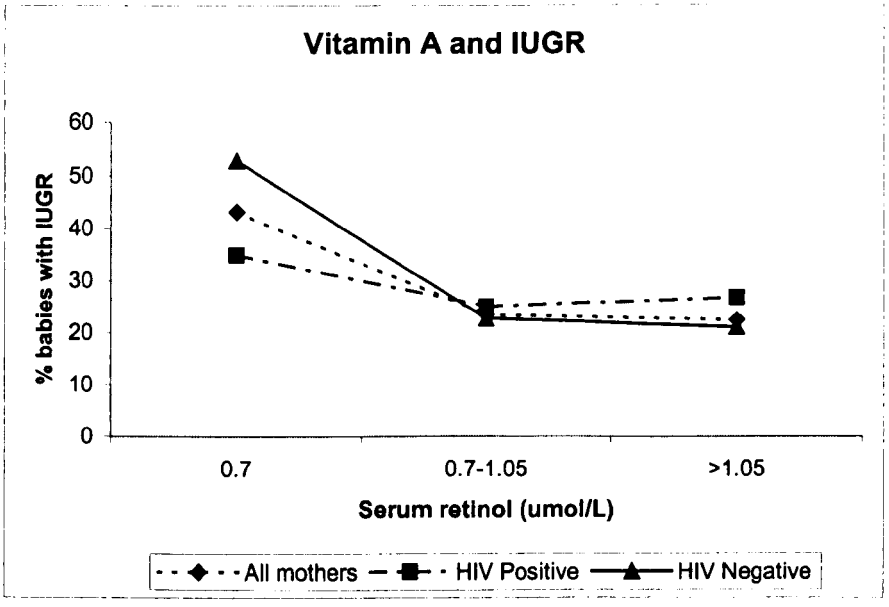
Antibiotic treatment during pregnancy and LBW (<2.5 kilograms) were the only factors associated with IUGR in babies born to HIV infected mothers.

Table 6.3 Risk factors for intra-uterine growth retardation

Factor	*Unadjusted relative risk (95% confidence limits)*		
	All mothers	*HIV non-infected*	*HIV positive*
Maternal HIV infection	1.19 (0.79-1.79)		
Father earns no income	0.76 (0.34-1.72	0.62 (0.21-1.81)	1.20-(0.37-3.92)
Maternal age < 20years	0.93 (0.51-1.70)	1.06 (0.55-2.03)	0.56 (0.90-3.43)
Primigravida	0.99 (0.63-1.54)	1.10 (0.65-1.86)	0.77 (0.31-1.96)
Primiparity	0.95 (0.61-1.47)	1.05 (0.53-2.14)	0.70 (0.35-1.70)
Previous H/o of pre-term delivery	0.83 (0.37-0.84)	1.02 (0.42-2.47)	0.47 (0.07-2.98)
H/o malaria treatment during pregnancy	1.10 (0.71-1.71)	1.27 (0.75-2.14)	0.80 (0.35-1.82)
H/o antibiotic treatment in pregnancy	**2.06 (1.19-3.57)**	1.52 (0.67-3.5)	**2.93 (1.36-6.35)**
H/o tuberculosis in pregnancy	1.79 (0.83-3.82)	**2.61 (1.22-5.64)**	0.85 (0.15 (4.79)
Positive RPR during pregnancy	1.55 (0.91-2.66)	1.89 (0.99-3.60)	1.11 (0.44-2.84)
Positive post-partum RPR	1.55 (0.93-2.58)	1.50 (0.75-3.01)	1.54 (0.72-3.32)
Antepartum haemorrhage in pregnancy	1.31 (0.57-3.01)	2.09 (0.76-5.78)	0.86 (0.24-3.01)
H/o alcohol intake during pregnancy	0.70 (0.31-1.59)	1.07 (0.40-2.91)	0.41 (0.11-1.55)
Maternal weight < 45kg	0.90 (0.33-2.47)	-	4.34 (0.67-28.0)
Antenatal maternal haemoglobin <11g/dl*	0.65 (0.25-1.73)	0.77 (0.23-2.55)	0.54 (0.10-3.04)
Post-partum maternal haemoglobin <11g/dl	1.16 (0.79-1.72)	1.17 (0.72-1.91)	1.05 (0.53-2.1)
Post-partum serum retinol < 0.7μmol/L	**1.91 (1.23-2.95)**	**2.47 (1.46-4.19)**	1.34 (0.65-2.76)
Post-partum serum retinol < 1.05μmol/L	1.37 (0.92-2.04)	1.48 (0.91-2.43)	1.13 (0.57-2.22)
CD4 cell count <500 cells/mm^3	1.11(0.73-1.69)	1.10 (0.60-2.01)	0.95 (0.49-1.85)
CD4 cell count < 25%	1.36 (0.89-2.15)	0.36 (0.05-3.36)	1.32 90.65-2.68)
Birth weight <2.5 kilograms	**2.33 (1.60-3.38)**	**2.37 (1.48-3.79)**	**2.20 (1.18-4.11)**
Ponderal index < 10^{th} centile	0.93 (0.49-1.92)	1.04 (0.46-2.31)	0.84 (0.23-3.04)
Gestation < 37 weeks	**0.28 (0.13-0.62)**	**0.74 (0.10-0.52)**	0.65 (0.27-1.53)

Significant values in bold, RPR rapid plasma reagin

Figure 6.11



χ^2_{trend} All mothers 7.22 P= 0.027; HIV positive 0.61 P=0.55; HIV non-infected 8.49 P=0.018

6.3.5 Multivariate analysis of factors associated with pre-term delivery, LBW and IUGR

Tables 6.4 shows the factors that were included in the logistic regression models. Table 6.5 shows results of the multivariate analysis of factors independently associated with pre-term delivery, LBW and IUGR.

1) Pre-term delivery (table 6.5)

Low antenatal Hb < 11 g/dl at booking was independently associated with pre-term delivery in all babies regardless of maternal HIV status. This finding was also observed for babies of HIV non-infected but not infected mothers. Lack of a paternal source of income was the only independent association with pre-term delivery in HIV infected mothers.

Table 6.4 Factors selected for multivariate analysis

Factor	*Pre-term delivery*			*Low Birth Weight*			*Intra-uterine growth retardation*		
	All[1]	*Pos*[2]	*Neg*[3]	*All*	*Pos*	*Neg*	*All*	*Pos*	*Neg*
Father earns no income	**Yes**	**Yes**	No	No	**Yes**	No	No	No	No
Mother earns no income	No	No	No	No	No	No	No	**Yes**	No
Maternal age < 20years	**Yes**	No	**Yes**	**Yes**	No	**Yes**	No	No	No
Primigravida	**Yes**	**Yes**	**Yes**	No	No	**Yes**	No	No	No
Primiparity	**Yes**	No	**Yes**	**Yes**	No	**Yes**	No	No	No
Previous h/o of pre-term delivery	**Yes**	**Yes**	No	**Yes**	**Yes**	No	No	No	No
H/o malaria treatment during pregnancy	**Yes**	**Yes**	No	No	No	No	No	No	No
H/o antibiotic treatment in pregnancy	No	No	No	No	No	No	**Yes**	**Yes**	No
H/o tuberculosis in pregnancy	No	No	No	**Yes**	No	**Yes**	No	No	No
Positive RPR during pregnancy	No	**Yes**	No	**Yes**	No	No	No	No	**Yes**
Positive post-partum RPR	No	No	No	No	No	No	No	No	No
Antepartum haemorrhage in pregnancy	No	No	**Yes**	No	No	**Yes**	No	No	No
H/o alcohol intake during pregnancy	No	No	**Yes**	No	No	No	No	No	No
Maternal weight < 45kg	**Yes**	**Yes**	No	**Yes**	**Yes**	No	No	**Yes**	**Yes**
Antenatal maternal haemoglobin <11g/dl*	**Yes**	No	**Yes**	**Yes**	No	**Yes**	No	No	No
Post-partum maternal haemoglobin <11g/dl	No	No	No	No	No	No	No	No	No
Post-partum serum retinol < 0.7μmol/L	No	No	No	No	No	No	**Yes**	No	**Yes**
Post-partum serum retinol < 1.05μmol/L	No	No	No	**Yes**	No	**Yes**	No	No	No
CD4 cell count <500 cells/mm^3	No	No	No	No	No	No	No	No	No
CD4 cell count < 25%	No	No	**Yes**	**Yes**	No	No	No	No	No
Birth weight <2.5 kilograms	No	No	No	No	No	No	**Yes**	No	No
Ponderal index < 10th centile	**Yes**	No	No	No	No	No	No	No	No
Gestation < 37 weeks	No	No	No	No	No	No	**Yes**	No	No
Intra-uterine growth retardation	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	No	No	No

All[1] All mothers;Pos[2] HIV infected mothers; Neg[3] HIV non-infected mothers
Yes - included in analysis

2) Low birth weight (table 6.5)

Antenatal Hb<11 g/dl and IUGR were independently associated with LBW for all deliveries. Antenatal anaemia was also associated with increased risk of LBW in non-infected mothers. IUGR was significantly increased in HIV infected women.

Table 6.5 Multivariate analyses of factors associated with pre-term delivery, LBW and IUGR.

Factor	*Adjusted Odds Ratio (95% confidence limits)*		
	All mothers	***HIV non-infected***	*HIV positive*
Pre-term delivery			
Father earns no income			11.7 (1.23-111.70)
Antenatal maternal haemoglobin <11g/dl*	5.12 (1.15-22.80)	13.70 (1.3-143.42)	
Low Birth weight			
Antenatal maternal haemoglobin <11g/dl	5.08 (1.00-25.78)	16.89 (1.01-283.22)	
Intra-uterine growth retardation	5.24 (1.06-26.0)		3.59 (1.10-9.19)
Intra-uterine growth retardation			
H/o antibiotic treatment in pregnancy	4.59 (1.67-12.65)		5.85 (1.33-25.75)
Post-partum maternal vitamin A < 20μg/dl	3.10 (1.08-8.86)	6.23 (1.67-23.24)	
Birth weight <2.5 kilograms	58.78 (7.13-484.0)		
Gestation less than 37 weeks	0.01 (0.00-0.08)		

Only significant factors included

3) Intra-uterine growth retardation (table 6.5)

For all deliveries, a history of antibiotic treatment during pregnancy, low serum retinol (<0.7μmol/L) and LBW were independently associated with increased risk of IUGR. Pre-term babies were significantly less likely to have IUGR regardless of HIV status. For babies of HIV non-infected mothers, low serum retinol (< 0.7μmol/L) was the only factor independently associated with increased risk of IUGR. In HIV infected mothers, a history of antibiotic treatment during pregnancy was the only significant risk factor.

6.4 DISCUSSION

This is the first study at UTH to evaluate factors associated with pre-term delivery, LBW and IUGR in relation to maternal HIV infection. The importance of the distinction between IUGR and gestation duration is generally acknowledged because of differences in aetiological factors as well as clinical outcomes (Kramer, 1987).

These results should be interpreted within the context of the limitations of the study. The sample size was small especially for HIV infected women and the prevalence of some of the factors evaluated, resulting in wide confidence limits for some of the risk estimates. A large number of the factors were evaluated in the univariate analysis on the basis that each has an individual influence on risk. However, a number of these factors are likely not to act independently. In some, the effect may have synergistic rather than additive effects. The small sample size restricted combined evaluations.

The sample was drawn from UTH, a referral hospital catering for high-risk pregnancies from the surrounding primary care clinics and rural villages. The prevalence of pre-term delivery, LBW and IUGR, therefore, could be an overestimate in comparison to the general population. The effect of malaria on pregnancy outcome will be small since malaria prevalence has been estimated to be around 0.6 percent in pregnant women delivering at UTH (on going HHV8 study). Finally, the estimation of the baby's gestational age at birth is subject to error because it was based on the last menstrual period (LMP) and not physical assessment of the newborn. In a recent study by Verhoeff *et al* (1997) evaluating gestational assessment by nurses in Malawi, fundal height and LMP underestimated the gestational age of babies compared to the Ballard exterior method.

6.4.1 Pre-term delivery

The incidence of pre-term delivery was 27.9% in HIV infected and 22.0% in non-infected mothers which is higher than previously reported by Bhat *et al* in 1989 (17%). This increase is probably reflective of either increasing prevalence of pre-term births, better referral systems, or bias in the method for assessment of gestational age.

The prevalence of premature births may have increased in Zambia as a consequence of the impact of health sector reforms and the Structural Adjustment Programme (SAP) on the population. Although one of the aims of health reforms is to strengthen referral systems, introduction of cost sharing and user fees at primary care facilities has had the secondary effect of limiting access to health care (van der Geest *et al* 2000). In addition, the continued reduction of the work force with SAP, without social structures to support affected families, is likely to increase the level of poverty in Zambia, further limiting access to health care. Satisfactory antenatal care has been linked to improved birth outcome in the US (Turner *et al* 1996)

Brocklehurst and French (1998) in their meta-analysis reviewed 22 prospective studies to examine the impact of maternal HIV infection on the risk of prematurity. The majority of these studies were conducted in Africa. The weighted Odds Ratio for prematurity was 1.86 (95% CI 1.63-2.06). The authors, however, concluded that there was evidence of significant heterogeneity between the studies (χ^2 70.27, P<0.001) and the summary measure needed to be treated with caution.

The current study failed to demonstrate a relationship between maternal HIV infection and prematurity. Although premature rupture of membranes was not assessed and gestational age data in the current study was limited to reported LMP, the results are in

agreement with those findings by Hira *et al* in a much larger study involving 1,954 consecutive deliveries in 1989. The effect of HIV on prematurity may be associated with maternal HIV disease stage, co-infections, chorioamnionitis and other factors.

Lack of paternal income, primigravidae and primiparity, previous history of premature labour, low post-partum maternal weight and antenatal anaemia were all associated with increased risk of pre-term delivery on univariate analysis of all deliveries (table 6.1).

In HIV infected mothers, increased risk of pre-term delivery was observed when there was lack of paternal income, previous history of premature labour, low post-partum maternal weight and a history of malaria treatment in pregnancy (table 6.1). The relationship with post-partum maternal weight was negative and linear (figure 6.8). After correcting for confounding variables on multivariate analysis antenatal anaemia independently increased the risk of pre-term delivery in HIV non-infected mothers and lack of paternal income in infected mothers (table 6.5).

Studies on impact of anaemia in pregnancy on prematurity in developing countries are limited. Kramer (1987) reviewed 42 studies and because most correlated pregnancy outcome with haemoglobin concentration late in pregnancy, only five studies were selected for evaluation. There were no firm conclusions drawn relating maternal anaemia with increased risk of prematurity in the review by Kramer. However, none of the five studies were from a developing country.

Despite increased maternal erythropoesis during pregnancy, haemoglobin concentration falls progressively until about the 32nd week of gestation owing to greater increases in plasma volume (Kitay & Harbot 1975). Expanding blood volume and enhanced blood flow improves foetal growth. Failure for the plasma volume to expand and resulting

hyperviscosity can lead to restricted growth, resulting in the infant being small for gestational age or premature (Steer 2000). On the other hand, anaemia could interfere with oxygen delivery to the infant and thus result in pre-term delivery. Garn *et al* (1981) estimated the haemoglobin nadir for lowest risk of prematurity in blacks in the United States as 8.9-9.5 g/dl and in whites as 10.5-12.5 g/dl. In the United Kingdom, the lowest rate of pre-term birth occurred at maternal haemoglobin concentrations of 9.6-10.5g/dl (Steer *et al* 1995).

The influence of anaemia on prematurity in Africa may be quite different from what is reported from industrialised countries and further studies are needed. The commonest causes of anaemia in Africa are iron and folate deficiency, malaria and HIV infection (WHO 1993) and other nutritional deficiencies. The research trials in which women have been supplemented with iron have been reviewed in recent reports (US Preventive Services Task Force 1993; Mohammed 1998; Scholl & Rilley 2000). Although iron supplementation can improve maternal haematological indices, controlled trials have failed to demonstrate that changes in haematological indices actually improve clinical outcomes in the newborn.

Malaria is unlikely to impact on anaemia in women delivering at the University Teaching Hospital in Lusaka as the prevalence is low, although HIV infected women are at higher risk of *P. falciparum* malaria (Verhoeff *et al* 1999, ongoing Human Herpes Virus type 8 study). Malaria treatment in pregnancy was associated with increased risk of prematurity in HIV infected mothers but this effect was lost after controlling for other variables.

The only independent factor associated with increased risk of pre-term delivery in babies of HIV infected mothers was a lack of paternal income. This association probably reflects poverty related environmental and social factors. The current study did not fully evaluate the extent to which socio-economic status of HIV infected women affects their pregnancy outcome. It is also possible that the lack of income in the spouse may be as a result of ill-health from HIV, with resultant additional stress on the pregnant woman. Further research is required to validate this finding.

6.4.2 Low birth weight

Overall 54 (18.9%) of the 286 babies born were LBW (figure 6.1). The incidence of LBW was 23.3 percent in HIV infected and 17 percent in non-infected mothers. As observed for prematurity, this estimate was higher than previously reported (Davis 1977; Watts *et al* 1990).

The majority of the LBW babies (61.1%) were born prematurely and 48.1% were growth retarded. These finding are not in agreement with previous reports in which the contribution of IUGR to LBW in developing countries was estimated to be higher (Stein & Ellis 1974; Villar & Bellizani 1982). The factors discussed previously in relation to prematurity may explain the difference.

Maternal HIV infection did not increase the risk of LBW (table 6.2), although the mean birth weight was significantly lower in babies born of HIV infected mothers. Most studies in Africa have demonstrated an association between low or mean birth weight and HIV infection (Hira *et al* 1989; Lepage *et al* 1991; Temmerman *et al* 1994; Sukwa 1996; Leroy *et al* 1998; Castetbon *et al* 1999). In two previous studies in Zambia both

LBW and low mean birth were significantly associated with maternal HIV infection (Hira *et al* 1989; Sukwa *et al* 1996). Both these studies were much much larger sample sizes than the current study.

Although univariate analysis in HIV non-infected mothers showed an increased risk of LBW with young maternal age, primigravidae, primiparity, history of previous pre-term delivery, ante-partum haemorrhage, low post-partum maternal serum retinol and IUGR, the only independent factor after correcting for confounders was antenatal maternal anaemia (table 6.2 & 6.5).

Birth weight is generally reduced amongst adolescents. Age is closely related to parity and maternal weight. Young mothers are likely to be primiparous, to have lower maternal weight and to have nutrient deficiencies, such as vitamin A, because they are still growing. Controlling for these confounders, therefore, is essential.

The confidence limits of the Odds Ratio for the association of antenatal anaemia with low birth weight in the multivariate analysis were very wide (1.01-283.22), however, similar results were found for premature delivery. This emphasises the importance of control of anaemia in pregnancy in urban Zambia. A recent review by Allen (2000) concluded that there is substantial evidence that iron deficiency and anaemia increase

Using data from non-malarious and malarious settings in Papua New Guinea, Brabin and Piper (1997) calculated that, severe maternal anaemia (Hb<7g/dl) in primiparous women was responsible for 8 percent of the LBW and malaria for 18 percent. In a malaria endemic rural area in Malawi, 33% of babies born to primigravidae mothers were LBW (Verhoeff *et al* 1999). The current study did not evaluate the effect malaria or anaemia according to parity although there was a significant negative linear

relationship between increasing parity and incidence of low birth weight. A similar linear relationship was found with post-partum maternal vitamin status and pre-term delivery. Maternal anaemia was increased when maternal serum retinol was low as reported in Chapter 5.

In HIV infected mothers, a history of previous pre-term delivery, positive post-partum RPR, low post-partum maternal weight and IUGR were found to increase the risk of LBW (table 6.2). On multivariate analysis, only IUGR remained significantly associated with LBW (table 6.5).

The increased risk of LBW in IUGR babies in HIV infected women might be related to co-infections. In Zambia, sexually transmitted diseases, are associated with adverse pregnancy outcome (Hira *et al* 1990, UNICEF 1995). All the women in this study were screened for syphilis at enrolment. Positive post-partum maternal RPR was associated with increased risk of LBW on univariate analysis in HIV infected mothers although the effect was lost after correcting for other variables. Hira *et al* (1990) found a relative risk of 7.8 for LBW in syphilitic pregnancies in a cohort of 230 women at the University Teaching Hospital. In Kenya, Temmerman *et al* found that prematurity was significantly associated with maternal HIV infection independent of positive RPR. In a different study in Kenya, Temmerman *et al* (1995) used intramuscular ceftriaxone, active against many of the common sexually transmitted diseases in a randomised controlled trial. Four percent of women who had taken antibiotics compared to 9.2 percent of women who had taken placebo, had LBW babies. Mean birth weight was also significantly lower in women who took placebo.

In sub-Saharan Africa, bacterial vaginosis probably affects 20-50 percent of women and has been associated with chorioamnionitis and premature rupture of membranes and pre-term delivery (Mayaud *et al* 1997). Although bacterial vaginosis was not studied, this should be evaluated in future studies in Zambia. This might help explain the association of LBW with a history of previous premature delivery observed in this study.

6.4.3 Intra-uterine growth retardation

Over a quarter (25.9%) of the babies had IUGR (figure 6.1). Of babies born to HIV infected mothers 29.1% were growth retarded compared to 24.5% for non-infected mothers. Only one tenth of the IUGR babies had asymmetrical growth retardation, which suggest that the majority of the IUGR babies had chronic exposure to the insult during pregnancy.

The major determinants of IUGR in developing countries include poor gestational nutrition, low pre-pregnancy weight, short maternal stature and malaria (Kramer 1987). Appropriate weight gain in pregnancy is critical to foetal growth. Optimal weight gains varies depending on the nutritional status at the start of pregnancy. The combination of low pre-pregnancy weight and low weight gain during pregnancy puts women at the greatest risk (Shah 1972; Winkoff *et al* 1981; Rosso 1985). The effect of improved weight gain during pregnancy and reduction of IUGR has been shown to be greater for undernourished women and for women during times of acute nutritional stress or famine (Kramer 1987; Prentice *et al* 1987)

Post partum maternal weight was evaluated but was not found to be associated with IUGR (table 6.3). The lack of effect could be related to the timing of the weight measurement. Kelly *et al* (1996) in their meta-analysis evaluating anthropometric measurements that are predictive of pregnancy outcome concluded a single measurement for attained weight at 16-20 or 24-28 weeks was the most useful. The strongest effect (OR 4.0) was attained at 24-28 weeks for IUGR when applied to women of below average pre-pregnancy weight.

Low post-partum maternal serum retinol (<0.7μmol/L) was found to be associated with significant increase in the risk of IUGR in babies born to HIV non-infected women after controlling for other factors (table 6.5). However, low vitamin A status has not been shown to increase the risk of IUGR in previous studies (Azaïs-Braesco & Pascal 2000). Moreover, Fawzi *et al* (1998) reported that multivitamins and not vitamin A supplementation in pregnancy decreased the risk of foetal death, LBW, prematurity and IUGR in a cohort of HIV infected women enrolled in randomised trial in Tanzania. The reasons for the conflicting results from the current study are unclear but the mechanism could still be through micro-nutrient deficiency.

Circulating retinol concentration in newborns is 50 percent lower than their mother (Azaïs-Braesco & Pascal 2000). Children born from deficient mothers, therefore, will be at higher risk of vitamin A deficiency, with consequence of increased infectious morbidity and mortality (Humphrey *et al* 1992). Adequate vitamin A status in pregnant and lactating mothers is therefore critical for child survival.

Infectious morbidity in HIV infected mothers may increase the risk of IUGR, a factor not evaluated in the review by Kramer (1987) but important in low resource settings

highly affected by HIV. Intra-uterine growth retardation (IUGR) was found to be associated with history of antibiotic treatment during pregnancy in HIV infected mothers both on univariate and multivariate analysis (table 6.3 & 6.5).

It is surprising that tuberculosis treatment during pregnancy increased the risk of IUGR in HIV non-infected but not infected women, although the effect was lost on multivariate analysis (table 6.6 & 5). This result should be interpreted with caution. The duration of tuberculosis treatment before delivery was not evaluated and yet this information is important before conclusions can be drawn from the above results. Chronic illness during pregnancy is more likely to result in IUGR. It should also be pointed out that there were only 9 mothers with tuberculosis, 5 HIV non-infected and 4 infected. Small effects probable could not be measured because of the small numbers.

A two-fold increase in IUGR was observed for LBW babies for all deliveries on multivariate analysis (table 6.5) whereas gestational age less than 37 weeks was not associated with increased risk of IUGR. This suggests that most IUGR is occurring late in pregnancy. The mechanisms to explain this are unknown, but could relate to nutritional deficiencies late in pregnancy, although this was not confirmed in the HIV infected group, probably due to the small sample size.

6.5 CONCLUSION

Low birth weight, prematurity and IUGR were common in women at UTH, although there were not associated with maternal HIV infection. Factors associated with these pregnancy outcomes differed depending on maternal HIV status. Whereas antenatal anaemia was found to be independently associated with LBW and prematurity, low

post-partum serum retinol concentration was associated with increased the risk of IUGR in HIV non-infected mothers. In infected mothers, the risk of prematurity was increased with lack of paternal income and IUGR with a history of antibiotic treatment during pregnancy. The public health implications of the observations in this study are obvious. The factors associated with the poor birth outcomes are probably modifiable and require emphasis in order to improve the efficiency of Safe Motherhood and MTCT of HIV prevention programmes.

CHAPTER 7

MOTHER TO CHILD TRANSMISSION OF HIV

7.1 INTRODUCTION AND OBJECTIVES

The general objectives of this chapter were to estimate the incidence and timing of HIV infection in the first year of life in children born of HIV infected mothers at University Teaching Hospital (UTH) and to determine associated risk factors for mother to child transmission (MTCT).

7.1.1 Rate and timing of transmission

In Lusaka, approximately 40 thousand children are born every year and over a quarter (27.5%) of the mothers are infected with HIV (Bhat *et al* 1998). About 12,000 of the deliveries occur at University Teaching Hospital (UTH). Hira and colleagues in 1989 estimated that the rate of mother to child transmission (MTCT) of HIV at UTH was 39%. This estimate was determined using HIV antibody ELISA results of child sera taken at 18 months of age. Mother to child transmission of HIV can occur in utero, intrapartum and postnatally, through breastfeeding. The Hira study, could not be used to determine the relative contribution of the three modes of transmission, as the child infection status was determined at 18 months.

The Ghent Working Group on Mother to Child Transmission in 1992 defined criteria for determining HIV infection in children in order to standardise estimation of MTCT rates (described in Chapter 2). HIV antibody results at 15 months and related clinical

signs, symptoms and deaths were used to determine infection status in the Ghent classification. However, early infection cannot be clearly defined by this classification. Early diagnosis of HIV infection in children is essential for decision making on medical and social support needs. Qualitative polymerase chain reaction (PCR) using proviral DNA and RNA in peripheral blood leukocytes is an excellent test for determining HIV infection in young children and is as reliable as or perhaps better than viral culture (Bremer *et al* 1996; Simonds *et al* 1998). Soon after birth, PCR specificity is over 95% although sensitivity is below 40%. However, after the first week of life sensitivity improves to 90% and above and specificity is almost 100% (Dunn *et al* 1995; Bremer *et al* 1996; Simonds *et al* 1998).

A definition for the time of infection in non-breast fed children based on consecutive blood samples tested for viral markers was proposed by the AIDS Clinical Trials Group (ACTG) in 1992 (Bryson 1992). A child would be considered to have in utero or early transmission if HIV genome was detected by PCR or if HIV was isolated from blood within 48 hours of birth with confirmation of positive results by at least one other sample obtained after the neonatal period. Late in utero or intrapartum infection would be considered if children originally negative in the first week of life became positive during the period from day 7 to 90 days.

This definition, however, is not appropriate in populations where breastfeeding contributes 14-16% of HIV infections (Dunn *et al* 1992; Nduati *et al* 2000). HIV infection risk is high in the first 90 days of life, particulary in non-exclusively breastfed children and when duration of breast-feeding is long (Tess *et al* 1998; Coutsoudis *et al* 1999). This chapter addresses the incidence as well as timing of HIV infection

determined by DNA PCR in children, in the first year of life, born to predominantly breastfeeding HIV infected mothers.

7.1.2 Risk factors for mother to child transmission

Various studies have shown that transmission of HIV from mother to child is affected by a number of factors. The significance and relative contribution of the individual factors, however, varies from site to site. It is generally agreed that high maternal viral load increases the risk of MTCT of HIV (European Collaborative Study 1999; O'Shea *et al* 1998; O'Donnovan *et al* 2000) and that elective caesarean section is protective (European Mode of delivery Collaboration, 1999). On the other hand, premature delivery has been found to be associated with MTCT of HIV in some (The European Collaborative Study 1996&1999; Kuhn *et al* 1997 &1999) but not all studies (St Louis *et al* 1993). Increase risk of low birth weight has been reported (Mofenson *et al* 1999; Temmerman *et al* 1994; Sheldon *et al* 1996), duration of rupture of membranes and labour (Burns *et al* 1994; Temmerman *et al* 1994; Minkoff *et al* 1994; Kuhn *et al* 1997), low maternal CD4 cells (European Collaborative Study 1996 & 1999, Kuhn *et al* 1997; O'Shea *et al* 1998; Garcia *et al* 1999) and low serum retinol (Semba *et al* 1994; Burger *et al* 1997).

With the limited available literature, factors significantly associated with HIV MTCT remain ill defined in Zambia. This information is essential for planning of care interventions for pregnant women and their children.

7.2 METHODS

7.2.1 Subject management

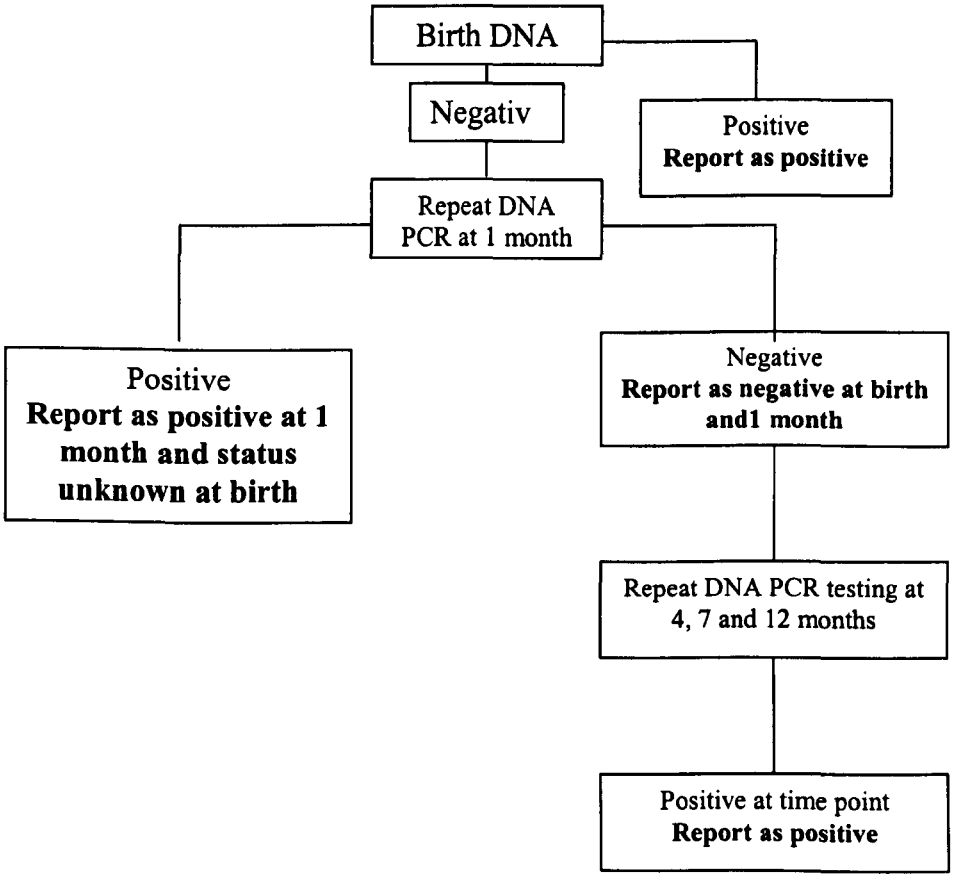
After delivery, a follow up appointment was given to each enrolled mother, scheduled at 1 month initially and later 4, 7, 10 12 and 18 months. A study clinic was set up in the Department of Paediatrics and any mother who did not come for scheduled appointments was followed up actively at home. Pre and post test counselling was conducted by a trained nurse counsellor at the 1 month visit for mothers who wished to know their HIV status. A morbidity (mother and child) and infant feeding questionnaire was completed in at each visit by a study midwife (appendix 3.1), followed by a complete physical examination of the mother and child. One ml of capillary blood was collected in an EDTA tube from each attending child by finger or heel prick. Ten ml of venous blood was collected from the mother and separated into a plain and an EDTA bottle. The plain bottle was immediately covered with foil to avoid light exposure. The plain blood was used for measuring RPR, hepatitis B surface antigen (HBsAg), hepatitis C and serum retinol by methods described in chapter 3 and 4. A full blood count and absolute CD4 subsets (CD4 and CD8) were immediately measured by a Coulter Counter (Coulter Counter; Coultronics, Margancy) and Facsscan (Becton Dickenson, San Jose, USA). The remainder of the maternal EDTA specimens and the children's samples were frozen at –70 to be shipped on dry ice to Yamanashi University in Japan for quantitative RNA and qualitative HIV DNA PCR testing respectively.

7.2.2 PCR testing

The child's qualitative DNA PCR was measured using an in house nested DNA PCR developed for testing Zambian whole blood samples at Yamanashi Medical College in collaboration with the Virology laboratory at UTH described in chapter 3. The sensitivity and specificity of this method on adult samples (Chapter 3) was 94% and 100%, using antibody serology as a gold standard (Handema *et al* 1999). The lower limit of detection of viral DNA for this assay was 3.5 copies per ml of blood. Maternal viral load was determined by reverse transcriptase polymerase chain reaction (Amplicor Monitor version 1.0, Roche diagnostic systems, Basel, Switzerland). This method of detection was described in Chapter 3.

Definitions

Figure 7.1 Child PCR testing algorithm



The algorithm (figure 7.1) for determining child HIV infection was developed based on the evidence that PCR specificity for child samples was greater than 95% at all time points and sensitivity was less than 40% at birth and greater than 90% at subsequent time points (Dunn *et al* 1995; Bremer *et al* 1996; Simonds *et al* 1998).

HIV DNA PCR positivity at any one of the testing points was considered as positive. A negative test at birth was confirmed with a subsequent negative HIV DNA PCR test at the one-month visit. Beyond one month, a previously negative child with a subsequent negative HIV DNA PCR result was defined as negative if HIV DNA PCR repeated at 12 months or antibody serology done at 18 months were negative. HIV status was defined as unknown if the sample at birth was negative and there was no subsequent sample. A child negative at birth who became positive at 1 month was defined as indeterminate at birth.

HIV infection in children was classified as early transmission (intrauterine, intrapartum and early postnatal transmission) if HIV DNA PCR was positive at birth and/or at 1 month of age. Late transmission was defined as negative both at birth and one month but positive on subsequent samples (4-12 months).

1) Maternal CD4 and RNA viral load

The CD4 cell count was categorised according to the CDC classification (< 200, 200-499, ≥ 500 for the cell count). A modified CD4 percentage classification (<15%, 15-24% and >25%) to that used by Sheldon et al (1996) was used because of the few number of infected mothers in the higher cut off level. In Rwanda, in a population of women whose RNA viral load was below 55,000 copies/ml, no association was

observed between viral load and MTCT of HIV. In the present analysis, RNA viral load was, therefore, divided into <10,000, 10-49999 and ≥50,000 copies /ml.

2) Other definitions

Other definitions are described in Chapter 4.

7.2.4 Statistical analysis

1) Estimation of MTCT of HIV

The following calculations were made:

Proportion of children with HIV infection at birth

$$\frac{\text{Number of children with positive DNA PCR at birth}}{\text{Total number of children tested at birth}}$$

Proportion of children with HIV infection at 1 month

$$\frac{\text{Number of children with positive DNA PCR at 1 month}}{\text{Total number of children untested or with negative DNA PCR at birth tested at 1 month}}$$

Proportion of children with late postnatal infection

$$\frac{\text{Number of children with positive DNA PCR after 1 month}}{\text{Total number of children with negative DNA PCR at 1 month followed up to 12 months}}$$

Cumulative incidence HIV infection at 1 month

$$\frac{\text{Number of children with positive DNA PCR at birth and 1 month}}{\text{Total number of children untested or with negative DNA PCR at birth tested at 1 month + positive children at birth}}$$

Cumulative incidence of HIV infection at 1 year

$$\frac{\text{Total number of children with positive DNA PCR in the first year of life}}{\text{Total number of children tested with confirmed status (negative or positive)}}$$

Distribution of timing of HIV infection in the first year life

$$\frac{\text{Number of positive children at defined time points}}{\text{Total number of children with positive results in the first year of life}}$$

With children with HIV infection as the dependent variable, univariate and stratified analyses for socio-demographic, medical, obstetric , nutritional and laboratory risk factors were undertaken (Pearson's X^2 and X^2 for trend and Fishers exact test). For continuous variables the independent student's t-test to compare means of normally distributed data and the Mann Whitney U test for non-normally distributed data were calculated.

Multiple logistic regression was used to determine factors independently associated with child HIV infection, correcting for other confounders. The analysis was performed using SPSS (SPSS Inc. 1989-1999) and EPI-INFO (Centers for Disease Control and Prevention, Atlanta, GA) statistical packages. All P values were 2 sided.

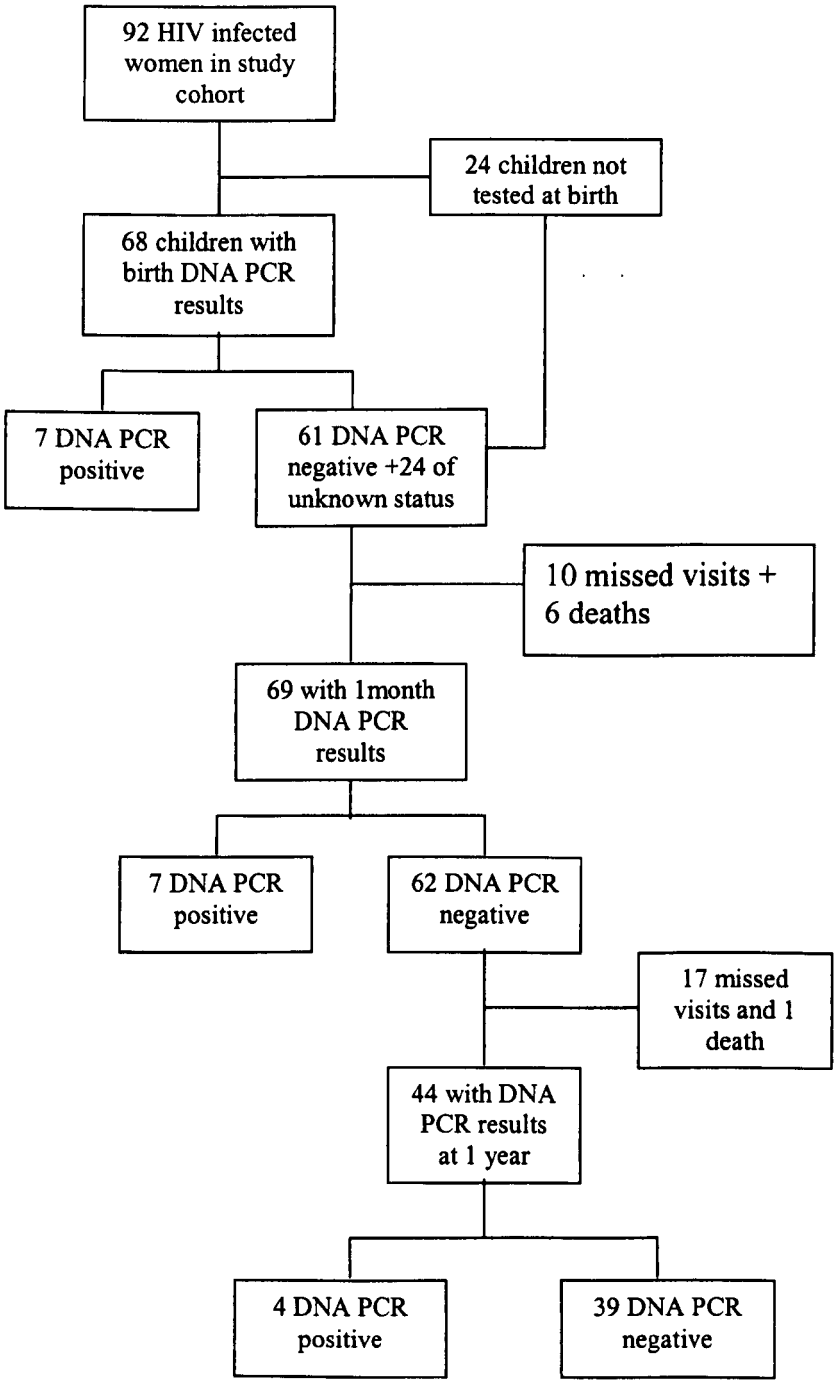
7.3 RESULTS

7.3.1 Study sample and estimation of rate of MTCT of HIV

Ninety-two (30.1%) of 306 women enrolled were HIV infected. Interpretation of DNA PCR results at different time points in children born to HIV infected women is illustrated in figure 7.2. DNA PCR results at birth (within 48 hours of delivery) were

undertaken for 68 (73.9%) children and 7 (10.3%; 95% CI 3.1-17.5%) were positive. Of the 24 children whose HIV status was not confirmed at birth, 7 were subsequently confirmed positive at 1 month..

Figure 7.2 **Flow diagram of DNA PCR testing and results**



The characteristics of the remaining 16 children are described in table 7.1. One of the children was too ill to have blood taken at birth and died within four days of delivery. Five more deaths occurred in the neonatal period before the one-month visit. Nine of the mothers could not be traced as the home addresses given were incorrect, although one of the mothers difficult to trace came back at 5 months to report her child was dead and for HIV counselling. One mother was a 'late refusal' because her husband did not wish her to continue.

At the one-month visit 69 (81.2%) of 85 negative or children of unknown status at birth had DNA PCR measured. Seven (10.1%; 95% CI 3.0-17.3%) were confirmed HIV infected (figure 7.2)

Table 7.1: One month visit characteristics of children with undefined HIV status both at birth and 1 month

Characteristic	*n(%)*
Blood not taken at birth due to ill health (died in perinatal period)	1 (6.2)
No subsequent DNA testing to confirm negative result because of death	5 (31.3)
Loss to follow up (incorrect address)	9 (56.3)
Late refusal	1 (6.2)
Total	16(100)

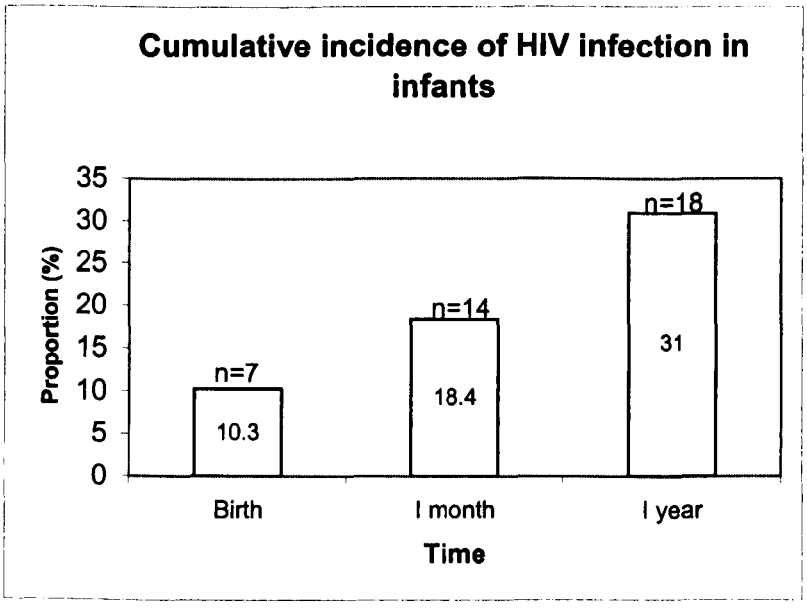
Four (9.1%; 95% CI 6.0-17.6%) more HIV infections were confirmed out of 44 children, negative at 1 month, on subsequent visits at 4 months, 7 months and a year. Table 7.2 describes the characteristics of 18 children, negative at 1 month, whose HIV

infection status was not determined subsequently. The majority (61.1%) of the children did not come back for review because the mothers and in some cases the father did not see any potential benefit for continuing participation.

Table 7.2: ***Characteristics of children with undefined HIV status at 4 to 12 months***

Reason for loss to follow up	n(%)
Child Death	1 (5.6)
Wrong address or transfer	6 (33.3)
Late refusal	11 (61.1)
Total Number	18 (100)

Figure 7.3



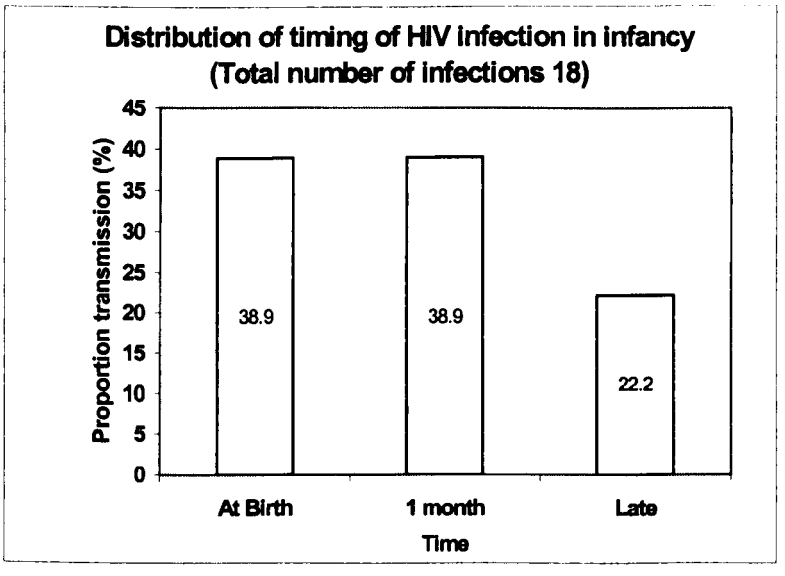
7.3.3 Characteristics of children with undetermined and known HIV status

For this comparison all children with known HIV status at by 1 month (early transmission) and 1 year (all transmissions) were compared to those whose status was undetermined by the end of these time points (table 7.3).

7.3.4. Incidence and timing of HIV infection in the first year of life

The cumulative incidence of HIV infection in infancy was: (7/68) 10.3% (95% CI 3.1-17.5% at birth); (14/76) 18.4% (95% CI 9.7-27.1) at 1 month and (18/58) 31.0% (95% CI 19.1-42.9%) at 1 year. 77.8% of the children were DNA PCR positive by 1 month of age (figure 7.4).

Figure7.4



In the first category (early transmissions), children born of mothers aged less than 20 years and mothers who were primiparous were more likely to be of undetermined status

at 1 month; 25% versus 1.8% for age less than 20 (P value 0.009) and 50% versus 25% for primiparous mothers (P value 0.047). In the second category (all transmissions), primiparity and postpartum maternal RNA viral load of 50,000 copie/ml and above were associated with an undetermined HIV status of the child at the end of the one-year follow up; 43.8% versus 16.3% for primiparity (P value 0.027) and 50% versus 14.8% for viral load of 50,000 and above (P value 0.043). The distribution of the other variables was similar between both groups.

7.3.5 Univariate analysis of risk factors for MTCT of HIV at birth and 1 month

1) At birth

Single marital status and postpartum maternal weight of less than 45 kilograms were associated with increased risk MTCT of HIV on samples taken at birth. Two (28.6%) of 7 mothers of children with confirmed HIV infection at birth compared to 1 (1.7%) of 59 mothers with negative children were single (P value 0.028). Postpartum maternal weight less 45 kilograms was found in 2 (28.6%) of the 7 mothers with confirmed HIV infection compared to 1 (1.9%) of 53 mothers with negative children (P value 0.034). The other variables were not associated with increased risk of transmission at birth.

2) At 1 month

At one month, 5 (83.3%) of 6 mothers with HIV infected children at 1 month and 10 (25.6%) of 39 mothers with negative children had a postpartum viral load of 50,000 copies or more.

Table 7.3 Characteristics of children with known and undetermined HIV status

Variable	*1 month HIV status*			*1 year HIV status*		
	Undetermined n/N (%)	*Known n/N (%)*	*P value*	*Undetermined n/N (%)*	*Known n/N (%)*	*P value*
Socio-demographic						
Father has no income	2/16 (12.5)	4/71 (5.6%)	0.304	1/16 (6.3)	1/43 (2.3)	0.4530
Mother has no income	14/15 (93.3)	52/68 (75.5)	0.286	13/16 (81.3)	32/40 (80.0)	0.616
Maternal illiteracy	3/15 (20.0)	16/71(22.5)	1.0	4/16 (25.0)	8/43 (18.6)	0.718
Single marital status	1/16 (6.3)	4/73 (5.5)	1.0	1/17 (5.9)	0 (0)	ND
Maternal age <20 yrs	4/16 (25.0)	2/72(2.8)	**0.009**	1/16 (6.3)	0 (0)	ND
Obstetric and Medical						
Postpartum maternal weight <45kg	1/14 (7.1)	4/66 (6.1)	1.0	0(0)	1/39 (2.6)	ND
Primiparous	8/16 (50.0)	18/72 (25.0)	**0.047**	7/16 (43.8)	7/43 (16.3)	**0.027**
Primigravida	5/16 (31.3)	13/72 (18.1)	0.237	4/16 (25.0)	6/43 (14.0)	0.261
Spontaneous vaginal delivery	10/15 (66.6)	58/70 (82.9)	0.155	12 (80.0)	35/42 (83.3)	0.713
Tuberculosis treatment	1/13 (7.7)	3/63 (4.8)	0.536	0 (0)	1/37 (2.6)	ND
Antibiotic treatment	2/9 (22.2)	8/46 (17.4)	0.661	1/10 (10.0)	4/ 29(13.8)	1.0
Rupture of membranes >4 hours	4/15 (26.7)	25/62 (40.3)	0.387	7/15 (46.7)	14/37 (37.8)	0.56
Preterm (<37 wks)	5/16 (31.3)	21/72 (29.2)	0.869	6/16 (37.5)	10/43 (23.3)	0.329
Birth weight <2.5 kg	4/15 (26.7)	16/72 (22.2)	0.740	4/16 (25.0)	7/43 (16.3)	0.468
Maternal postpartum laboratory findings						
Retinol<0.7μmol/L	6/16 (37.5)	15/75 (20.0)	0.131	7/18 (38.9)	7/43(16.3)	0.092
Haemoglobin<11g/dl	11/16 (68.8)	45/76(59.2)	0.477	12/18 (66.7)	27/44 (61.4)	0.695
Positive RPR	2/16 (12.5)	11/76 (14.5)	1.0	1/18 (22.2)	5/44 (11.4)	0.427
CD4 cell count <200 cells/mm^3	2/16 (12.5)	11/76 (14.5)	1.0	2/18 (11.1)	2/44 (13.6)	1.0
RNA viral load >50000 copies/ml	4/12 (33.3	23/58(39.7)	0.755	6/12 (50.0)	4/27 (14.8)	**0.043**

RPR rapid plasma reagin, Significant values in bold, ND not done due to empty cells.

The difference in the two proportions was statistically significant (P value 0.012). None of the socio-demographic, obstetric and medical factors were associated with MTCT of HIV at 1 month.

7.3.6 Description of late infections

Table 7.4 describes the characteristics of 4 children who were HIV negative at 1 month after delivery but became positive at subsequent visits, 2 at12 months and for the remaining 2 positives at 4 and 7 months. All 4 mothers reported having lost a child previously, two had lost 4 children each and the other 2 had lost 1 and 2 respectively. Two mothers were anaemic, one being severely anaemic, with a haemoglobin of 4.5g/dl. Postpartum maternal weight and serum retinol was more than 50 kilograms and over 0.7μmol/L respectively.

Three mothers had RNA viral loads of 50,000 copies/ ml or more and one had 4,101.1 copies/ ml. All 4 mothers were still breast feeding at the time of acquisition of HIV by the child and two of the mothers exclusively breast fed up to 6 months. Breast abscesses were reported, at the one-month, visit by one of the mothers who exclusively breast-fed. The CD4 count of this mother was 88 cells/mm^3 and the viral load 70,219.8 copies per ml.

7.3.7 Univariate analysis of risk factors for MTCT of HIV (early or all infections in infancy)

Because of the small number of HIV infections in children subsequent analyses were conducted with all early infections (birth and 1 month) or all infections during the first year of life as dependant variables.

Table 7.4 ***Maternal and child characteristics in late postnatal transmission (after 1 month of age)***

Variable	*Case Number*			
	1	**2**	**3**	**4**
Paternal occupation	Barman	Caretaker	Police Officer	Hotelier
Parity	5	5	2	3
H/o previous child death	1	1	1	1
Postpartum weight (kg)	80	64	52	58
Postpartum serum retinol (μmol/L)	1.58	-	0.97	0.96
Birth weight (kg)	2.6	2.5	3.0	2.4
Postpartum maternal viral load (copies/ml)	70219.8	93913.0	82017.0	4101.1
Postpartum maternal CD4 cell count (cells/mm^3)	88	213	441	635
Postpartum maternal CD4%	6.6	14.3	22.2	24.7
Postpartum haemoglobin (g/dl)	10.1	4.5	12.3	13.3
Timing of transmission (months after delivery)	7	12	12	4
Exclusive breastfeeding at 4 months	Yes	Yes	No	Yes
Breastfeeding at time of transmission	Yes	Yes	Yes	Yes

1) Socio-demographic factors

Single marital status was associated with increased risk of early but not all HIV infections in the first year of life (P value 0.024). There were 23.1% (3/13) mothers with HIV infected children and 1.7% mothers with non-infected children by 1 month of age who were of single marital status. Apart from maternal income all the other socio-demographic factors were more frequent in the children who were infected although the differences were not statistically significant.

Table 7.5: Socio-demographic factors associated with HIV infection in infancy

Variable	*DNA PCR at birth and 1 month*			*DNA PCR by 1 year*		
	Infected n/N (%)	*Non-infected n/N (%)*	*P value*	*Infected n/N (%)*	*Non-infected n/N (%)*	*P value*
No paternal income	2/12 (16.7)	2/59 (3.4)	0.130	2/16 (12.5)	1/39 (2.6)	0.2
No maternal income	8/12 (58.3)	45/56 (80.4)	0.136	10/15 (66.7)	29/37 (78.4)	0.377
Maternal illiteracy	4/12 (33.3)	12/59 (20.3)	0.448	5/16 (31.3)	8/40 (17.9)	0.278
Single marital status	3/13 (23.1)	1/60 (1.7)	**0.016**	3/17 (17.6	0/40 (0)	ND
Maternal age <20 yrs	1/13 (7.7)	1/59 (1.7)	0.331	1/17 (5.9)	0/40 (0)	ND
Primiparity	3/13 (23.1)	10/59 (16.9)	0.725	4/17 (23.5)	7/39 (17.9)	0.719
Primigravidae	4/14 (30.8)	14/59 (23.7)	0.692	3/17 (17.6)	6/39 (15.4)	1.0
Any alcohol intake	2/11 (18.2)	9/55(16.4)	1.0	3/14 (21.4)	5/36 (13.9)	0.670

Significant values in bold, ND not done due to empty cells

2) Obstetric and medical characteristics

Antibiotic treatment for suspected bacterial infection during pregnancy the only social demographic factor that was found to be significantly associated with increased risk MTCT of HIV by one month of age but not for all infections in infancy (table 7.6). Mothers with HIV infected children were more likely to have received antibiotic

treatment (42.9%) than mothers (12.8%) with non-infected children (P value 0.026). Previous history of abortion in past pregnancies and in the preceding pregnancy treatment for tuberculosis and malaria, premature delivery, and low birth weight were more common in early infected than non-infected children, although these differences were not statistically significant.

Similar analysis including all HIV infections indicated that previous history of abortions and child death in past pregnancies and in the preceding pregnancy treatment for tuberculosis, malaria and suspected infection, premature delivery and low birth weight were more frequent in infected children. These differences were also not statistically significant.

3) Nutritional and laboratory factors

Postpartum maternal weight less than 45 kilograms was associated with increased risk of early MTCT of HIV but not overall infections in infancy (table 7.7). A quarter (3/12) of the mothers with infected children compared to 1.9% (1/54) with non-infected children weighed less than 45 kilograms (P value 0.017). The other factors not found to be of statistical significance occurred more commonly in non-infected children apart from maternal postpartum mid-upper arm circumference and positive maternal postpartum RPR.

4. Maternal CD4 cell count and percentage

CD4 cell count levels were significantly lower (P value = 0.028) in mothers of HIV infected than in mothers of non-infected children in infancy, median 371 versus 519

cells/mm^3 (figure 7.5). This relationship was absent in children with early HIV infection (figure 7.6).

Table 7.6 Obstetric and medical factors associated with early and all HIV infections in infancy

	DNA PCR at birth and 1 month			*DNA PCR by 1 year*		
	Variable	***Non-infected n/N (%)***	***P value***	***Infected n/N (%)***	***Non-infected n/N (%)***	***P value***
Previous history of:						
Prematurity	0/6 (0)	5/59 (8.5)	-	0/18 (0)	5/39 (12.8)	ND
Abortion	3/13 (23.1)	10/59 (16.9	0.692	5/17 (29.4)	5/39 (12.8)	0.136
Child death	5/13(38.5)	24/59 (40.7)	1.0	9/17 (55.6)	14/39 (35.9)	0.233
Preceding pregnancy						
Elective caesarean section	0/13 (0)	1/57 (1.8)	ND	0/17 (0)	1/38 (2.6)	ND
Tuberculosis treatment	2/10 (27.3)	1/53 (1.9)	0.063	2/14 (14.3)	1/34 (2.9)	0.200
Malaria treatment	6/13 (46.2)	15/52 (28.8)	0.233	6/16 (37.5)	11/33 (33.3)	1.0
Antibiotic treatment	3/7 (42.9)	5/39 (12.8)	**0.026**	3/10 (30.0)	4/26 (15.4)	0.370
Antepartum haemorrhage	1/10 (10.0)	6/51 (11.8)	1.0	2/14 (14.3)	4/31 (12.9)	1.0
Rupture of membranes >4 hours	4/10 (40.0)	21/52 (40.4)	1.0	4/13 (30.8)	14/34 (41.2)	0.739
Premature (<37weeks)	5/13 (38.5)	16/59 (27.1)	0.415	6/17 (35.3)	9/39 (23.1)	0.349
Low birth weight (<2.5 Kg)	5/13 (38.5)	11/59 (18.6)	0.120	6/17 (35.3)	6/39 (15.4)	0.095

Significant values in bold, ND not done due to empty cells

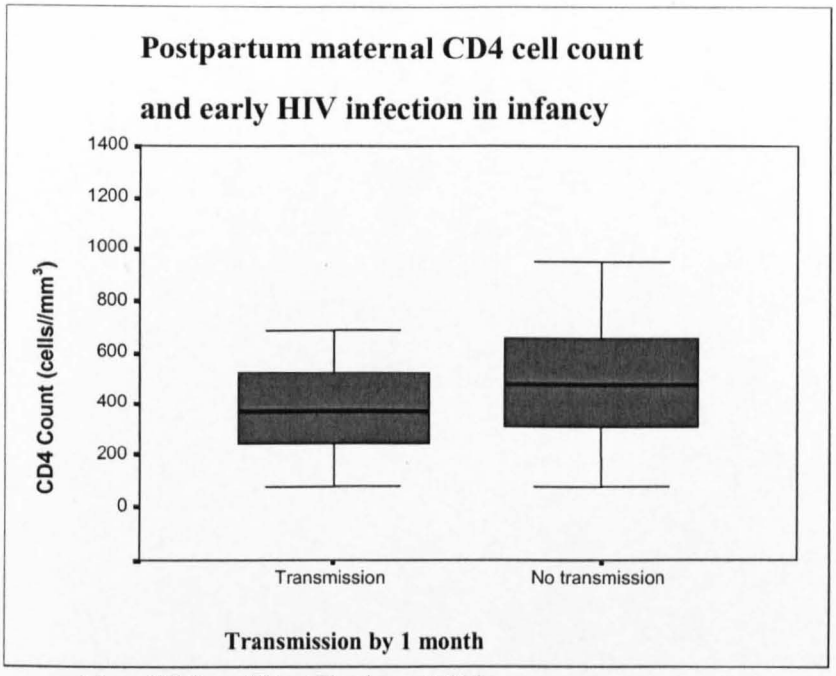
Table 7.7 Maternal nutritional and laboratory factors associated with MTCT of HIV

*Variable**	*DNA PCR at birth and1 month*			*DNA PCR by 1 year*		
	Infected n/N (%)	*Non-infected n/N (%)*	*P value*	*Infected n/N (%)*	*Non-infected n/N (%)*	*P value*
Weight <45kg	3/12 (25.0)	1/54 (1.9)	**0.017**	3/16 (18.8)	1/35 (2.9)	0.086
MUAC <23cm	3/13 (23.1)	4/59 (6.8)	0.106	3/17 (17.6)	2/39 (5.1)	0.158
Anaemia (<7 g/dl)	0/14 (0)	8/62 (12.9)	-	1/18 (5.6)	5/40 (12.5)	0.655
Anaemia (<11 g/dl)	6/14 (42.9)	39/62(62.9)	0.168	8/18 (44.4)	25/40 (62.5)	0.199
Lymphocyte count (<1,500)	2/14 (14.3)	17/61 (27.9)	0.496	4/18 (22.2)	10/39 (23.1)	1.0
Retinol <0.7μmol/L	1/14 (7.1)	14/61 (23.0)	0.2760	1/17 (5.9)	7/40 (17.5)	0.413
Retinol <1.05μmol/L	4/14 (28.6)	21/61 (50.9)	.390	6/17 (35.3)	18/40 (45.0)	0.48
Antenatal RPR	4/11 (36.4)	6/35 (17.1)	0.220	5/14 (35.7)	4/23 (17.4)	0.255
Postpartum RPR	2/14 (14.3)	9/62 (14.5)	1.0	2/18 (11.1)	5/40 (12.5)	1.0
Positive HBsAg	1/10 (10.0)	5/49 (10.2)	1.0	1/13 (7.7%)	4/32 (12.5)	1.0

HBsAg hepatitis B surface antigen, MUAC mid-upper circumference, RPR rapid plasma reagin

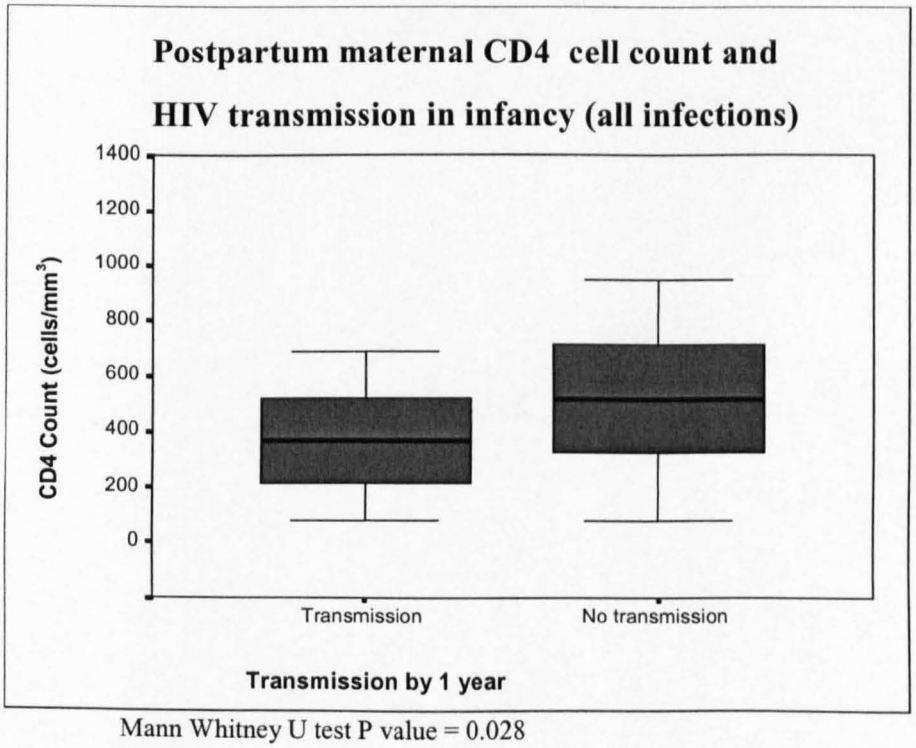
All variables taken postpartum except for antenatal RPR., significant values in bold.

Figure 7.5



Mann Whitney U test P value = 0.099

Figure 7.6



Mann Whitney U test P value = 0.028

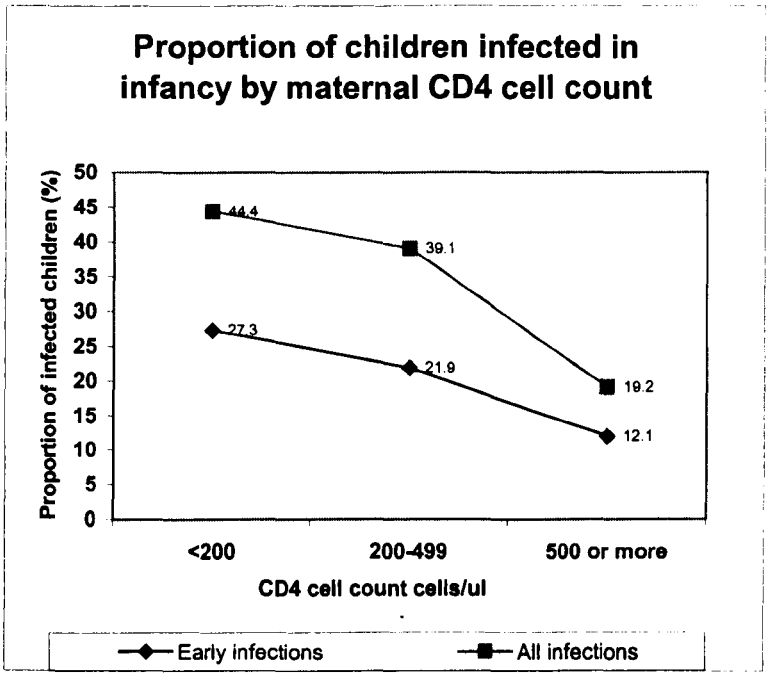
Absolute CD4 cell count at specific cut off points of less than 200 and 500 cells/mm^3 (table 7.8) were both not associated with increased HIV infection in infancy. However, CD4 percentage at cut off points of 15% and 25% did show a positive association for all but not early HIV infections. A significant negative linear association was observed between CD4 cell count and percentage and all HIV infections in infancy, although not with early infections (figure 7.7 and 7.8)

Table 7.8: CD4 cells and HIV in infancy

Variable	*DNA PCR at birth and 1 month*			*DNA PCR by 1 year*		
	Infected n/N (%)	*Non-infected n/N (%)*	*P value*	*Infected n/N (%)*	*Non-infected n/N (%)*	*P value*
CD4 cell count <200 cells/mm^3	3/14 (21.4)	8/62 (12.9)	0.414	4/18 (22.2)	5/40 (12.5)	0.438
CD4 cell count <500 cells/mm^3	10/14 (71.4)	33/62 (53.2)	0.248	13/18 (72.2)	19/40 (47.5)	0.080
CD4 % <15%	6/14 (42.9)	13/62 (21.0)	0.088	8/18 (44.4)	7/40 (17.5))	**0.034**
CD4% <25%	12/14 (85.8)	37/62 (59.7)	0.058	16/18 (88.8)	19/40 (47.5)	**0.002**

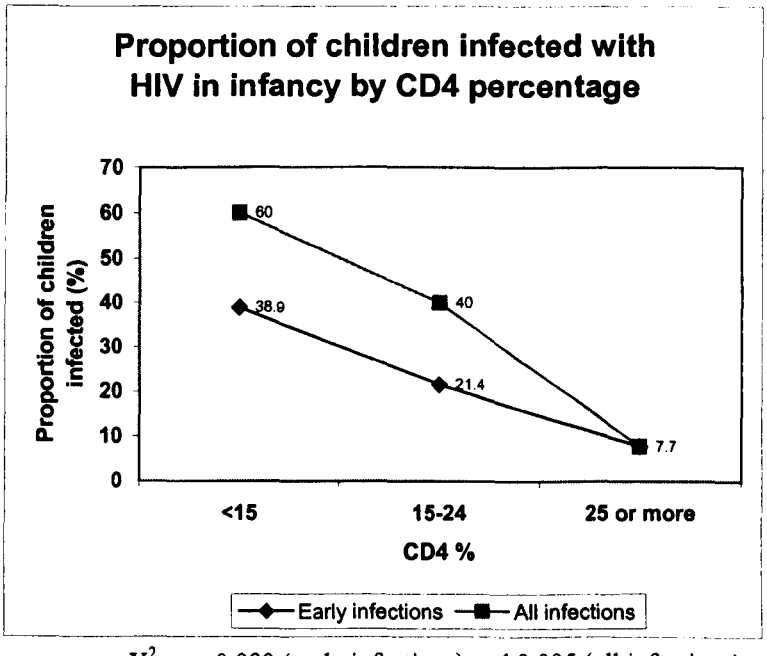
Significant values in bold

Figure 7.7



X^2_{trend} = 0.202 (early infections) and 0.094 (all infections)

Figure 7.8

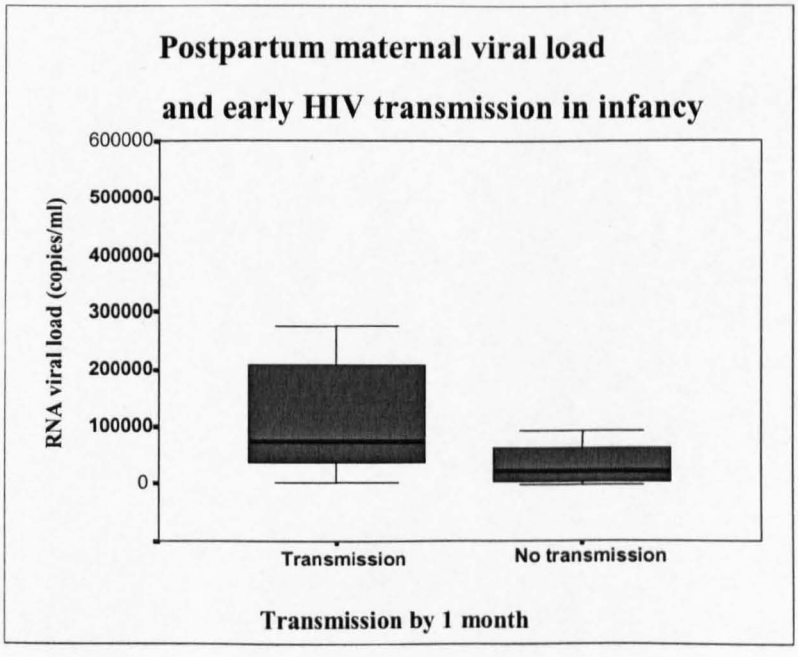


X^2_{trend} = 0.099 (early infections) and 0.005 (all infections)

5) Maternal Viral load

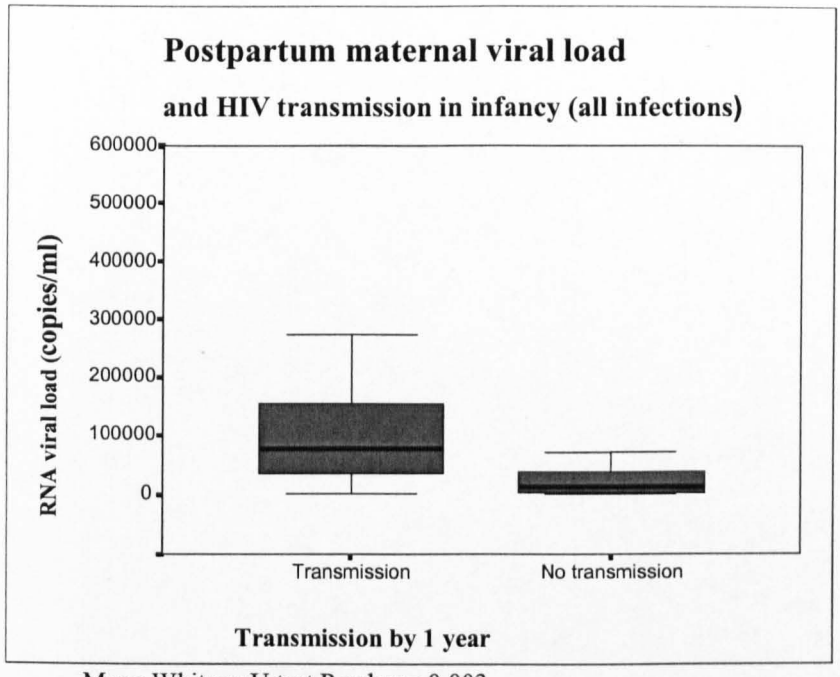
As shown in figures 7.9 and 7.10 postpartum maternal RNA viral load levels were higher in mothers of HIV infected children, both for early and all infections in infancy than mothers of non-infected children. The median viral load was 76,176.5 versus 22,034.5 infections for early infections (P value 0.020) and 76,171.5. Versus 14,924.5 for all infections (P value 0.003) and there was a positive linear trend (figure 7.11) of increasing RNA viral load with increased rate of transmission in infancy (P value .0.015 for early and 0.001 for all infections). This relationship was also observed for RNA viral load at a cut off point of 50,000 and above (P value 0.005 for early and 0.000 for all infections). One of the mothers who transmitted infection to her child had a viral load of 611.8 copies per ml. RNA viral load of 50,000 copies per ml or more was not associated with low CD4 count (< 500 cell/mm^3) or percentage (<25%).

Figure 7.9



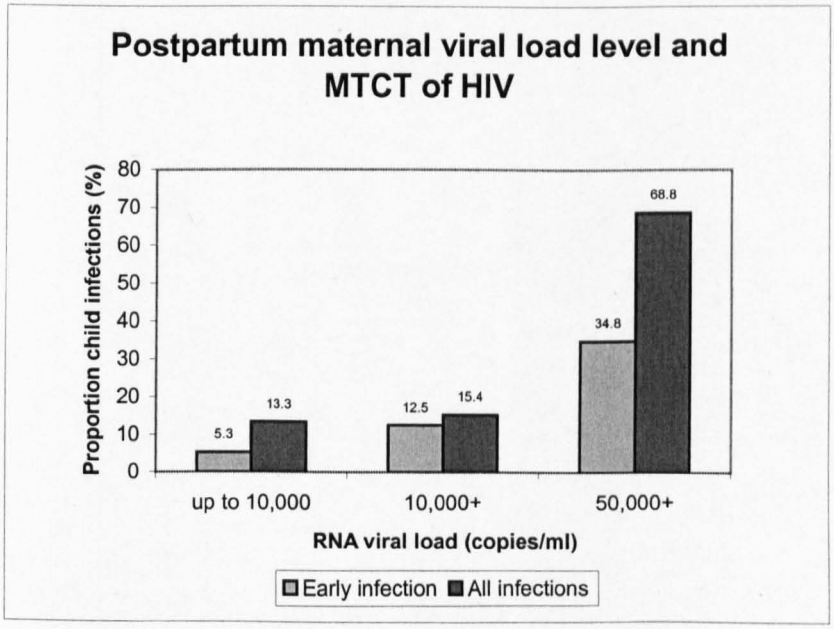
Mann Whitney U test P value = 0.020

Figure 7.10



Mann Whitney U test P value = 0.003

Figure 7.11



X^2_{trend} 0.015 for early infections and 0.001 for all infections

7.3.8 Multivariate analysis of factors associated MTCT of HIV

Multiple logistic regression models were used to describe variable significantly associated with increased transmission of early and all HIV infections in infancy on univariate analysis at P value < 0.05 (table 7.9). RNA viral load was the only factor found to be independently associated with early and all HIV transmissions in infancy (P value 0.005 for early and 0.001 for all infections).

Table 7.9: Variables selected for inclusion in the logistic regression model

Variables	*Early HIV infection*	*All HIV infections*
	Unadjusted P value	*Unadjusted P value*
Single marital status	0.016	-
Postpartum weight < 45kg	0.017	0.086
Antibiotic treatment in pregnancy	0.026	0.370
Postpartum CD4 % (<25%)	0.058	0.002
Postpartum RNA viral load <50,000	0.015	0.000

7.4 DISCUSSION

7.4.1 Study sample

This one-year cohort study, involved 92 HIV infected mothers and their children. Although small, the study has important implications for Zambia, in its efforts to control MTCT of HIV. The study involved a select sample of mothers who consented to HIV testing during labour with follow-up pre and post-test counselling at one month after delivery. This algorithm could be used for MTCT interventions during labour in cases where the woman missed counselling and testing opportunity during pregnancy.

Although there were no major obstacles in the enrolment process, there are important observations to be noted from the study cohort experience. HIV status could not be determined for 34 (36%) of the 92 children mainly because the mothers never returned to the clinic despite active follow-up in their homes. This observation is not unusual in cohort studies and is similar to the findings by Ioannidis *et al* (1999)in a cohort in Malawi. The HIV infection status could not be determined for 797 (36.9%) of 2156 children born to HIV infected mothers because most (653) mothers never came back to the clinic (Ioannidis *et al* 1999). In the current Zambian cohort, 16 of the 34 mothers who were lost to follow up did not return for the first visit at 1 month. Six of the children of the 16 mothers died before determining the status.

Despite setting up a study clinic, open to all mothers whenever they faced problems with the children or themselves, the drop out rate was still high in the subsequent visits. One of the main reasons why some mothers did not come back was fear of their male partners. It has been discussed earlier in Chapter 4 that, although these mothers were literate, most of them were economically dependent on their partners. These findings indirectly reaffirm the importance of partner involvement not only in future studies but interventions involving married women and their children.

Many mothers gave incorrect addresses. The fact that enrolment to the study cohort took place when the mother was in labour could have put pressure on the mothers to consent to testing and participation for fear that their care during labour being compromised. The midwives in this study were trained not to be coercive and on the consent process but in reality these precautions may still not adequate in hospital based studies in low-resource settings and require evaluation. The other possible explanation

could have been that mothers who did not wish to know their HIV results gave incorrect addresses due to lack of understanding and confidence that HIV results would only be discussed with mothers who requested for them voluntarily. If this explanation is correct, the question that arises is then is whether if study enrolment was done after HIV post-test counselling, follow up rates would have improved. This enrolment strategy would result in selection bias and study would have to take that into consideration for the results to be generalisable.

Missing data also pose a major challenge of representativeness of the study findings. Lost information could curtail the power of the study to detect small differences in stratified analysis. To assess the magnitude of this problem, a comparative analysis of characteristics of children, who had known and undetermined HIV status by 1month and 1 year, was conducted. The results indicated that mothers less than 20 years and were more likely to drop out early and those with a viral load of 50,000 and above later during follow up. On the other hand, primiparous women dropped out early or later. One explanation for young mothers dropping out could be that they are more likely to be influenced by their husbands. In addition, it could be that children of young mothers have higher neonatal mortality, resulting in loss to follow up.

High RNA viral load occurs during acute infection or later in symptomatic HIV (WHO/UNAIDS 1999) and will increase the risk of MTCT of HIV. Even though the status of the children of the mothers with high viral load who dropped out could not be determined, it is plausible that mortality due to HIV infection was responsible for the loss to follow up.

7.4.2 HIV transmission infection in children

1) Estimation of MTCT of HIV rate

The overall transmission rate (31%) found in this study is lower than that estimated in of an earlier study at the same hospital by Hira *et al* (39%) in 1989 . This variability has been observed in other MTCT of HIV cohort studies in Africa. The estimated rate in a cohort in Nairobi Kenya (42.8%) was similar to that in Abidjan, Cote d'Ivoire (40%) but was higher than the estimates in Burkina Faso (25.5%), Rwanda (25.7%), South Africa (34%) and Tanzania (21.7%) (Lepage *et al* 1993; Datta *et al* 1994; Bredberg-Raden *et al* 1995; Prazuck *et al* 1995; Bobat *et al* 1996; Ekpini *et al* 1997). The fact that MTCT of HIV occurs at various time points creates methodological difficulties. Differences in definitions of child HIV infection and timing of estimation, high loss to follow up and infant mortality all have an effect on the rate of MTCT of HIV estimated. Hira *et al* (1989) determined HIV infection in the children at 18 months of age using HIV antibody tests and clinical criteria in undetermined children who died. In the current study DNA PCR was used and HIV transmission was estimated using consecutive test results up to 12 months of age. Out of the initial cohort of 205, only 109 (53%) mother child pairs were followed up to 2 years in the Hira study. In the current study, the HIV status was not known in 34 (37%) of the 92 children. The children who died were not included in the estimation of the transmission rate as their last available PCR was negative and the deaths were not thought to be HIV related. Their cause of mortality is discussed in chapter 8.

2) Timing of transmission

An important issue in the understanding and planning of effective interventions for the prevention of MTCT of HIV is the timing of transmission. In non-breast fed children, the late in utero and intrapartum periods appear to be the time during which most MTCT of HIV occurs (Brossard *et al* 1995; Rouzioux *et al* 1995; Kuhn *et al* 1997). The finding 4 of the 18 child HIV infections occurred after 1 month reaffirms the importance breast feeding transmission in Zambia, as these infections were as a result of breast feeding.

It can be assumed that a child with a positive PCR at birth was probably infected in utero. It is possible that children who have virus detectable by DNA PCR or culture in the first few days of life have detectable HIV virus because of replicating virus acquired in utero. There are, however, no laboratory tests to confirm this assumption. Rouzioux et al (1995) used a modified Markov model formulation to estimate the timing of transmission in non-breast fed children of mothers enrolled in the French Prospective Study on Paediatric HIV Transmission. Timing of infection was dependant on the results of at least 2 of the following tests; viral culture, PCR, antigenaemia (p24) and Western blot antigen testing to proteins produced by the HIV *pol* and *gag* gene, of which one of them had to be the Western blot result. The results of this study suggested that MTCT of HIV in non-breast fed children occurred late in utero in about 30% of cases and intrapartum in 70%.

The excellent correlation between PCR and culture results in the first few days of life in the study by Bremer *et al* (1996) further supports the concept that that approximately 30% of children acquire infection in utero. With this understanding, HIV cultures were

not done in the Zambian study and of the 18 DNA PCR positive children 7 (38.9%) were determined at birth. It should however be noted that, MTCT of HIV is a continuum with no distinct demarcation between infection in utero and infection during delivery (Rouzioux *et al* 1995). This phenomenon or differences in populations studied might explain the lower proportion of HIV infection at this time found in Zambia compared to that by Hira et al in 1989.

It is reasonable to suggest that the child infections (10.1%) determined at one-month after birth in the Zambian study included both intrapartum and early breast-feeding transmissions because of the limited sensitivity of both DNA and RNA PCR-in identifying intrapartum transmission (Dunn *et al* 1995; Bremer *et al* 1996; Simonds *et al* 1998). The median period between birth and emergence of viral markers is believed to be around 10 days (Rouzioux *et al* 1995).

Beyond one month of age 4 (9.1%) of 44 children with DNA PCR results negative at 1 month became infected as a result of breast-feeding in the current cohort. Confusion has surrounded the contribution of breast feeding to MTCT of HIV and most studies are unable to quantify very early transmission due to breast-feeding. The current estimate of 9.1% in children above one-month is lower than that reported by Dunn *et al* in an early meta analysis. They estimated 14% additional risk of postnatal transmission from breast-feeding (Dunn *et al* 1992). The difference in estimate might be due to the fact that the current estimate did not include children below 1 month.

More recent data are available from a pooled meta-analysis and a study in Malawi (Leroy *et al* 1998; Miotti *et al* 1999). Absolute transmission rates were estimated at 3.2 per 100 person years of breast-feeding among children aged 2.5 to 6 months (Leroy *et*

al 1998). The cumulative risk of late transmission increased with duration of breast-feeding, with a 9.2% risk of late transmission in children breast feeding at age 36 months. Miotti *et al* (1999) reported cumulative transmission risk from breast feeding in Malawi to be 3.5% at 6 months 7% at 12 months and 10.3% at 24 months, although children below 2 months were not included in this estimate.

Results from a trial in Nairobi, Kenya in which children born to HIV infected mothers were randomised to breast-feeding or formula showed that 40% of all MTCT of HIV infections could be attributable to breastfeeding (Nduati *et al* 2000). Most of these infections were acquired during the first few months of life

Breast-feeding transmission is significant in Zambia and needs to be considered when planning and evaluating MTCT of HIV interventions. Policy guidelines on infant feeding and HIV have been published by international agencies (UNAIDS 1997; UNAIDS 1999; WHO 1998). Mothers with HIV infection should be guided and supported in choosing the most appropriate feeding option for their children by having complete and accurate information. Access to safely prepared nutritionally adequate breast milk substitutes (replacement feeding) will reduce MTCT secondary to breastfeeding. However, artificial feeding may be associated with increased risk of morbidity and mortality from infectious diseases and malnutrition if the mother is not adequately advised and supported (WHO 2000). In addition breast feeding ordinarily provides complete nutrition up to 4 to 6 months of age and about half from 6 months to 12 months and up to a third from 12 months to 24 months.

The issues raised are complex and interventions difficult to implement. Provision of formula in the first 6 months of life is expensive and in a society where breast-feeding is

the norm might result in social stigma and discrimination. Retrospective evaluation of breast feeding patterns, in a cohort study in South Africa designed to study the efficacy of vitamin A supplementation in pregnancy on MTCT of HIV, suggested that exclusive breast-feeding up to 12 to 14 weeks might not increase the risk of HIV transmission to the child (Coutsoudis et 1999). This might be the only option for most mothers in Zambia but requires more studies to validate the results.

7.4.3 Risk factors for MTCT of HIV

A number of potential targets for MTCT of HIV interventions in Zambia were examined.

1) Viral Load

The results in this study reaffirm observations from other studies in industrialised countries as well as Africa that high maternal RNA viral load is associated with MTCT of HIV (Sperling *et al* 1996; Garcia *et al* 1999; Mofenson *et al* 1999; The European Collaborative Study Group 1999, O'Donovan *et al* 2000). The median RNA viral load was significantly higher (over 3 times) in mothers who transmitted the infection to their children that those who did not and this relationship was seen for early as well as all infections in infancy on univariate analysis.

When RNA viral copies increased (from <10,000 to 50,000 or more), the percentage of infected children increased from 5.3 to 34.8% for early and 13.3 to 68.8% for all HIVinfections. After adjusting for other significant variables on multivariate analysis mothers with RNA viral load of 50,000 and above were more likely to transmit to their children and this effect was present both for early and all HIV infections. There was

only one mother with RNA viral load less than 1,000 that transmitted HIV to her child, the level being 611.8 copies per ml. It has been previously reported MTCT of HIV will occur even at very low viral load and that there no threshold at which transmission will not occur (The European Collaborative Study Group 1999). With anti retroviral treatment in pregnancy neither the change in the plasma RNA from entry to delivery nor a critical plasma level at delivery can explain the observed substantial reduction in MTCT of HIV (Sperling *et al* 1996; Mofenson *et al* 1999).

One of the limitations in our study was, because samples had to be transported to Japan, only 70 (76.1%) of the samples were suitable for quantitative RNA analysis, reducing the sample size further. In the remaining samples, different handling and processing of the specimens could influence the results although it has been reported previously that sample collection, storage conditions and specimen processing only marginally affect RNA quantification (Ginnochio *et al* 1997).

2) Maternal immunological status

In univariate analysis, this study indicated that median CD4 cell count was lower in the mothers in the mothers that transmitted HIV to their children in infancy although not for early infection and that transmission increased linearly with decreasing CD4 percentage. None of the mothers with CD4 cell count above 700 cells per μl transmitted infection to their children. Several studies have reported increased transmission with decreased maternal immune status reflected by low CD4 counts, low CD4 percentage and low CD4/CD8 ratios although they all reported different degrees of association (Lepage *et al* 1993; St Louis *et al* 1993; Thomas *et al* 1994; Mayaux *et al* 1995; Fowler

& Rogers 1996; Landesman *et al* 1996; The European Collaborative Study Group 1996).

In this study, CD4 percentage was not found with MTCT of HIV in infancy when significant factors in the univariate analysis (RNA viral load, marital status, maternal weight and history of antibiotic treatment) were accounted for in multivariate analysis. Reduction in CD4 cell count s, percentages or CD4/CD8 ratios occur with advanced HIV disease and the observed increased risk of MTCT of HIV may be postulated to be a factor of high viral load associated with progressive HIV disease. In previous logistic regression models that have not included maternal viral load, it would, therefore, not be surprising that CD4 indices remain independently associated with the risk of MTCT of HIV (Lepage *et al* 1993; St Louis *et al* 1993; Landesman *et al* 1996; Simonds 1999). Depressed immunity predisposes the mother other to infections and increased risk of MTCT of HIV is associated with some of these these infections. In this study cohort, tuberculosis (TB) an AIDS defining illness was not common and the children of mothers on treatment for TB were not at increased risk of HIV infection. On the other hand, antibiotic treatment for other suspected infections was found to be associated with increased the risk of early MTCT of HIV but not all HIV infections in infancy on univariate analysis. The infections treated with antibiotics were not defined in this study but there is some evidence that chorioamnionitis and hospitalisation for pneumonia is associated with increased risk MTCT of HIV (St Louis *et al* 1993; Thomas *et al* 1994; Landesman *et al* 1996; Van Dyke *et al* 1999). Similar to the observations for CD4, the increased risk of MTCT observed with antibiotic treatment was lost on multivariate analysis.

More recent studies that have taken viral load into account when evaluating the effect of depressed immunity, have not found CD4 indices to independently increase the risk of MTCT of HIV (Garcia *et al* 1999; Katzeinstein *et al* 1999; Mofenson *et al* 1999; Shaffer *et al* 1999; The European Collaborative Study 1999).

2) Maternal Nutritional Factors

As stated in chapter 4, serum retinol has been widely used to measure vitamin A status and is a useful tool a t population level for measuring changes in vitamin A status secondary to an intervention (Sommer &West 1996). The earliest manifestation of clinical dysfunction secondary to vitamin A deficiency can occur at levels at levels above 1.05μmol/L, though these are uncommon and mild. A study in Malawi in 1994 evaluated the association of serum retinol levels in HIV infected mothers with the risk of MTCT of HIV. Mothers with serum retinol below 1.05μmol/L had an increased risk of transmission, which dropped with increasing levels (Semba *et al* 1994).

The current cohort did not show any relationship between low serum retinol and transmission despite the fact that 48.4% and 23.1% of HIV infected mothers had serum retinol less than 1.05 and 0.7 μmol/L respectively (Chapter 4). This finding is similar to that of Burger *et al* (1997) in 95 HIV-1-infected mothers of different ethnic and social backgrounds living in the New York and LosAngeles metropolitan areas in the United States. Sixteen of the 95 mothers transmitted HIV-1 to their children.

An important issue is that of stability and validity of serum retinol as an index of vitamin A status. Blood is merely a transport medium for serum retinol from liver stores to target cells. A variety of factors influence serum retinol concentrations. In general vitamin A stores are depleted by liver dysfunction, other organ disease, low protein status, inadequacy of other nutrients, infection and other metabolic insults (Sommer & West 1996). Fever, infection and other factors can cause transient changes in serum levels unrelated to any change in vitamin A stores. Filteau *et al* (1993) observed that that malaria eradication could dramatically impact on serum retinol distribution in Ghanaian children by influencing serum concentration regardless of vitamin A status. These are important caveats in interpreting study results based on serum retinol measurements. One might achieve more precision by using more than one method to measure vitamin A status. Measurement of acute phase proteins such as acute phase reactants, α_1-anti chymotrypsin and α_1-antiglycoprotein might be essential for adjusting for the effect of infection (Paracha *et al* 2000).

Randomised studies are useful in confirming a 'causal link' when a specific factor associated with an end point is studied as the intervention arm in a controlled design. Vitamin A supplementation in pregnancy has been studied in a controlled randomised fashion. Vitamin A supplementation as 5,000 IU retinyl palmitate and 30 mg beta carotene during the third trimester of pregnancy and 200,000IU retinyl palmitate at delivery was not effective in reducing MTCT of HIV although it reduced the incidence of premature delivery (Coutsoudis *et al* 1999). Another study in Tanzania assessing the effects of vitamin A and multivitamins' supplementation, between 12 and 27 weeks gestation, of 1075 HIV infected pregnant mothers on birth outcomes also found no

effect of vitamin A on MTCT of HIV. However, multivitamins decreased the risk of low birth weight (<2500 g) by 44%, severe prematurity (<34 weeks of gestation) by 39% and intrauterine growth retardation by 43% and a significant increase in CD4, CD8, and CD3 counts was noted postnatally (Fawzi *et al* 1998). The existing data, therefore, does ascertain whether between populations it is low serum retinol alone or a combination of nutritional factors that have an effect on MTCT of HIV. In this study postpartum maternal weight less than 45 kilograms was associated increased risk of early MTCT but not MUAC less than 23cm and anaemia (haemoglobin less than 7 and 11g/dl). Because of the small number of transmitting mothers, it was not possible in the current study to evaluate the effect of a combination of factors (low maternal weight low MUAC, anaemia with low MCV and MCHC) on MTCT of HIV.

3) **Medical factors**

A number of other factors were also evaluated. Syphilis might be expected to damage the placenta and also activate lymphocytes thus enhancing viral replication and increasing the chances of transmission (St Louis *et al* 1993; N'gom *et al* 1997). A similar hypothesis has been postulated for malaria. In Malawi placental *P. falciparum* infection was associated with poorer survival in the children born to HIV infected mothers which may represent increased risk of transmission (Bloland *et al* 1995). Both RPR positivity and reported treatment for malaria were not found to be associated with MTCT of HIV. The malaria cases were reported treatments with no clear evidence of laboratory confirmation of the malaria and the mothers found to be RPR positive in this

study were treated well before delivery through the syphilis programme. It is, therefore, not surprising that no association with transmission was found. Although 8 (4.7%) of the 275 mothers tested at delivery were negative antenatally, only 4 were HIV infected and could not be evaluated further.

The prevalence of hepatitis B surface antigen (HBsAg) and positive mothers were not at increased risk of MTCT of HIV. There is no evidence in the current literature demonstrating an association between hepatitis B infection and MTCT of HIV. However, hepatitis B infection is still a potential problem in MTCT interventions as it could interfere with admisnistration anti-retroviral drugs that are metabolised in the liver.

4) Obstetric factors

Events during delivery can influence early MTCT of HIV more especially intrapartum transmission. Premature delivery, low birth weight, intrapartum haemorrhage, duration of rupture of membranes of more than 4 hours, vaginal delivery and obstetric procedures have been shown to increase the risk of MTCT of HIV although some of the results are not consistent (Datta *et al* 1994; Dunn *et al* 1994; Mayaux *et al* 1994; European Collaborative Study 1994, 1996 & 1999; Landesman *et al* 1996; Mandelbrot *et al* 1996; Simonds *et al* 1998; Garcia *et al* 1999; Kuhn *et al* 1999; Mofenson *et al* 1999). None of these factors were associate with MTCT of HIV in the current study. Apart from low birth weight and prematurity, the prevalence rates of all the other variables in the study population were low. There was also high number of women lost

to follow up, reducing the power of the study to detect small differences. It is, however, difficult to explain why no increased risk of MTCT was observed with prematurity and low birth weight.

5) Socio-demographic factors

These were evaluated in the current study as they have an important role to play both in acquisition of new infection as well as disease progression. Apart form maternal age less than 20 being associated with early MTCT of HIV in univariate analysis, none of the others studies were found to be of any significance. This finding might be reflective of a ripe epidemic where there is a co-existence of new and old infections in defined populations.

6) Late Infection (Breast feeding)

Prevention of MTCT of HIV from breast-feeding is one of the major challenges currently facing researchers and policy makers in low resource settings. Transmission of HIV through breast-feeding can occur during the early lactation (colostrum/early milk) as well as later in lactation. In the current Zambian study it was difficult to establish the actual timing of infections detected in the first month of life but 4 new HIV infections were acquired between 1 and 12 months of age. Because of the small number of children stratified analysis of associated risk factors was not possible. However, description of the 4 cases revealed that all the 4 mothers had lost a child before. The cause of death for these children was not established in the study, although HIV infection could have been a contributory factor. All these mothers had CD4 cell counts

and percentage below 700 cells per mm^3 and 25% and the HIV RNA viral load was above 50,000 in three of the mothers.

In many low resource settings, the risks of not breastfeeding on overall infant morbidity and mortality have to be carefully weighed against the benefits of avoidance of HIV exposure and possible transmission during lactation. Breast-feeding is essential for child survival and a recent meta-analysis of recent studies in developing countries demonstrated this, even when deaths from non-infectious causes were included (WHO 2000). There are several mothers in Zambia who persistently are seeking a healthy child despite past history, as was the case in the 4 mothers in this study. This dilemma is a reality in many resource poor settings highly affected by HIV and where mothers have few feeding options. Two children interestingly escaped HIV infection until they were 12 months of age (table 7.4). At 12 months these children could have been saved through alternative feeding. However, it would be difficult at programmatic level to specifically target these children at that age. The use of low cost anti-retrovirals in children during lactation, passive (immunoglobulin) and active (vaccine) immune agents to protect children during early (colostrum) and /or late breastfeeding and early weaning (at 3-6 months) requires further consideration and research.

7.5 CONCLUSION

This study concludes that of HIV infected mothers delivering at UTH in Lusaka 31% will transmit infection to their children; 38.9% will be detected at birth, 38.9% at 1 month of age and 22.2% between 4 months and 12 months. Although univariate analysis indicated that MTCT of HIV might be a multifactorial event, viral load appears

to be the major factor independently associated with increased transmission risk. Strategies to prevent MTCT of HIV will need to focus on primary prevention of HIV infection on 'would be' mothers and reduction of maternal viral load through preventative anti-retroviral therapy in mothers. Breast feeding transmission also requires addressing through counselling and support on feeding choices and in future other interventions targeting lactating mothers of proven benefit.

CHAPTER 8

GROWTH, MORBIDITY AND MORTALITY IN HIV INFECTED AND NON-INFECTED INFANTS

8.1 INTRODUCTION AND OBJECTIVES

Human immuno-deficiency virus transmission from mother to child increases child mortality and the number of sick children who present to health services in many parts of Africa. The hard won gains in reduction of infant and child mortality achieved by successful child survival strategies such as infant immunisation and promotion of breast-feeding, are being rapidly undermined by the burden of HIV infection (USAID 1990; Boerma *et al* 1998).

Physical growth, neurodevelopment, infectious complications and death have been described as the 4 cardinal indicators defining HIV clinical progression and HIV disease outcome in children (Moye *et al* 1993). Measurement of these indicators is of fundamental importance in the understanding the impact of the disease and development as well as strengthening of prevention, care and support programmes. Collection of this data is also important for the monitoring effectiveness of mother to child transmission (MTCT) of HIV intervention programmes, as well as other child survival interventions such as the Integrated Management of Childhood Illness (IMCI), Infant Feeding Programmes and the Expanded Programme of Immunisation.

The objectives of this chapter were to describe in HIV infected and non-infected infants;

1) growth,

2) mortality patterns,

3) causes mortality and

4) factors associated with infant mortality

8.1.1 Child growth

Anthropometry is an important tool for assessing the health of children and can be used for evaluating the impact of health programmes (Dibley *et al* 1987). To evaluate growth, reference curves are required to compare observed anthropometric measurements with expected reference values for age and sex. A number of reference curves have been developed (Dibley et al 1987). However, the National Centre for Health Statistics (NCHS) growth reference curves were identified as the most suitable international standards by a working group convened by WHO in 1977 (WHO 1995).

In order to overcome the problems associated with expressing reference anthropometric indicators as a percentage of the reference median, Z-score were proposed (Waterlow *et al* 1977). They measure the deviation of the observed anthropometric measurement from the reference median in terms of standard deviations. With Z-scores, it is possible to compare the growth status of groups children of different ages in population studies as well as surveillance and monitoring of programmes (Waterlow *et al* 1977, Dibley et al 1986).

Studies on impact of maternal HIV infection on infant growth in sub-Saharan Africa are limited and child growth patterns remain poorly defined in these children. In a previous study in Rwanda, the weight, height and head circumference for age mean Z-scores

were lower among HIV infected children than non-infected ones (Lepage *et al* 1996). Similarly in The Democratic Republic of the Congo (DR Congo), Bailey et al (1999) found that by 3 months of age HIV infected children were shorter than both uninfected children and those whose mothers were negative.

Several studies in industrialised countries have demonstrated lower anthropometric measurements in children exposed to maternal HIV infection than in those whose mothers are non-infected (Mckinney *et al* 1993; European Collaborative Study 1995; Saavedra *et al* 1995). There is also an indication of post-natal catch up growth in uninfected infants exposed to maternal HIV infection (Mckinney *et al* 1993; Moye *et al* 1993; Saavedra *et al* 1995; Agostoni *et al* 1998).

In sub-Saharan Africa many children are born in poor families with an increased risk of low birth weight, under-nutrition and infectious diseases. These conditions in themselves lead to poor growth. Malnutrition is a major problem in Zambia with 42.4% of children estimated to be stunted (DHS 1996). When the mother has had no formal education (estimated to be 13.3% of the Zambian population), the prevalence of stunting is as high as 50%. This Zambian Demographic Health Survey (DHS) data, was not stratified by maternal and child HIV status but the major causes of morbidity 2 weeks preceding the survey were diarrhoea and cough with rapid breathing.

8.1.2 Infant morbidity and mortality

The clinical presentation of paediatric HIV infection in Africa consists of non-specific manifestations that also commonly occur among non-infected children (Spira et al 1999). However, HIV infection increases infant and child mortality (Blanche *et al* 1989;

Lallemant *et al* 1989; Braddick *et al* 1990; Ryder *et al* 1990; European Collaborative Study 1991; Miotti *et al* 1992; Taha *et al* 1995). Prospective studies of infected children in industrialised countries have suggested that the cumulative risk of clinical progression increases rapidly in the first year of life (Mayaux et al 1995; Ziegler et al 1996). It is estimated that up to 20% of children develop severe immuno-deficiency in the first year and die within 4 years (Ziegler et al 1996).

HIV infected children generally die earlier in Africa (Marum et al 1997; Spira et al 1999). Infant mortality rates of up to 39% have been reported from African studies (Boerma et al 1998). In a 5-year prospective cohort in Rwandan of HIV infected children, mortality rates were 26% and 65% by 1 year and 5 years respectively. The median survival was 12 months from the estimated time of infection. In comparison, the 5-year survival in Europe and America was between 25-30% and the median survival time was 6 to 10 years, before anti-retroviral therapy became the standard of care (Tovo et al 1992; European Collaborative Study 1994; Barnhart et al 1996).

The severity of maternal HIV disease at delivery is a major factor affecting duration of survival in an HIV infected child (Tovo *et al* 1992; Blanche *et al* 1994; Abrams *et al* 1995). Other factors include LBW, prematurity and co-infections such as syphilis (Bloland et al 1996).

Infant mortality in Zambia increased substantially during the 1980s (Timæus 1998) and has continued to rise (DHS 1996). In the study by Hira et al (1989), although 42% of the children infected with HIV died by 18 months, it was not clear whether HIV was the main contributing factor to the high mortality. Timæus (1996) in his review on the impact of HIV on mortality concluded that there may be other factors in Zambia.

8.2 METHODS

8.2.1 Study population

HIV infected and non-infected mothers and their children enrolled at delivery were followed up post-natally. The mother-child pairs had follow-up appointments at 1, 4, 7, 10 and 12 months. At every visit a standard questionnaire was administered evaluating interim medical problems and a physical examination of both the mother and the child was also done with the assistance of trained clinical assistants (appendix 3.1). At each follow up except at the10 month visit, a blood sample was collected from the baby in an EDTA bottle by heel prick for HIV PCR. The blood was processed as described in Chapter 3.

Mothers who did not attend the scheduled visit were actively followed up at home within a week of defaulting. Any illness at the time of the scheduled visit was treated in the follow up clinic by the medical staff following standard management guidelines. Mothers were requested to report any illness between visits to the research midwives at the follow up clinic or if possible to bring the sick child to the research clinic for treatment. Information on whether currently breast-feeding was obtained.

The cause and timing of death were abstracted from hospital or clinic records if available. If not available, the information was obtained from the mother using a standard morbidity definition (see next page). Where information was not clear, an opinion of the doctor was sought.

Weight and recumbent height were measured at each visit. Anthropometric measurements were completed as described in Chapters 3 and 6.

8.2.2 Definitions

Reported morbidity was defined as any illness requiring treatment at the health centre, by a traditional healer or at the hospital To define cause of mortality the following clinical descriptions were used:

1) Pneumonia

Cough and fast breathing or chest in-drawing, requiring admission and treatment with antibiotics

2) Malnutrition or failure to thrive

Failure to gain weight (FTT) or continued weight loss for two or more under 5 clinic visits, resulting in flattening or decline in the growth curve

3) Diarrhoea

Two or more loose stools in 24 hours.

4) Tuberculosis

Tuberculosis (Tb) was substantiated from hospital records. The criteria used, were developed at UTH over the past 20 years as the presence of three of the following:

1) Symptoms and signs suggestive of Tb
2) Radiological features suggestive of Tb
3) Response to antituberculous treatment
4) History of close contact with a case of Tb

Or

Sputum smear or gastric washings positive for acid fast bacilli

Or

Lymph node or other tissue biopsy revealing acid fast bacilli or suggestive of Tb

5) Septicaemia

A young infant who was feverish or cold skin with associated either lethargy, irritability or inability to drink, requiring treatment or responding to antibiotics.

6) Malaria

Fever with no associated rapid breathing requiring treatment or responding to anti-malarials.

8.2.3 Statistical Analysis

Median weight for age (W-A), height for age (H-A) and weight for height (W-H), including median Z-scores (WAZ, HAZ, WHZ) were calculated using the Epinut anthropometric software in Epi-Info version 6.04b (CDC Atlanta). Epinut uses growth reference data from the National Centre for Health Statistics (NCHS) recommended by the WHO Working Group (1986) as international standards. Epinut calculates Z-scores or standard deviation (SD) scores by subtracting the median weight height or weight for height of the reference population at the child's age from the child's weight and dividing by the SD of the weight for the reference population at that age.

Three HIV child-mother categories were defined for comparison of anthropometric values: Child and mother non-infected (group 1); child and mother infected (group 2) and child non-infected and mother infected (group 3). Infants were classified as stunted, underweight or wasted if their corresponding Z-score were below –2 that of the reference population. Severe malnutrition was classified as Z-score below –3.

Mortality was analysed using Kaplan Meier survival analysis and the log rank statistic was used to compare the probability of survival in relation to different risk factors. The indicators for the risk factors studies were as defined in Chapter 4, 5, 6 and 7. Cox's proportional hazards model was used to analyse multiple factors potentially associated with reduced survival. All factors thought to be of public health significance were included in the model followed by a backward step selection with a significance level of $P<0.05$ to arrive at the final model. To investigate the effect of maternal HIV infection more completely, additional Kaplan Meier log rank analyses were performed adjusting for LBW, prematurity and IUGR. Data was analysed using SPSS version 10.

8.3 RESULTS

8.3.1 Background maternal characteristics and reported infant morbidity

Table 8.1 summarises the maternal characteristics of the three HIV child-mother groups (1, 2, & 3) included in the analysis.. Low CD4 cell count (<500 cells/mm^3) and percentage (25%) was significantly associated with group 2 and 3, in which the mother was HIV infected. Post-partum anaemia (Hb< 11 g/dl) was significantly associated with HIV infection in the mother but not the child (group 3) whereas positive antenatal RPR was significantly associated with group 2, in which both the mother and child were HIV infected. The rest of the characteristics were similar in all 3 groups.

The proportion of infants that were reported unwell in between follow up appointments was between 34.7% and 100%. From one month of age ≥ 50% of the children were reported unwell during the period preceding the next appointment. There were no significant differences in reported illness between the 3 child-mother groups.

Table 8.1 Background characteristics

Variable	*HIV group* [1]	*n/N (%) with variable*	*Relative risk (95% CI)*	*P value*
Male sex	1 C-M-	104/192 (54.2)	reference	
	2 C+ M+	8/15 (53.3)	1.08 (0.36-2.57)	0.95
	3 C-M+	22/37 (56.4)	1.08 (0.61-1.92)	0.80
Maternal age <20 years	1 C-M-	32/207 (15.5)	reference	
	2 C+ M+	1/17 (5.9)	0.36 (0.05-2.64)	0.48
	3 C-M+	0/41 (0)	-	-
Maternal illiteracy (cannot read)	1 C-M-	56/203 (27.6)	reference	
	2 C+ M+	5/16 (31.2)	1.18 (0.43-3.25)	0.75
	3 C-M+	8/41 (19.5)	0.68 (0.33-1.40)	0.28
CD4 <500 cells/mm^3	1 C-M-	38/201 (18.9)	reference	
	2 C+ M+	12/17 (70.5)	**8.06 (2.98-21.80)**	**<0.001**
	3 C-M+	17/41 (41.5)	**2.41 (1.40-4.15)**	**<0.001**
CD4 < 25%	1 C-M-	12/201 (6)	reference	
	2 C+ M+	14/16 (88.2)	**51.42 (12.38-213.56)**	**<0.001**
	3 C-M+	17/41 (41.4)	**5.20 (3.20-8.45)**	**<0.001**
Pregnancy malaria treatment	1 C-M-	59/182 (32.4)	1	
	2 C+ M+	5/16 (31.3)	0.95 (0.35-2.62)	0.92
	3 C-M+	12 /37 (34.3)	1.07 (0.57-2.03)	0.83
Serum retinol < 0.7μmol/L	1 C-M-	64 /207 (30.9)	reference	
	2 C+ M+	6/10 (37.5)	1.31 (0.57-2.03)	0.58
	3 C-M+	17/41 (41.5)	1.46 (0.83-2.56)	0.19
Antenatal haemoglobin <11g/dl	1 C-M-	14/36 (38.9)	reference	
	2 C+ M+	0/2 (0)	-	-[2]
	3 C-M+	6/9 (66.7)	2.50 (0.71-8.77)	0.16
Postpartum haemoglobin <11g/dl	1 C-M-	86/208 (41.3)	reference	
	2 C+ M+	8/17 (47.1)	1.24 (0.50-3.09)	0.65
	3 C-M+	26/41 (63.4)	**2.12 (1.18-3.80)**	**0.01**
Antenatal positive RPR	1 C-M-	17/140 (12.1)	reference	
	2 C+ M+	5/13 (38.5)	**3.72 (1.34-10.34)**	**0.01**
	3 C-M+	5/25 (20)	1.63 (0.68-3.88)	0.29
Postpartum positive RPR	1 C-M-	18/208 (8.7)	reference	
	2 C+ M+	2/15 (11.8)	1.37 (0.34-5.55)	0.66
	3 C-M+	6/41 (14.6)	1.61 (0.75-3.43)	0.23

[1] C-M- Child-mother negative, C+M+ Child-mother positive, C-M+ Child negative-mother positive. [2]RR relative risk not calculated due to empty cells, RPR rapid plasma reagin
Significant values in bold

Table 8.2 Reported morbidity (all illnesses) during infancy

Age (months)	*HIV category*[1]	*n/N (%) illness*	*Relative Risk (95% CI)*	*P Value*
1	1 C-M-	58/167 (34.7)	reference	
	2 C+ M+	5/11 (45.5)	1.52 (0.48-4.79)	0.47
	3 C-M+	10/27 (37.0)	1.09 (0.53-2.25)	0.82
4	1 C-M-	45/85 (52.9)	reference	
	2 C+ M+	4/7 (57.1)	1.17 (0.28-4.94)	0.83
	3 C-M+	10/20 (50)	0.91 (0.41-2.00)	0.81
7	1 C-M-	83/104 (79.8)	reference	
	2 C+ M+	3/6 (50)	0.31(0.07-1.47)	0.15
	3 C-M+	22/27 (81.5)	5.24 (0.74-37.04)	0.08
10	1 C-M-	85/103 (82.5)	reference	
	2 C+ M+	7/8 (87.5)	1.45 (0.19-11.08)	0.72
	3 C-M+	12/13 (92.3)	2.35 (0.32-17.2)	0.69
12	1 C-M-	88/117 (75.2)	reference	
	2 C+ M+	7/7 (100%)	-	-[3]
	3 C-M+	21/28 (75)	0.99 (0.46-2.14)	0.98
Overall[2]	1 C-M-	All proportions	reference	
	2 C+ M+	included in	1.33 (0.60-2.87)	0.64
	3 C-M+	weighted	1.12 (0.74-1.61)	0.73
		analysis		

[1] M-C- Child-mother negative, C+M+ Child-mother positive, C-M+ Child negative-mother positive. [2] Overall Mantel Haenszel weighted relative risk analysis [3]Relative risk not calculated due to empty cells

8.3.2 Median weight for age and Z-scores

Table 8.3 describes median weight Z-scores for age for the three groups of children. For all the 3 groups, median birth-weight Z-score was below the NCHS reference median. In the first four months, all 3 groups exhibited an increase in median *z*- score values. After 4 months, the median birth-weight Z-scores declined progressively to below the NCHS reference median. In HIV-infected children, the median Z-score at 12 months of age was -3.46.

Over 95% of the children were breast-fed from birth until one year of age; 243 of 250 children (97.1%) at 1 month, 125 of 127 children (98.4%) at 4 months and 147 of 152 children (96.7%) at 12 months.

8.3.3 Median height for age and Z-scores

At birth, all 3 groups had median height for age above the NCHS reference median. After birth, all had sustained marked reduction in growth attainment to 12 months (table 8.4 & figure 8.2). At 12 months, the median height for age Z-score in HIV infected infants (group 2) was –4.4 SD compared to -2.2 SD in group 1 and -2.22 SD in group 3.

Table 8.3 Median weight (kg) for age and Z-scores in infants

Age	*Mother and child HIV negative (group1)*			*Mother and child HIV infected (Group 2)*			*Mother HIV infected but child negative (Group 3)*		
	N	*Median[1] (IQR[2])*	Z-score[3] *(IQR)*	*N*	*Median (IQR)*	Z-score *(IQR)e*	*N*	*Median (IQR)*	Z-score *(IQR)*
0	206	2.9 (2.6-3.2)	-0.67 (-1.39-0.06)	17	2.6 (2.2-2.9)	-1.68 (-2.9-0.90)	41	2.9 (2.6-3.2)	-0.89 (-1.39- 1.70)
1	154	4.4 (3.9-4.9)	0.31 (-0.47-1.06)	10	3.9 (3.5-4.7)	-0.63 (-0.86-1.31)	26	4.2 (3.7-4.4)	0.01 (-0.72-0.49)
4	96	7.0 (6.2-7.6)	0.89 (-0.18-1.55)	8	6.7 (5.6-7.1)	-0.45 (-1.01-0.73)	22	6.8 (6.2 –7.2)	0.81 (-0.06-0.89)
7	106	8.0 (7.1-8.6)	0.11 (-1.01-0.84)	6	7.3 (7.0-9.4)	-0.78 (-1.12-1.89)	29	7.8 (7.4-8.3)	-0.34 (-0.76-0.10)
10	82	8.6 (7.8-9.2)	-0.65 (-1.57-0.06)	6	7.9 (7.8-8.4)	-1.09 (-1.47-0.37)	11	7.6 (7.5-8.6)	-1.68 (-2.03-1.06)
12	112	9.0 (8.3-10.0)	-0.64 (-1.65-0.03)	6	7.4 (5.0-8.3)	-3.46 (-5.52-0.70)	29	8.8 (8.3-9.8)	-1.16 (-1.84- 0.15)

Median[1] median weight in kg; *IQR[2] 25-75[th]* centile; Z-score[3] Median weight in standard deviations from reference population

Figure 8.1

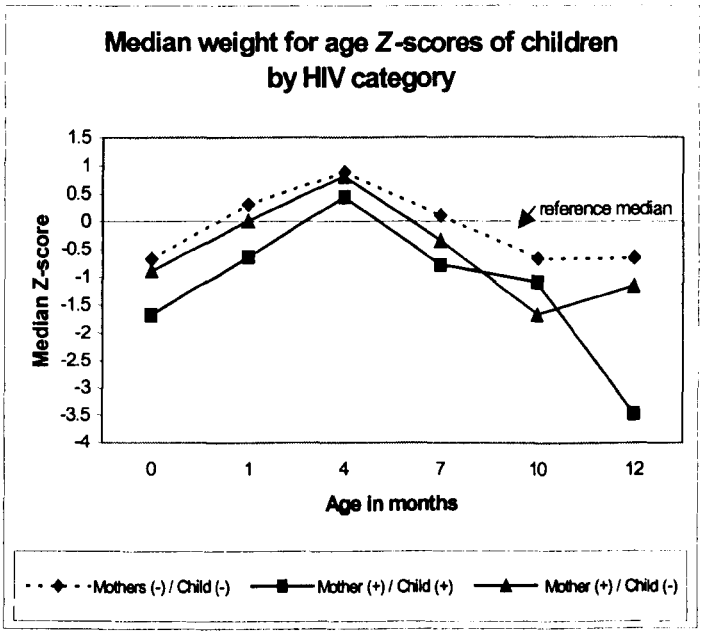
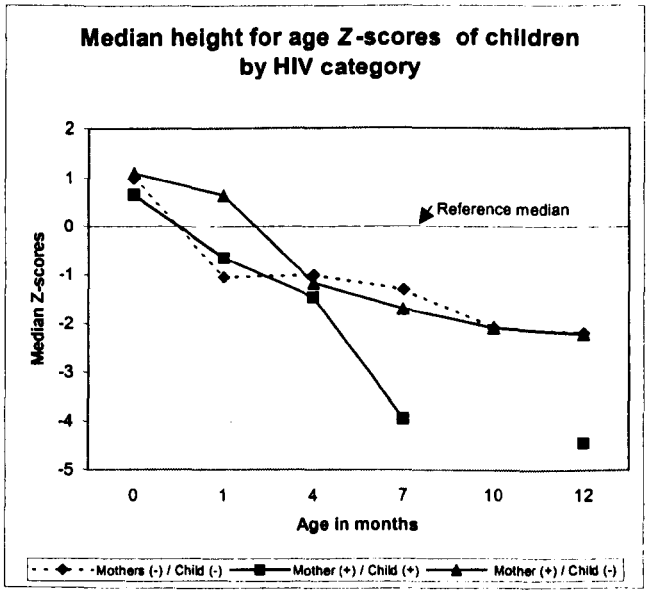


Figure 8.2



10 month value for HIV infected children possible outlier

Table 8.4 Median height (cms) for age and Z-scores in infants

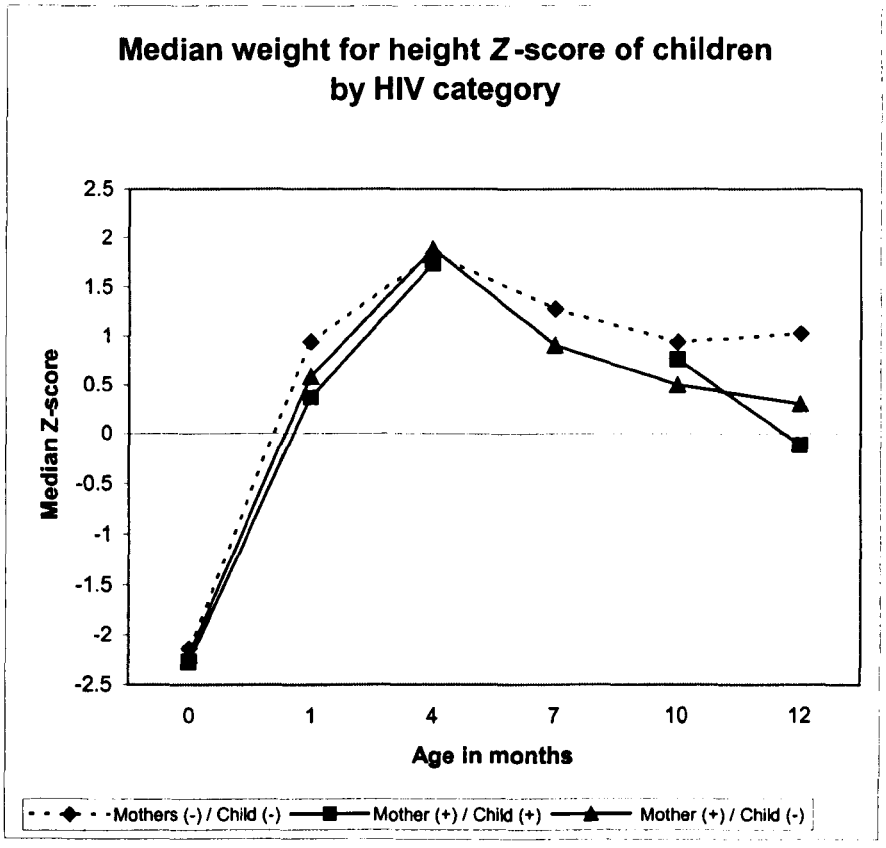
Age	Mother and child HIV negative (Group 1)			Mother and child HIV infected (Group 2)			Mother HIV infected but child negative (Group3)		
	N	*Median[1] (IQR[2])*	*Z-score[3] (IQR)*	*N*	*Median (IQR)*	*Z-score (IQR)*	*N*	*Median (IQR)*	Z-score *(IQR)*
0	171	52 (50-54.2)	1.10 (0.06-3.74)	15	51 (45-53)	0.66 (-1.52-2.41)	59	52 (51-54)	1.10 (0.23-1.97)
1	154	52 (50-54)	-1.04 (-1.98-0.18)	10	53 (52-53)	-0.64 (-1.04--0.24)	27	53 (50.5-54)	-0.06 (-1.33--0.23)
4	96	60 (57-63)	-1.0 (-2.3-0.11)	8	59 (56.5-61.5)	-1.46 (-2.15--0.82)	22	59 (57-61)	-1.17 (-2.12--0.63)
7	106	65 (63-67)	-1.3 (2.08--0.21)	6	58.5 (57-64)	-3.95 (3.95--1.34)	29	64 (62-67)	-1.71 (-2.83--0.56)
10	83	67 (65-74)	-1.09 (-2.88--0.6)	6	67 (62-69)	-1.19 (-2.89--1.0)	11	67 (66-68)	-2.09 (-2.88--1.37)
12	112	69.5 (67-73)	-2.20 (-3.0--1.14)	6	65 (61-66)	-4.44(-6.71--2.23)	29	69 (67-72)	-2.22 (-3.0--1.51)

Median[1] Median length in cm *IQR[2]* 25-75th centile Z-score[3] median length in standard deviations from reference population

Table 8.5 Median weight for height (%) and Z- scores in infants

Age	Mother and child HIV negative (Group1)			Mother and child HIV infected (Group 2)			Mother HIV infected but child negative (Group 3)		
	N	*Median (IQR)*	*Z-score (IQR)*	*N*	*Median (IQR)*	*Z-score (IQR)*	*N*	*Median (IQR)*	*Z-score (IQR)*
0	166	56.6 (51.9-62.8)	-2.14 (-2.9-1.30)	13	52.8 (50-54.7)	-2.27 (-3.08--2.06)	31	55.1 (23.01-60)	-2.20 (-3.24--1.27)
1	148	83.2 (75-90.4)	0.94 (-0.25-1.82)	10	74.8 (71.4-88.7)	0.37 (0.30-1.58)	22	81.2 ((70.5-87.1)	0.58 (-0.41-1.06)
4	92	116.1 (105.2-125.7)	1.84 (0.59-2.87)	8	112.6 (92.4-116.6)	1.74 (0.30-1.58)	20	116 (105-121.8)	1.89 (1.03-2.69)
7	106	122.8 (111.9-134.4)	1.28 (0.13-2.48)	6	125.8 (116.7-166.7)	3.57 (0.03-7.55)	25	120 (116.7-127.2)	0.91 (0.03-2.29)
10	82	125.1 (117.7-137.5)	0.94 (0.02-1.99)	6	121.3 (118.3-125.9)	0.77 (-0.23-1.80)	10	119.1 (106.1-127.3)	0.51 (-0.35-1.27)
12	112	131 (118.5-141.3)	1.03 (-0.29-2.06)	6	108.1 (90.9-127.9)	-0.11 (-2.05-2.6)	22	126.6 (117.7-135.1)	0.31 (0.02-2.01)

Figure 8.3



7 month value for HIV infected children not plotted – possible outlier

8.3.4 Median weight for height and Z-scores

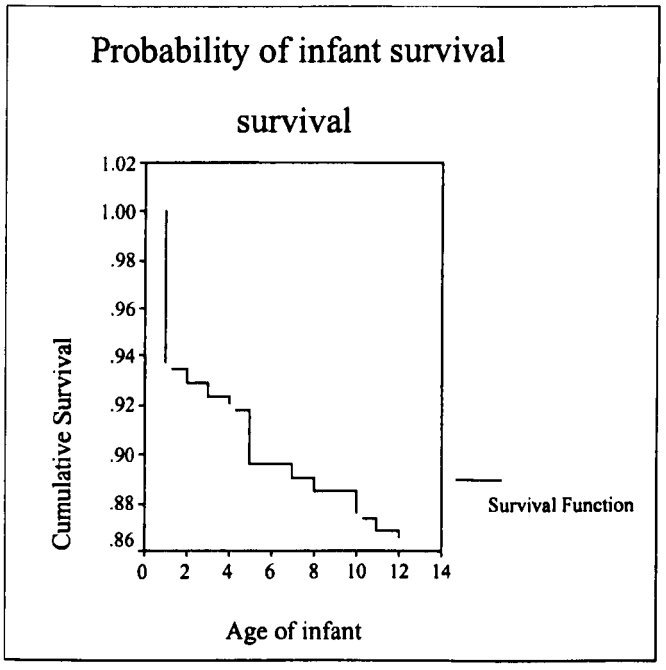
At birth, median weight-for-height Z-scores was < –2 in all 3 groups (table 8.5 & figure 8.3). Until 4 months of age, growth curves for the 3 groups were similar and showed marked improvement, with weight for height scores peaking at 4 months, at 1.84 (group 1), 1.74 (group2) and 1.89 (group 3). After 4 months of age, there was a consistent fall in the Z-scores in all groups, although values remained above the NCHS reference median except in HIV infected children.

8.3.5 Infant mortality

1) Overall infant mortality

Of 183 children, with outcome data at one year of age, 25 children died. Twelve of these deaths (48%) were in neonates. Figure 8.4 illustrates the probability of child survival at 1 year by Kaplan Meier survival analysis. The estimated survival probability was 86.3%. From this survival estimate the infant mortality rate was projected to be 136 per1000 live births.

Figure 8.4



2) Mortality and HIV infection

Of the 25 deaths, survival curves are shown in relation to maternal and child HIV status, prematurity, low birth weight and intrauterine growth retardation (figure 8.5 to 8.12).

Eleven of 130 children of HIV non-infected mothers compared to 14 of 53 infected mothers, with known outcome at 1 year died. In children of HIV positive mothers, the probability of survival at 1 year was 72.8% compared to 91.5% in children of non-infected mothers. The estimated infant mortality rate for infants born to HIV infected and non-infected mothers was 272 and 85 per1000 live births.

Of the 53 children born to infected mothers: 14 were HIV infected; 31 were non-infected and 8 were of indeterminate status. There were no reported deaths amongst negative children of HIV infected mothers. Six of the infants of the 14 HIV infected infants died. The 8 infants of indeterminate status died before their HIV status was confirmed, of which 6 died early in the neonatal period.

The probability of survival by Kaplan Meier analysis in infants born of HIV positive mothers according to child HIV status is illustrated in figure 8.6. The one-year survival probability by HIV status was 54.6% in infected, 100% in uninfected and 0% in infants of indeterminate status. From these estimates, the estimated projected infant mortality rate when the child was HIV infected was 454 per 1000 live births.

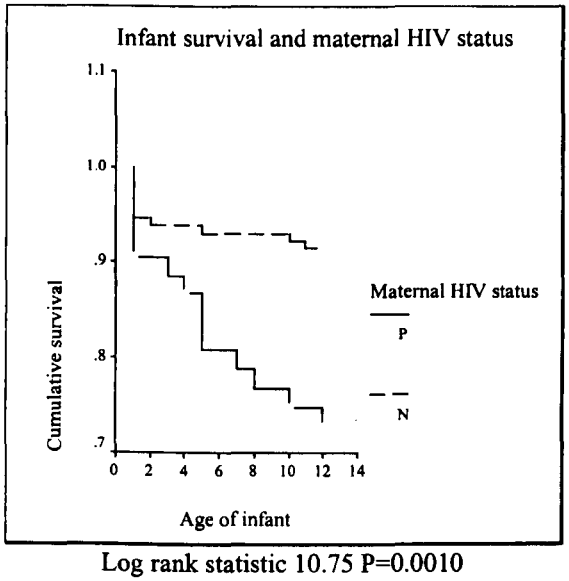
Table 8.6 shows the timing of death in the 6 HIV infected infants in relation to timing of HIV transmission. Four out of 5 children, HIV infected within the first month of life, died before 6 months of age.

None of the uninfected children with positive mothers who completed follow up died in the first year. On Kaplan Meier univariate analysis, maternal HIV infection was

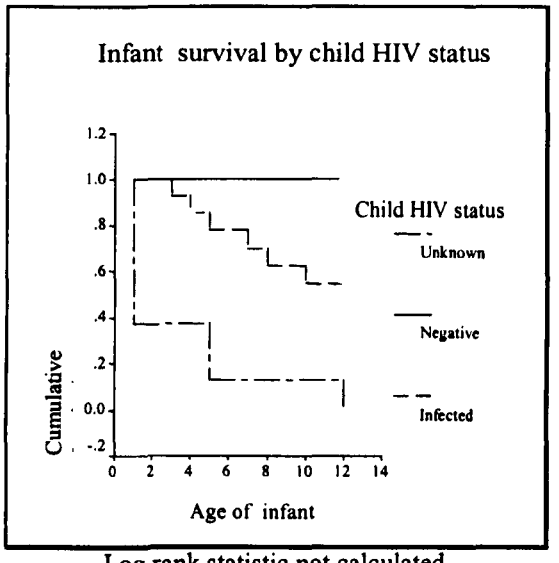
significantly associated with reduced probability of survival in the first year of life (table 8.7). After adjusting for birth weight, gestational age and IUGR, maternal HIV infection was significantly associated with reduced probability of survival in normal birth-weight, term and IUGR babies (figure 8.7 to 12).

3) Infant mortality factors

Figure 8.5 & 8.6 Infant survival by maternal and child HIV status



Log rank statistic 10.75 P=0.0010

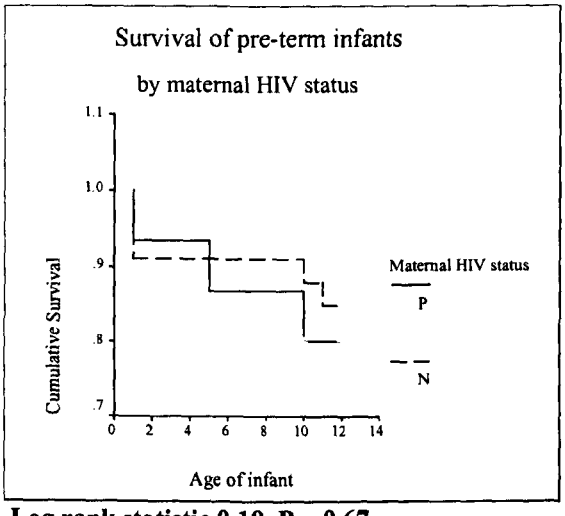


Log rank statistic not calculated

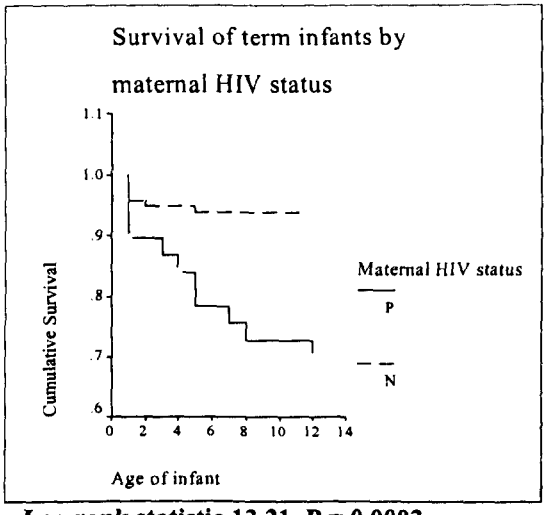
Table 8.6 Mortality in HIV infected infants by timing of HIV detection

Case	*Age (months) when HIV PCR positive*	*Age (months) at time of death*
1	1	3
2	0	4
3	0	5
4	0	5
5	1	7
6	7	10

Figure 8.7& 8.8 Survival in pre-term and term infants by maternal HIV status

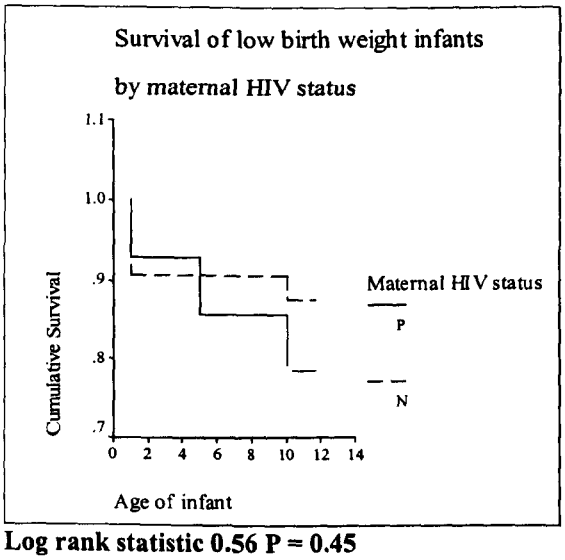


Log rank statistic 0.19 P = 0.67

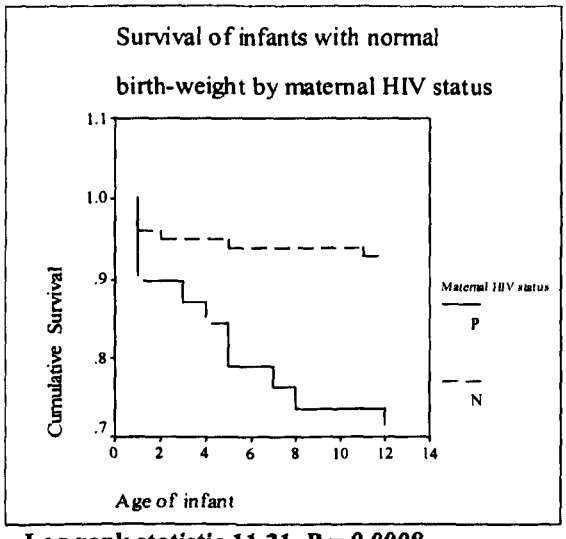


Log rank statistic 13.21 P = 0.0003

Figure 8.9& 8.10 Birth-weight, maternal HIV status and infant survival

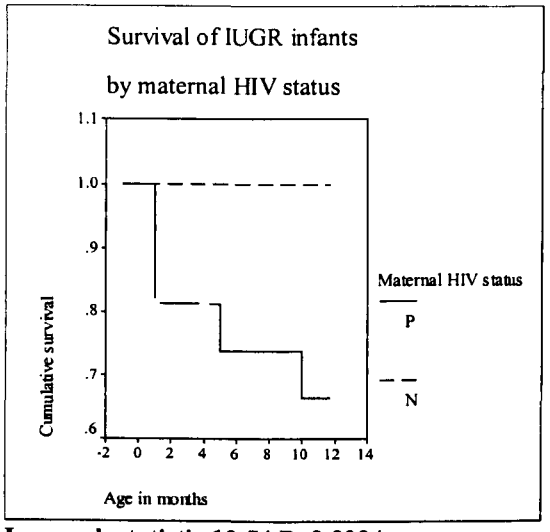


Log rank statistic 0.56 P = 0.45

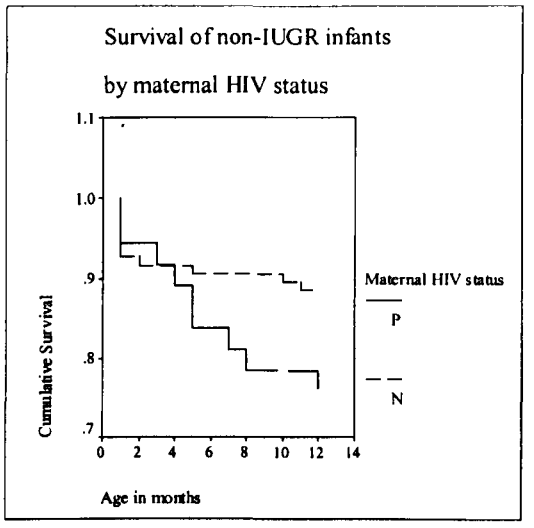


Log rank statistic 11.31 P = 0.0008

Figure 8.11 & 8.12 Intra-uterine growth, maternal HIV status and infant survival



Log rank statistic 12.54 P=0.0004



Log rank statistic 4.32 P=0.643

2) Multivariate analysis

Maternal factors: HIV infection; age, gravidae, post-partum serum retinol, CD4 percentage, RPR and Hb and infant factors: gestation, birth-weight and intra-uterine growth were included in the Cox regression proportional hazards model (table 8.8). Child HIV status was not included because of the small number of children.

Maternal HIV infection, primigravidae were independently associated with reduced probability of survival, whereas the risk was increased with maternal age less than 20 and post-partum Hb less than 11g/dl (table 8.9). The mothers of 10 of the 25 children who died were primigravidae and 5 of their children died in the neonatal period.

Table 8.7 Univariate analysis of risk factors for infant mortality

Variable	*Number of cases*	*Censored*	*Number of deaths*	*Log rank statistic (P value)*
Maternal HIV				
Positive	53	119	11	
Negative	130	39	14	10.75 (0.001)
Maternal age (years)				
<20	25	23	2	
20-34	142	120	22	
≥35	14	13	1	1.56 (0.46)
Gravidae				
1	50	40	10	
>1	132	117	15	2.2 (0.14)
Parity				
1	56	46	10	
>1	126	111	15	1.09 (0.3)
CD4 percent				
<15	17	15	2	
15-24	32	4	4	
≥25	148	129	19	0.1 (1.0)
Antenatal Hb (g/dl)				
<11	14	13	1	
≥11	18	15	3	0.70 (0.40)
Postpartum Hb (g/dl)				
<11	94	84	10	
≥11	88	73	15	1.57 (0.21)
Post-partum maternal serum retinol (μmol/L)				
<0.7	70	57	13	
≥1.05	111	99	12	2.26 (0.14)
Antenatal RPR				
Positive	22	18	4	
Negative	108	91	17	0.9 (0.760)
Postpartum RPR				
Positive	21	18	3	
Negative	162	140	22	0 (0.94)
Birth weight (kg)				
<2.5	46	39	7	
≥2.5	136	118	18	0.10 (0.75)
Gestation				
<37	48	40	8	
≥37	134	117	17	0.42 (0.52)
IUGR				
<10th Centile	49	44	5	
≥10th Centile	134	114	25	0.60 (0.44)

IUGR intr-uterine growth retardation

Table 8.8 Variables included in Cox regression proportional hazards model

Variable	*Indicator*
Maternal factors:	
HIV infection	Positive HIV
Age	< 20 years
Gravidae	Primigravidae
Post-partum serum retinol	<1.05μmol/L
Post-partum CD4 percentage	<200
Antenatal RPR	Positive RPR
Post partum Hb	<11g/dl
Infant factors:	
Gestation	Pre-term
Birth-weight	<2.5kg
Intra-uterine growth	<10th centile

RPR rapid plasma reagin

Table 8.9 ***Proportional Hazards ratio of infant mortality (Multivariate analysis)***

Variable	*Proportional hazards ratio (95% CI)*	*P value*
Maternal HIV infection	0.28 (0.11-0.68)	0.005
Maternal age <20 years	9.7 (1.71-54.96)	0.0103
Primigravidae	0.20 (0.07-0.63)	0.0056
Post-partum maternal Hb< 11g/dl	3.17 (1.16-8.64)	0.0242

4) **Causes of mortality**

The causes of mortality were septicaemia, pneumonia, diarrhoea and failure to thrive. Table 8.10 shows the causes of mortality according maternal and child HIV status categories. Pneumonia (44%) was the main cause of mortality, affecting mainly children born to HIV positive mothers. Septicaemia (36%) was the second commonest cause of mortality. Eight of the 9 septicaemia deaths occurred in the neonatal period, 5 of which were perinatal deaths. Three of the neonates who died perinatally were HIV PCR negative at birth but the status was unknown at the time of death. The remaining 2 perinatal deaths were in neonates of HIV non-infected mothers.

Table 8.10 Causes of infant mortality and HIV status

Disease	*M -C -*[1]	*M +C+*[2]	*M + CI*[3]	*Total (%)*
Pneumonia	4	4	3	11 (44)
Septicaemia	5	0	4	9 (36)
Diarrhoea	2	1	1	4 (16)
Failure to thrive	0	1	0	1 (4)

[1] Mother negative-child negative [2] Mother positive-child positive [3] Mother positive-child indeterminate

8.4 DISCUSSION

8.4.1 Background and morbidity

For evaluation of growth patterns Z-scores were used in order to compare deviations across different ages as well as growth parameters using a uniform reference median

and cut-off points. Medians and inter-quartile ranges rather than means and standard errors were calculated for our sample because of the small numbers in some of the categories, with the loss of normality in the measurements especially in the later months of follow up.

Three child-mother group categories were defined to compare Z-scores: Child negative –mother negative, group 1; child positive-mother positive, group 2; child negative-mother positive, group 3. Maternal CD4 cell count and percentage were lower in group 2, where only the mother was HIV infected. Post-partum Hb was associated with group 3 and positive antenatal RPR in group 2. Although these factors might cause poor growth in children, they were not adjusted for in the analysis because of sample size restriction.

Overall morbidity was defined as reported treatments for illness in the first year of life. It was not part of the analysis of this chapter to define morbidity more explicitly because of limitations in diagnostic capacity such as laboratory and radiology facilities. The finding that the number of reported treatments in the first year of life was similar across the 3 groups is not surprising. The impact of HIV infection on morbidity was probably masked by the high prevalence of reported morbidity (≥ 50% after 1 month of age) and HIV related mortality. HIV infected children usually present with spectrum of clinical syndromes similar to HIV uninfected children, although chronic symptoms and failure to thrive are more common (Chintu et al 1945; Vetter et al 1996; Marum et al 1997; Spira et al 1999).

In Abdijan, a prevalence study in 1992 among children, aged 1 month to 15 years, malnutrition and ARI were associated with sero-positivity amongst hospitalised

children (Vetter et al 1996). At UTH in Lusaka, Zambia, in sero-positive between 6 months and 59 months, the two leading causes of admission were malnutrition and pneumonia.

8.4.2 Growth patterns

Whilst malnutrition and wasting are characteristic of symptomatic HIV infection in adults, it is not clear whether the same phenomena exists in children. Similar to previous findings by Lepage et al (1996) in Rwanda and Bailey et al (1999) in the Democratic Republic of Congo (DR Congo), the current study demonstrated sustained growth impairment in weight, height and weight for height, with HIV infected children being more affected (figure 8.1, 8.2 & 8.3).

In Durham in North Carolina, Mckinney et al (1995) found that by 24 months HIV infected children were abnormally lean and wasted. They were proportionately smaller both in weight and height, making them normal weight for height. A cohort study by Saveedra et al (1995) of 59 HIV infected children and 50 uninfected children from birth to 70 months in Baltimore Maryland, had similar results. In a longitudinal study over 4 years, the European Collaborative Study Group (1994) found that infected children were on average 6% lighter and 2% shorter.

Although the median weight for age Z-scores, in the current study, progressively increased to above the reference median in the first 4 months of life, there was subsequently a steady decline observed till 12 months of age (figure 8.1). The pattern was more severe in HIV infected children, in whom by 12 months the median weight for age Z-score was < -3, the range for severe stunting.

The observed catch-up growth in weight in the first 4 months was probably the influence of breast-feeding and the faltering thereafter could have been due to inadequate supplementary feeding, reflecting the typical weaning effect on growth at the time of introduction of supplemental feeds. Another contributory factor, in the current study, could have been illness as reported morbidity the also increased sharply after 4 months (table 8.2).

The observed weight patterns are similar to other African urban and rural communities (Bailey et al 1999), with the rural poor being more affected. In a cross-sectional survey in rural Zambia, Hautvast (2000) found that the mean weight for age *Z*- scores in breastfeeding children of unknown HIV status were constantly below the NCHS reference median from 1 to 12 months of age. The children had no initial catch-up growth and were within the under-nutrition range by10 months of age.

The children in the current study had severe impairment in linear growth. Although, at birth, the median height Z-score was above the reference, after birth, there was a persistent decline in Z-scores in all 3 groups, HIV infected children being most affected. By 7 months HIV infected children were severely stunted with median weight for height *Z* score being < -3.

The stunting observed in the first 4 months did not reduce total body mass, as the weight for height Z-scores increased linearly during this time due to catch-up in weight (figure 8.3). After 4 months growth in both weight and height declined markedly although growth was proportionate, with normal weight for height values at 12 months.

Waterlow (1988) in his review of growth patterns study cohorts in developing countries examined length increments over 3 monthly periods. He concluded that in developing

countries linear growth begins to fall off after the third month of life after supplementary feeding is introduced. The average increments in length remain low at about 80% of the reference until the end of the second year and then there is a suggestion of catch-up. For public health purposes, Waterlow suggested a cut off in deficit of linear growth increment, of 65% of the reference median would result in significant stunting beyond infancy.

The pathogenesis of malnutrition in HIV infected children is not clear but is likely to be influenced by many factors acting synergistically. Inadequate oral intake has yet to be documented in children especially in association with acute infections although Miller et al reported that nutrient intake is both similar and adequate for growth in HIV infected and non-infected children (1991). Investigations in adults have suggested that the resting energy expenditure of HIV infected patients, including those without symptoms may be greater than that of control subjects (Melchior et al 1993). It is clear that malabsorption exists, although a strong correlation between malabsorption and growth problems has not been found (Miller et al 1991; Grunfeld et al 1992; Kelly et al 1999). Two children with demonstrable growth hormone deficiency were treated with growth hormone and increased growth velocity was observed in one of the patients (Jospe & Powell 1990; Laue et al 1990), suggesting that hormonal factors could also play a role.

There is some evidence to suggest that poor growth precedes and may contribute to the onset of immuno-deficiency and opportunistic infection (Saavedra et al 1995). Growth failure preceded the diagnosis of AIDS by a median of 12 months in 9 children with haemophilia and HIV infection (Brettler et al 1990). Adult data indicates that loss of

lean body mass is strongly predictive of death in HIV infection (Kotler et al 1989). Thus routine evaluation of body composition may be another indicator that programme managers and clinicians can use to determine eligibility for and response to interventions.

8.4.2 Infant mortality

Studies focusing on mortality differences between infected and uninfected children are difficult to conduct in Africa. Our study, although contributing to mortality data in Africa, had limitations that have a bearing on the interpretation of the results. The initial sample size of 300 mother-child pairs was small for mortality evaluation. Only 92 of the mothers were HIV infected and 18 passed on the infection to their babies. In addition there was high level of loss to follow up, both amongst children born to HIV infected and uninfected mothers. A significant proportion of children died in the neonatal period without a definate HIV diagnosis and could not be included as HIV infected cases according to the classification by the Ghent Working Group (Dabis *et al* 1992). These limitations restricted stratifying children by HIV status in the risk factor analysis for survival probability.

Our estimated infant mortality of 136 per1000 live births is higher than that of the 1996 DHS (109/1000) and is probably related to sampling bias rather than a true estimate. The children were born at UTH, a referral hospital dealing with high-risk pregnancies. The women that deliver in Lusaka also have higher HIV prevalence than rural areas (MOH/CBoH 1999). The estimated infant mortality for uninfected infants born to HIV

infected and non-infected mothers was 272 and 85 per 1000 thousand and when the child was also infected mortality rose to 454 per 1000.

The population attributable risk percentage from this data can be calculated as shown in the table 8.11. 41.3% and 24.9% of deaths in infants born at UTH in Lusaka can be attributed to HIV infection in the mother and child respectively. It is estimated, therefore, that out of the 12,000 infants born at UTH, 13.6% will not reach their first birthday. HIV infection in the mother and the child is responsible for 41.3% and 24.9% of the mortality respectively.

Table 8.11 Population attributable risk percentage

	Children of HIV infected mothers	
	HIV infected	***HIV infected and non-infected***
% children in population (Pe)	10%	30%
Infant mortality rate (Ie)	424/1000	272/1000
Attributable risk (AR)	339/1000	187/1000
Population attributable risk (PAR)	33.9	56.1
Population attributable risk % (PAR%)	24.9%	41.3%

MTCT rate: 31%

Infant mortality rate (IMR): 136/1000 live births

Infant mortality rate in children of HIV uninfected mothers: 85/1000 (Io)

AR=Ie-Io ; PAR=(AR) Pe; PAR%= PAR(100)/IMR

Prospective cohort studies in Africa have yielded variable infant mortality estimates in HIV infected children. In Rwanda, the estimated risk of death at one year of age was 260 per 1000 among infected children and 20 per 100 among uninfected children born to HIV positive mothers (Spira et al 1999). The risk in uninfected children did not differ from the risk of death at one year among uninfected children born to HIV non-infected

mothers. In cohorts in Uganda and South Africa, estimated risk of infant death were 330 and 350 per 1000 (Lepage 1998), compared to infant mortality rates of 107 and 59 per 1000 respectively in the general population. Recent results from the zidovudine intervention trials in Abidjan, Cote d'Ivoire and Bobo Dioulasso, Burkina Faso indicated that death at 1 year was 531 per 1000 among infected children whose mothers received peripartum zidovudine and 492 per 1000 among infected children whose mothers received placebo (Dabis et al 1999). The mortality was 44 and 58 per 1000 in Abidjan and Bobo Dioulasso in uninfected children.

These high mortality rates reaffirm the need for developing comprehensive programmes aiming at reducing the impact of HIV on children in Africa. Although the reduction of HIV MTCT remains an important issue in Africa, the organisation of paediatric care services deserves special attention if improved child survival is to be achieved. The causes of infant mortality in this study were pneumonia, septicaemia, diarrhoea and failure to thrive. Similar results have been reported in other previous African studies (Lepage et al 1998; Bobat et al 1999). Death from septicaemia was common in our study and most of these children died in the neonatal period, with equal distribution between children born of HIV non-infected and infected mothers. This finding is important as septicaemia can be prevented with simple and cheap interventions such as chlorhexidine vaginal cleansing (Biggar et al 1996). However, the suitability of the definition of septicaemia for neonates was limited and could easily be confused with other diseases.

After adjusting for confounding factors, maternal HIV infection and first pregnancy, independently accounted for reduced probability of survival in infancy. First

pregnancies are classified as high-risk pregnancy and might be associated pregnancy related complications such as pre-eclampsia. Surprisingly, post-partum anaemia and maternal age less than 20 years on the other hand increased the probability of survival. This association should be evaluated further. Possible explanations could be better follow care with recognition of post-partum anaemia and high loss to follow up in the young mothers below 20.

8.5 CONCLUSION

Growth faltering is a problem in HIV infected children, with the most impact being on linear growth. Although reported infant morbidity does not seem to be affected by HIV infection, infected children are more likely to die. The increase in infant mortality is also observed in HIV uninfected children of infected mothers. Apart from HIV infection, infant mortality is also associated with primigravidae. There is need for further evaluation of the interaction between post-partum anaemia and young maternal age and infant mortality.

CHAPTER 9

SUMMARY AND CONCLUSIONS

A prospective study at the University Teaching Hospital of 306 mothers and infants, enrolled at the time of delivery, was conducted in 1997. The primary aim was to define the magnitude and effects of maternal HIV infection on maternal morbidity and infant outcome. The thesis was divided into 5 broad themes: The characteristics of the women; the magnitude and risk factors for post-partum anaemia; prevalence and risk factors for adverse birth outcomes; characteristics of HIV mother to child transmission (MTCT) and infant growth patterns, morbidity and mortality.

9.1 STUDY SAMPLE

The women were mainly over 19 years (87.3%), literate (73.7%) and married (91.4%), with no formal income (75.7%). Although the women appeared to be of fair nutritional status, in terms of post-partum (PP) weight, mid-upper arm circumference (MUAC) and vitamin A status, 48.2% and 46.7% had antenatal or PP anaemia (PPA) and of these 1.8% and 6.2% were severely anaemic. Common problems were malaria treatment during pregnancy (32.6%), previous child death (32.4%), previous abortion (16.4%) and hypertension in pregnancy (13.7%).

The main limitation of the study is the small sample size, missing values and the high number of women lost to follow up. These limitations are, likely to influence the generalisability of the results and also to mask small associations. This concern is discussed in the individual chapters and an attempt has been made compare the characteristics of those lost and not lost follow up.

9.2 MATERNAL HIV, SYPHILIS AND HEPATITIS B INFECTION

The results demonstrate that maternal HIV infection is major health problem in Lusaka. With the 30.1% prevalence of maternal HIV infection found in this study, it is estimated that 3,612 of the 12,000 annual deliveries at UTH occur in HIV infected women.

In addition to HIV, the women were also exposed to other sexually transmitted diseases (STDs), syphilis and hepatitis B. The prevalence rates of post-partum RPR and HBsAg positivity were 10.1% and 4.5%. Although these estimates were lower than that of HIV infection, an important finding in this study was the evidence of exposure to these STDs during pregnancy. Of RPR negative women at antenatal screening, 4.7% were RPR positive on repeat testing post-partum. These women would have been missed during routine RPR screening, as normally this is done only at the first antenatal visit.

These findings have programmatic implications. They highlight the importance of targeted primary prevention of HIV in married pregnant women through effective voluntary counselling and testing models that integrate couple counselling with condom promotion in antenatal clinics (Allen et al 1999). The package of care for pregnant women should aim to reduce the risk of exposure both to HIV and other STDs in negative women.

9.3 RISK FACTORS FOR MATERNAL HIV INFECTION

The association of low PP CD4 cell count (RR 10.63), PPA (RR 3.99), HBsAg carriage (RR 27.45), ante-partum haemorrhage (RR 5.89) and alcohol intake (RR 5.67) with maternal HIV infection raises important research, preventive, and care and support questions in Zambia.

The interaction between alcohol intake and maternal HIV infection presents an interesting paradigm. Does alcohol consumption influence behaviour, putting the women at risk of HIV infection or is alcohol consumption a marker of other social factors associated with HIV infection? Although the association of HIV infection with alcohol has been previously reported (Boerma *et al* 1999; Morrison *et al* 1997; Kapiga *et al* 1998; Mnyika *et al* 1996), there appears to be no evidence of possible successful intervention approaches.

The association of maternal HIV infection with low CD4 cell count in a population of HIV women who had a low prevalence of opportunistic infections, such as tuberculosis (5.3%), is an indication that asymptomatic women are still at high risk of HIV disease progression. These women need to be monitored and screened for opportunistic infections.

The need for detection and management of anaemia as a continuum to cover the antenatal, post-partum and post-natal period is highlighted by the findings of this study. The prevalence of anaemia in the antenatal period and post partum was 48.2% and 49.7%. The prevalence of PPA was significantly higher in HIV infected (60.9%) than non-infected women (40.7%). Monitoring and management of anaemia is particularly essential in pregnant women on anti-retroviral therapy preventive therapy. Very few

women had antenatal anaemia screening (18.3%) probable due to lack of screening facilities.

The cost effectiveness of vaccinating HIV exposed babies with hepatitis B vaccine at birth requires discussion in Zambia. The evaluation of hepatitis B in this study was limited to HBsAg screening of mothers, not their infants. A previous study in Lusaka showed a higher prevalence of hepatitis B e antigen in HIV infected women, although this association was not confirmed in a study in Malawi (Oshitani et al 1996; Ahmed et al 1998). A more comprehensive study of hepatitis B in pregnancy including anti HBc antibody, and HBeAg would be important in defining exposure and risk of transmission to the infant.

The association of maternal HIV infection with ante-partum haemorrhage suggests that maternal HIV impacts on common obstetric emergencies. Although this association has been reported previously (Mcintyre 1993; Braddick et al 1990), the reason for this association is unclear. However, the data suggests that consideration of HIV infection is important in obstetric management guidelines. One recommendation could be to offer HIV counselling and testing to all women with ante-partum haemorrhage.

Primigavidae (RR 0.3) was independently associated with lower risk of HIV infection after allowing for the confounding effect of other factors, such as age. HIV prevalence in primiparous women should approximate to HIV incidence because these younger women would have only recently become sexually active. Targeting and reinforcing HIV prevention efforts in primiparous women through antenatal clinics, such as couple counselling, will help ensure the women remain negative during their reproductive

years. This, however, should not undermine preventive efforts before pregnancy, before women become sexually active.

9.4 POSTPARTUM ANAEMIA AND HIV INFECTION

The association of PPA with caesarean section (RR 9.95) has serious implications concerning recommending caesarean section for prevention of HIV MTCT in Zambia. Due to the high HIV prevalence in adults in Zambia, blood transfusions are limited to symptomatic anaemic patients in a few hospitals with HIV screening facilities. Blood transfusions are not regularly used or encouraged during caesarean section and blood loss in these women is complicated by post-partum anaemia.

This study showed that PPA was associated with post-natal anaemia (RR 1.98). An association of anaemia with HIV disease progression and mortality in adults has also been previously reported (Mocroft et al 1999). Screening for PPA provides an opportunity for identifying women in need of closer post-natal monitoring and support. In Zambia, where monitoring CD4 cell count is not affordable, identifying anaemia during pregnancy and post-partum could help identify those women at risk of disease progression.

Post-partum anaemia was associated with vitamin A deficiency, another potential cause of morbidity in pregnancy, in low resource settings (Huffman et al 1998). This finding demonstrates the complexity of pregnancy related morbidity. It highlights the need for micronutrient supplementation in pregnant and lactating women, ideally within a comprehensive package of care for prevention of MTCT and Safe Motherhood.

9.5 HIV INFECTION AND BIRTH OUTCOMES

This is the first study in Zambia to provide data on the interaction of HIV infection with pre-term delivery, low birth weight (LBW) and intra-uterine growth retardation (IUGR). The prevalence estimates of these outcomes in this population were high, 23.8% pre-term delivery, 18.9% LBW and 25.9% IUGR. The study, however, failed to demonstrate any association of maternal HIV infection with pre-term delivery, LBW or IUGR, although mean birth weight was significantly lower in babies of HIV infected mothers.

In HIV uninfected women, antenatal anaemia was independently associated with pre-term delivery and LBW, and post-partum vitamin A deficiency with IUGR. These results re-affirm the problem of nutritional deficiencies in pregnancy.

Factors independently associated with poor birth outcomes in HIV infected mothers were, lack of paternal income for pre-term delivery (RR 11.7), IUGR for LBW (RR 3.59) and history of antibiotic treatment in pregnancy for IUGR (RR 5.85). The reasons for the relationship between lack of paternal income and pre-term delivery were not studied but could be related to the soci-economic status of the women. These women require psychosocial support and close monitoring during pregnancy.

Although IUGR was associated with LBW, only one tenth of the children were asymmetrically growth retarded, meaning the majority of the IUGR in HIV infected children had chronic exposure to the insult. The association of IUGR with antibiotic treatment during pregnancy, further suggests that the chronic insult might be chronic infection.

9.6 MOTHER TO CHILD TRANSMISSION OF HIV

The HIV MTCT rate was 31%. Out the 3,612 children born to HIV infected women at UTH annually, 1,120 are HIV infected. Approximately one tenth (9.3%) of the annual births at UTH, are HIV infected.

The timing of detection of HIV infection was 38.9% at birth, 38.9% at 1 month and 22.2% between 4 and 12 months of age. These proportions correspond to in utero, intrapartum or early breast-feeding and late breast-feeding transmission. Since the majority of infections are detected at birth and the early post-natal period, targeting and concentrating anti-retrovirals to late pregnancy and this period might prevent up to 77.8% of the paediatric HIV infections in this population. Biological efficacy, service utilisation, acceptance of and adherence to the intervention and breast-feeding patterns, however, will influence the impact of the interventions at population level.

Previous studies in Zambia have not examined the effect of biological factors such as high maternal HIV viral load, low CD4 cell count or percentage, low serum retinol and anaemia on MTCT. This study showed that maternal viral load was the only factor associated with MTCT after controlling for confounding variables. Anti-retroviral therapy for pregnant mothers to reduce maternal HIV viral load is, therefore, recommended for prevention of MTCT and these drugs are urgently needed in Zambia.

9.7 HIV INFECTION AND INFANT GROWTH

The study suggests that HIV infected children have marked growth impairment, the deficit in linear growth being more marked. The median weight and height Z-scores were below –2 and –3 at 12 months of age respectively. The children initially showed a

progressive increase in weight up to 4 months of age, but this was followed by a continuous decline through to 12 months. For linear growth, a steady decline was observed from birth and by 7 months the children were severely stunted. Stunting observed in the first 4 months did not reduce total body mass. Instead, an increase in weight for height Z-scores was observed in the first 4 months secondary to catch up in weight. After 4 months, weight for height Z-scores were normal, as both weight and height proportionately declined.

9.8 MORBIDITY AND MORTALITY IN INFANCY

Morbidity patterns were similar in HIV infected and non-infected children but mortality was much higher when the child was infected. The total infant mortality was estimated at 136 per 1000 live births. In HIV uninfected children born to uninfected mothers, the infant mortality rate was 85 per 1000 live births. This estimate rose over 3-fold (272 per 1000) in HIV uninfected infants of infected mothers and 5-fold (424 per 1000) in infected infants. Accordingly, 41.3% or 24.9% of infant mortality in children born at UTH could be attributed to maternal HIV infection or infection in infants respectively. With the high level of deaths associated with HIV infection in infants, there is need for developing care and support guidelines for children born to HIV infected mothers in order to improve their outcome. The main cause of mortality in HIV infected children was pneumonia and although the causes of pneumonia were not sought in this study, this finding raises two important programmatic questions. Should infants receive antibiotic chemoprophylaxis for *Pneumocystis carinii* pneumonia and other common causes of pneumonia in Zambia? Would immunisation of infants with the new

pneumococcal conjugate vaccines be a useful strategy? These questions require testing in intervention trials.

The lower infant mortality in babies of younger mothers (< 20 years) and those with PPA is difficult to explain. One explanation could be the impact of the Safe Motherhood Programme which classifies pregnancy in an adolescent as a high-risk pregnancy at antenatal booking and consequently all cases are referred to UTH for closer monitoring. Similarly anaemic women could have been followed up more closely, especially following caesarean section. It is also possible that in the young mothers, the high rate of loss to follow up could have biased the results.

9.9 IMPLICATIONS FOR FURTHER RESEARCH

1) Maternal HIV infection

Maternal HIV infection is a major problem in women. Research on primary prevention of maternal HIV infection should include:

(a) Intervention studies targeting HIV non-infected women with an aim to maintain their negative status. Interventions to be assessed should include: Assessing the impact of health education in antenatal clinics using different methods (wall charts, leaflets, group talks and discussion, audio-visual methods); counselling women alone versus couple counselling with or wothout condom promotion; STD screening with active or passive contact tracing contact tracing. These interventions could be assessed individually or in combination.

(b) There is need for an in-depth community rapid appraisal of factors influencing sexual behavioural patterns and uptake of interventions at the household and

community level and qualitative studies using participatory approaches to gain insight into interventions that are acceptable at community level.

2) Hepatitis B

A comprehensive study on MTCT of hepatitis B including an economic evaluation of post-partum infant immunisation should be undertaken.

3) Anaemia

Post-partum anaemia was associated with maternal HIV infection, caesarean section and post-natal anaemia. Further studies should include:

(a) Evaluation of PPA as tool for assessing HIV disease progression in infected mothers

(b) Evaluation of different approches of providing iron, folate and micronutrient supplementation post-partum and postnally in HIV infected and non-infected women

(c) Longitudinal assessment of impact of malaria in pregnancy in an urban setting with high HIV prevalence.

(d) Evaluation of indications and risk factors for caesarean section including blood transfusion patterns and clinical outcome

(e) Assessment of impact of post-partum and post-natal micronutrient supplemention in lactating mothers on anaemia and HIV disease progression.

4) Care issues

Infant mortality in HIV infected and non-infected children was extremely high. Many of the causes of mortality are preventable and mortality could be reduced with appropriate interventions. Further research questions should include;

(a) Evaluation of health seeking behaviours by the care givers

(b) Assessment of service delivery including referral systems and quality of care

(c) Development and implementation of guidelines that include HIV related morbidity. The current Integrated Management of Childhood Illnesses should be modified to include HIV related morbidity.

(d) Evaluation of efficacy of antibiotic preventive therapy in infancy

(e) Evaluation of efficacy of pneumococcal conjugate vaccine in infancy

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APPENDIX 3.1
MOTHER TO CHILD TRANSMISSION STUDY

QUESTIONNAIRE

Date [ ][ ][ ][ ][ ][ ] Study Number [ ][ ][ ][ ]

Interviewer [ ] Consent given (Y/N) [ ]

Mothers Name_____

Fathers Name _____

Address/Landmark: _____

_____

Fathers/Mothers Occupation: _____

Place of Work: (Mother/Father):

MATERNAL INFORMATION:

Maternal Age (years):

[ ][ ] 99 if NK

≤ 18 [ ]
19-34 [ ]
≥ 35 [ ]

Marital Status:

Married [m]
Widowed [w]
Single [s]

If married how long? [ ] years [ ] months

Is this your first husband? [ ] (Y/N)

If widowed how long ago did your husband die? [ ][ ]

Reason for death _____

Education Status:

illiterate [1]
literate [2]

Occupation

Office [1]
market/business [2]
houseboy (girl) [3]
[4]

of mother

[1]
[2]
[3]
[4]

of father

[1]
[2]
[3]
[4]

Do you own:

House 1
Car 2
Television 3
Bicycle 4
Radio 5
None 6

farmer

none 5 5

In the last 2 days how often have you had:

Meat Fish Chicken Green Veg. Fruit (mango/orange/pawpaw)

PREVIOUS OBSTETRIC HISTORY:

Gravida Para Preterm Labour StillBirth

Abortions

Number of children alive Number of children dead

MEDICAL HISTORY DURING PREGNANCY:

	Tb	hypertension	APH/Abruptio	Malaria	Other Infection	Syphilis Ab test (Y/N)
(Y/N)						

Results of syphilis test P N

If positive Rx received (No. of doses) 0 1 2 3

If other infection specify:

Smoke (Y/N)

LMP

Gestation (weeks)

EDD

Alcohol (Y/N) ☐

PROM: <4hrs ☐ 4-24hrs ☐ >24 hrs ☐

Mode of delivery:

SVD	1
Breech	2
Instrumental	3
C/S (elect)	4
C/S (emerg)	5

MATERNAL EXAMINATION

Post delivery weight (kgs) ☐☐

MUAC (cms) ☐☐☐

AIDS (Y/N) ☐

Hb (g/dl) (if done) ☐☐☐

ACTION TAKEN:

Blood for (Serology, PCR,CD4 HbsAb, RPR) (5MLS EDTA) ☐

Blood for Vit A (2MLS PLAIN) ☐

Colostrum for Vit A (PLAIN 1ML) ☐

INFANT RECRUITMENT

Age (hrs) ☐☐

Birth-weight ☐☐☐☐

Length (cm) ☐☐

Length (cms) ☐☐☐

Gestation

ACTION TAKEN
(Y/N)
Blood for PCR (2ML EDTA) ☐

APPENDIX 3.2

FOLLOW-UP FORM FOR MOTHERS AND THEIR CHILDREN

Date: _____/_____/_____ Study Number _____ Interviewer _____

Mothers Name : _____

Childs Name: _____

Fathers Name: _____

Address/Landmark: _____

Father/Mother's Occupation:_____ Tel:_____

Place of Work: (Mother/Father):_____

MATERNAL INFORMATION

Has the mother come?: (Yes_____/ No_____);

If no, the reason for the absence of the mother:_____

Maternal Age: (Not known_____/ Known_____) _____ years old

BREAST FEEDING HISTORY

Is the child currently breastfeeding?: (Yes_____/ No_____/ Unknown_____)

Exclusively breastfed (Yes_____/No_____/ Unknown_____)

If no, when stopped _____ months

Supplementary feed introduced at _____ months

History of sores/abscess on breast since last seen: (Yes_____/ No_____)

MATERNAL MEDICAL HISTORY IN THE LAST 3 MONTHS

Unwell since delivery? (Yes_____/ No_____)

If unwell, how many times? (Yes_____/ No_____)

Where were you treated?: (1. Hospital_____/ 2.Health Centre _____/

3. Private Clinic_____/ 4. Herbalist _____/ 5. Home _____)

If hospitalised how many times: _____

Treatment Received: (1. Oral_____/ 2. Injection_____/ 3.

Oral/Injection_____)

Specify treatment: _____

ILLNESS SINCE LAST SEEN

Recurrent fever of at least one month's duration: (Yes_____/ No_____)

Recurrent oropharyngeal candidiasis: (Yes_____/ No_____)

Recurrent respiratory infections (pulmonary infection, pneumonia, otitis media): (Yes_____/ No_____)

Chronic diarrhoea of at least one months duration: (Yes_____/ No_____)

Weight loss: (Yes_____/ No_____)

Persistent cough of at least one month's duration: (Yes_____/ No_____)

Tuberculosis: (Unknown_____/ Yes_____? No_____)

Pneumonia: (Yes_____/ No_____)

Meningitis: (Yes_____/ No_____)

Other illness: (Yes_____/ No_____)

If other illness, specify: _____

MATERNAL MEDICAL EXAMINATION

Weight: _____kg Mid arm circumference: _____ cm

Well? (Yes____/ No_____) If unwell, specify problems: _____

Generalised lymphadenopathy (more than 2 sites, more than 5 cm): (Yes___/No ____)

Skin lesions of Kaposi's sarcoma: (Yes_____/ No_____)

Non resolving herpes simplex Yes_____/ No_____)

Focal neurological signs (Yes_____/ No_____)

Dementia (Yes_____/ No_____)

SOCIAL SITUATION

Marital status: (Married_____/ Separated_____/ Widowed _____/Single_____)

If married how long?: _____ years _____months

Is this your first husband?: (Yes_____/ No_____)

If widowed how long ago did your husband die?: _____ years

Reason for death of husband: _____

Occupation of mother: (1. Office _____/ 2. Market or business _____/

3. Housegirl_____/4. Farmer_____/5. Housewife_____/ 6. None_____)

Occupation of father: (1. Office _____/ 2. Market or business _____/

3. Houseboy_____/ 4. Farmer_____/ 5. None_____)

In the last 2 days have you had: (1.Meat_____/ 2.Fish_____/

3. Nshima_____/Beans_____/ 5. Green Veg._____/ 6. Fruit_____)

Number of children alive: _____ Number of children dead: _____

ACTION TAKEN: Blood for Serology, CD4, Viral load (5ml, EDTA): _____

CHILD INFORMATION

Date:_____/_____/_____ Study Number:_____

Age: _____ months old Sex: (Male_____/ Female_____)

CHILD MEDICAL HISTORY SINCE LAST SEEN

Did your child have any illness since last seen? (Yes_____/ No_____)

If yes, how many times? _____

Where was the child treated?: (1. Hospital_____/ 2.Health Centre _____/

3. Private Clinic_____/ 4. Herbalist _____/ 5. Home _____)

How many times have you hospitalised since last seen?:_____

Is there any following history?:

1. Blood/blood products transfusion (Yes_____/ No_____)
2. Injection (Yes_____/ No_____)
3. Traditional scarifications (Yes_____/ No_____)
4. Dental treatment (Yes_____/ No_____)
5. Surgical procedures (Yes_____/ No_____)
6. I.V. medications/infusion (Yes_____/ No_____)

ILLNESS HISTORY IN BABY

Recurrent fever: (Yes_____/ No_____)

Recurrent oropharyngeal candidiasis: (Yes_____/ No_____)

Recurrent respiratory infections (pulmonary infection, pneumonia, otitis media):

(Yes_____/ No_____)

Chronic diarrhoea: (Yes_____/ No_____)

Weight loss or abnormally slow growth: (Yes_____/ No_____)

Persistent cough of at least one month's duration: (Yes_____/ No_____)

Tuberculosis: (Unknown_____/ Yes_____/ No_____)

Pneumonia: (Yes_____/ No_____)

Meningitis: (Yes_____/ No_____)

How many times have you hospitalised since last seen?:_____

Other illness: (Yes_____/ No_____)

If other illness, specify: _____

CHILD MEDICAL EXAMINATION

Weight: _____g Height: _____cm
Occipito-frontal circumference: _____ cm
Nutrition:
(1. Normal_____/ 2. Underweight _____/ 3. Protein-energy malnutrition_____)
Generalised lymphadenopathy (more than 2 sites, more than 5 cm): (Yes___/ No_____)
Skin lesion of Kaposi's sarcoma (Yes___/ No_____)
Non resolving herpes simplex (Yes___/ No_____)
Focal neurological signs (Yes___/ No_____)
Mental retardation (Yes___/ No_____)
HISTORY OF VIRAL INFECTION:
1. Measles (Yes____/ No_____)
2. Mumps (Yes____/ No_____)
3. Chicken pox (Yes____/ No_____)
4. Other (Yes____/ No_____)
If other viral infection, specify _____

IMMUNIZATION HISTORY
Source: 1. Children's clinic card (CCC)_____/ 2. Mother's recall_____/
3. Unknown_____)
BCG (Yes_____/ No_____/ Unknown _____)
BCG Scar (Present_____/ Absent_____)

ACTION TAKEN
Blood for PCR, Serology (3 ml, EDTA) _____
Blood for Vitamin A (2 ml, Plain): _____
Date..... Investigator.....

Appendix 6.1 Factors associated with pre-term delivery

Variable	All mothers	HIV non-infected	HIV infected
	n/N (%)	n/N (%)	n/N (%)
Father has no income	11/16 (16.7	6/43 (14.0)	13/153 (8.5)
Maternal age < 20years	15/68 (22.1)	12/44 (27.3)	3/24 (12.5)
Primigravida	24/68 (36.8)	23/44 (52.3	2/22 98.3)
Primiparity	29/68 (42.6)	23/44 (52.3)	6/24 (25)
Previous f pre-term delivery	10/68 (14.7	6/44 (13.6)	4/24 (16.7)
Malaria treatment during pregnancy	25/62 (40.3)	13/38 (34.2)	12/24 (50)
Positive RPR during pregnancy	9/45 (20)	3/29 (10.3)	6/16 (37.5)
Positive post-partum RPR	9/74 (12.2)	4/50 (16.7)	5/50 (10)
H/o alcohol intake during pregnancy	2/64(3.1)	ND	2/21 (9.5)
Maternal weight < 45kg	8/63 (12.7)	5/41 (12.2)	3/22 (13.6)
MUAC <23cm	8/68 (11.8)	6/44 (13.6)	2/24 (8.3)
Antepartum haemorrhage	5/58 (8.6)	2/36 (5.6)	3/22 (13.6)
Antenatal maternal haemoglobin <11gm/dl	10/58 (76.9)	5/6 (83.3	5/7 (71.4)
Post-partum maternal haemoglobin (<11gm/dl)	37/68 (54.4)	19/44 (43.2)	18/24 (75)
Post-partum maternal vitamin A < 1.05 μmol/L	29/68 (42.6)	16/44 (36.4)	13/24 (54.2)
CD4 cell count <500 cells/mm	20/68 (30.9)	10/44 (22.7)	10/24 (41.6)
CD4% < 25	20/70 (27)	14/24 (58.3)	6/50 (12)
Intra-uterine growth retardation	6/68 (8.8)	1/44 (2.3)	5/24 (20.8)

Appendix 6.2 Factors associated with low birth weight

Variable	All mothers	HIV non-infected	HIV infected
	n/N	n/N	n/N
Father has no income	3/53 (9.4)	2/34 (5.9)	3/19 (15.8)
Maternal age < 20years	13/54 (24.1)	10/34 (29.4)	3/20 915)
Primigravida	18/54 (33.3)	16.34 (47.1)	2/20 (10)
Primiparity	21/54 (33.32)	16/34 947.10	5/20 (25)
Previous pre-term delivery	21/54 (38.9)	7/34 (20.6)	2/20 (25)
Malaria treatment during pregnancy	15/48 (31.3)	8/29 927.6)	7/19 (36.8)
Positive RPR during pregnancy	7/39 (17.9)	3/25 (12)	4/14 (28.6)
Positive post-partum RPR	9/54 (16.7)	3/34 (8.8)	4/14 (28.6)
H/o alcohol intake during pregnancy	4/52 (7.7)	8/29 (27.6)	7/19 (36.8)
Ante-partum haemorrhage	4/46 (8.7)	3/28 (10.7)	1/18 95.6)
Post-partum maternal weight < 45kg	5/50 (10)	2/33 (6.1)	7/17 (17.6)
MUAC< 23cm	6/54 (11.1)	4/34 (11.8)	2/20 (10)
Antenatal maternal haemoglobin <11gm/dl	8/11 (72.7)	4/5 (80)	4/6 (66.7)
Postnatal maternal haemoglobin <11gm/dl	26/54 (48.1)	14/34 (41.2)	12/20 (60)
Post-partum maternal vitamin A <0.7 μmol/L	28/53 (52.8)	17/33 (51.5)	11/20 (55)
CD4 cell count (cells/mm^3) <500	20/54 (37.1)	10/34 (29.4)	38/64 (59.4)

Appendix 6.3 Factors associated with intrauterine growth retardation

	All mothers	HIV non-infected	HIV infected
	n/N	n/N	n/N
Father earns no income	5/72	3/48	2/24
Maternal age < 20years	9/74	8/49	1/25
Primigravida	19/74	15/49	4/25
Primiparity	21/74	15/49	6/25
Previous H/o of pre-term delivery	5/74	4/49	1/25
H/o malaria treatment during pregnancy	22/65	16/44	6/21
Positive RPR during pregnancy	11/52	7/35	4/17
Positive post-partum RPR	11/74	6/49	5/25
H/o alcohol intake during pregnancy	5/72	3/49	2/23
Maternal weight < 45kg	3/69	ND	3/22
MUAC<23cm	6/74	5/49	1/25
Antepartum haemorrhage in pregnancy	4/62	2/41	2/29
Antenatal maternal haemoglobin <11gm/dl*	5/13	3/3	2/4
Post-partum maternal haemoglobin <11gm/dl	38/74	22/49	16/25
Post-partum maternal vitamin A < 0.7 umol/L	31/72	19/48	12/24
CD4 cell count <500 cells/mm	24/74	10/47	14/25

Prevention of mother to child transmission of HIV in Africa

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Summary

Mother to child transmission (MTCT) of HIV can be interrupted through prophylactic antiretroviral therapy (ARV), avoidance of breast-feeding and elective caesarian section. Studies in Africa using cheaper ARV regimens in breast-feeding HIV infected mothers have demonstrated reductions in MTCT of HIV.
The most recently published results are those of niverapine therapy, a cheap drug given as a single dose to an HIV positive woman at delivery, and to her baby within 72 hours of delivery. With all of the available options, what remains an urgent priority in Africa is the strengthening of reproductive health care systems and community mobilization to effect delivery of the interventions to save the many children infected with HIV every day.

Introduction

Although HIV/AIDS remains a problem globally, the epidemic is concentrated mainly in Africa and has the most devastating consequences in Sub Saharan Africa. The United Nations Joint Program on HIV/AIDS (UNAIDS) estimates that there are approximately 1 600 children infected with HIV everyday and that nearly 600 000 new infections occur every year. Ninety percent of these children are living in Africa.[1] Nearly all of the infections in children are acquired through mother to child transmission (MTCT) during pregnancy, labour and breast-feeding. HIV/AIDS, therefore, is slowing down or reversing the decline in infant and child mortality observed in the 1970s and early 1980s, with the hard won gains in immunization, breast-feeding promotion and the control of diarrhoeal diseases.[2,3]

Even although Africa possesses only some 10% of the world's population, compared with industrialized countries the gap in the incidence of paediatric HIV infection is growing at an alarming rate. This trend is not surprising because, apart from the lower HIV prevalence rates in pregnant women in industrialized countries, the HIV positive women do not breast-feed in these countries and they are also offered anti-retroviral therapy (ARV) during pregnancy. This paper is a review of literature of HIV infection in pregnancy, mode and risk of transmission and possible interventions. It is hoped that this information will be useful to both practising physicians and programme managers.

Antenatal Prevalence of HIV in Africa

About 50% of HIV infected adults in Africa are women, most of whom are of child bearing age.[1] The HIV pandemic mainly affected East and Central Africa and parts of West Africa in the late 1970s and 1980s and subsequently spread to Southern Africa and Ethiopia.[1] In most urban cities in Southern African, and Cote d'Ivoire in West Africa, antenatal HIV prevalence rates are over 10% as shown in Table I.[4]

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Prevention of mother to child transmission of HIV in Africa

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Table I. HIV prevalence in antenatal clinics

Country	City	Million Inhabitants	VCT Centres	Sample (N)	HIV Prevelance	Year
Burkina Faso	Dobo Dioullasso	0.4	1	4000	9.2	1999
Cote D'Ivoire	Abidjan	2.5	1	2500	14.0	1995
Kenya	Nairobi	2.0	6	1807	15.0	1996
Mombasa		0.5	2	200	12.5	1995
Malawi	Blantyre	0.4	2	814	30.0	1997
South Africa	Soweto	3.0	1	15000	18.3	1997
Durban		2.0	8	3351	27.0	1997
Tanzania	Dar-es-Salaam	3.0	4	3000	12.0	1994
Thailand	Bangkok	8.0	20	40000	2.3	1996
Zambia	Lusaka	1.5	10	595	27.5	1994
Zimbabwe	Harare	1.5	0	1800	28.0	1996

The infection rates are still rising. In Botswana, the proportion of adults living with HIV has doubled over the last 5 years with 43% of pregnant women in Francistown testing positive in 1997.[5] An estimated prevalence of 59% has been reported from Beit Bridge in Zimbabwe.[5] Nigeria, the most populated country in Africa, is threatened with an emerging HIV epidemic and with ever increasing numbers of HIV infected children. In Ethiopia there has been a rapid increase in the prevalence of HIV in young women.[6] In the Muslim areas of North Africa and the Middle East, HIV infection of women and children does not yet appear to be a major problem.[1]

Despite the high levels of HIV infection in Africa, some countries are reporting reduced prevalence rates in antenatal mothers. Uganda was one of the first countries in Africa to have an open attitude about HIV/AIDS and is the first to report a dropping prevalence of infection amongst pregnant women as a result of behavioural changes.[7]

Mode of Transmission

Transmission of HIV can occur in utero, at the time of labour, and post-natally, during breast-feeding. The current understanding is that the majority (70-80%) of the infection occurs around the time of delivery.[8-13]

Evidence of in utero transmission[14,15]

- The HIV virus has been isolated in foetal tissue such as trophoblastic and haematological cells.
- In some babies, the possibility of viral isolation at birth indicates evidence of viral replication in the baby before delivery.
- Some babies are sick soon after birth implying that, at birth, immuno-suppression has already occurred.

Evidence of transmission at the time of labour[16-19]

- The first born twin has a higher risk of HIV infection. This may be as a result of the first born cleaning off the birth canal for the second twin during the process of delivery.
- Babies born by elective caesarian section have lower infection rates.
- Prolonged rupture of membranes of over four hours is associated with higher transmission.
- Over 25% of the babies born to HIV positive mothers have undetectable HIV by PCR at birth because PCR can only detect the virus when there are enough viral particles. The infection acquired at birth, therefore, is unlikely to be detected by PCR soon after delivery.

Evidence of transmission during breast-feeding[20-22]

- HIV has been isolated in both the cell free and cellular portions of breast milk.
- The additional risk of HIV transmission attributable to breast-feeding in

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women with established HIV infection during pregnancy over and above the transmission acquired in utero and during delivery, is between 7% to 10%.

- If HIV infection is acquired during the period of breast-feeding, transmission of infection to the baby is higher and has been estimated to be about 30%.

Factors affecting transmission

Before ARV became the standard of care for pregnant women infected with HIV, the MTCT of HIV rates in industrialized countries, where few women breast fed, were between 15% and 20% in Western Europe and 20% and 25% in the United States.[23] With the advances in care, the transmission rate in industrialized countries is now under 8%. In Africa, where most woman breast-feed, and where there has been virtually no ARV available, various studies indicate MTCT transmission rates of between 25% to 40%.[23] In non-breast feeding women in Thailand and Brazil, the rates are reported to be 18% and 13% without ARV.[21,24] Apart from increased risk in breast feeding populations, various studies have shown that a number of factors play a role in MTCT of HIV (as shown in Table II).[25]

Table II: Risk factors associated with MTCT

Strong evidence	Less evidence of effect
Maternal	
High Viral load	Viral strain
Clinical AIDS	Immune response
Poor immune status	Nutritional status
	Other diseases
Labour and delivery	
Pre-maturity	Obstetric procedures (e.g. episiotomy)
Vaginal delivery	Duration of rupture of membranes
	Duration of labour
Postpartum	
Breast-feeding	Washing the neonate

Interventions for MTCT of HIV

Voluntary counseling and testing (VCT)

The identification of HIV positive women through confidential counseling should be an integral part of preventive activities for MTCT of HIV. However, the minimum requirements for the implementation of good quality counseling are:

- Staff who are trained in counseling skills;
- Allocation of staff whose primary responsibility will be counselling;
- Laboratory capacity for testing including trained staff and availability of HIV test kits at all times;
- A private room or space designated for confidential pre and post-test counselling; and
- Antenatal facilities that are spouse or partner friendly in order to facilitate partner counselling.

Testing algorithms for HIV should be designed within the context of existing infrastructure. The service should ensure that women come back to get their test results. Rapid tests are becoming increasingly cheaper and easy to do and have the advantage of the woman receiving results the same day. A return rate of 100% was achieved in three ANC clinics in Lusaka, Zambia using pre-test group discussion and the rapid test algorithm.[26]

Benefits of Counselling

Good counselling has several positive benefits for the pregnant mothers and their spouses or partners:

- The couple can make informed reproductive decisions;
- It provides an opportunity for dialogue with the spouse and other members of the family or community;
- Preventive education can be reinforced for HIV negative women; and
- The overall management of the pregnancy for HIV positive women can be planned more efficiently.

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Anti-retroviral therapy (Table 3)

The Paediatric AIDS Clinical Trials Group 076 trial (PACTG 076)

In 1994, zidovudine (AZT) in non-breast feeding women was shown to reduce MTCT of HIV by 67.5% (25.5% in placebo compared to 8.3% in the AZT group). The pregnant women that were enrolled in this randomized placebo controlled study took the drug orally from 14 to 34 weeks gestation and intravenously during labour. The babies also had six weeks of oral syrup.[27] The HIV infected women enrolled were asymptomatic, anti-retroviral naïve and had CD4 counts above 200 cells/ul. Tolerance to the treatment was excellent for both mothers and babies and development of drug resistance was rare. Based on these findings, ARV immediately became the standard of care for preventing MTCT of HIV in industrialized countries. As a result MTCT rates in developed countries have reduced to less than 8%.

A follow up study in the US, the ACTG 185 in pregnant women with advanced immuno-suppression (CD4<200cells/ul) and treated with AZT prior to pregnancy, also demonstrated a reduction in MTCT of HIV to less than 6%.[28] Currently in the US, therefore, it is recommended that, regardless of CD4 count, viral load, or prior ARV therapy, as a minimum, the ACTG 076 or some modification should be given to all HIV infected pregnant women.[28] When possible, initiation of therapy should be delayed until after the first trimester (the time of organogenesis).

Thai trial

With the understanding that the majority of infections in children occur around the time of delivery, another study was conducted in Thailand to examine the efficacy of a short course of oral AZT in asymptomatic, non-breast-feeding women from 36 weeks gestation.[29] AZT was also given to the mother orally during labour, but no drug was given to the baby. Transmission in this study was reduced from 19% to 9%, a 50% reduction. The average estimated cost of this regimen was 50US$ compared to 800 US$ for the PACTG 076.

Cote d'Ivoire and Burkina Faso Trials

The results of studies in Cote d'Ivoire and in Burkina Faso, using short course AZT treatment in breast feeding populations, were reported in March 1999.[30,31] The study in Cote d'Ivoire involved women enrolled at 36 weeks and randomized to oral AZT twice a day until delivery. Oral AZT was also given during labour. The authors' report that the treatments were well tolerated with no side effects and the majority of the babies were breast-fed. The estimated risk of transmission was 21.7% and 12.2% at four weeks, and 24.9% and 15.7% at three months; placebo compared to the treatment group. Established reduction in transmission of HIV were 44% at four weeks and 37% at three months.

At the same time as the Cote d'Ivoire study, a multi-centre trial involving both Cote d'Ivoire and Burkina Faso, women were enrolled between 36 and 38 weeks and randomized to oral AZT or placebo twice a day until delivery. The dose was doubled at the onset of labour and, finally, AZT was given to the mother twice a day after delivery for seven days. No drug was given to the baby. The risk of HIV infection at six months was 18% in the AZT group and 27.5% in the placebo. The conclusion from this study was that AZT offered a 37% reduction in transmission. This regimen is estimated to cost US$ 70-210.

Petra Trial

Another ARV intervention study conducted in Africa is the UNAIDS sponsored PETRA study.[32] In this four armed trial, AZT and 3TC were administered to

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women using the following regimens:
Arm 1: From 36 weeks, during delivery and to the child for 1 week;
Arm 2: To women only during delivery and to the child for 1 week;
Arm 3: Only during delivery; and
Arm 4: No drug given.

Sixty percent of the women included in the analysis breast-fed and 33% delivered by elective ceasarian section. Transmission rates in arm 3 and 4 were 15.7% and 16.5% respectively, suggesting that treatment during delivery alone is not adequate for prevention of MTCT of HIV. On the other hand, the rate of transmission was 10.2% in arm 2 and 7.8% in arm 1. The maximum benefit from this study was a 50% reduction in transmission with the comprehensive regimen in arm 1.

HIVNET 012 (Niverapine trial)

On July 14, 1999, the results of the National Institute of Allergy and Infectious Disease (NIAID) sponsored study, HIVNET 012, were announced.[33] The clinical trial demonstrated that niverapine, given as a single dose to an HIV infected pregnant woman at the onset of labour followed by a single dose to the infant in the first 72 hours of life, reduced transmission by as much as 47%. This regimen was compared to one in which AZT was given during labour followed by twice a day oral treatment to the infant for the first week of life. At 14-16 weeks, 13.1% of the infants that received niverapine were infected compared to 25.1% in the AZT group. The estimated cost of niverapine is US $4. From the results above, niverapine has a clear advantage over all other regimens because it is not only cheap, but is also easy to administer. The main side effects that have been observed with niverapine therapy are allergic skin rashes. Resistance also develops relatively quickly.

Table III: Antiretroviral interventions to reduce MTCT of HIV of proven efficacy

Study	Drug	Antepartum dose	Intrapartum dose	Postpartum maternal dose	Postpartum infant dose	% reduction
PACT 076 (No BF)	AZT	100mg PO 5X/d from 14-34 wks	2mg/kg IV infusion over 1 hour followed by 1.0 mg/kg/hr	No	2mg/kg PO 6 hrly for 6 wks	68% at age 6 months
Thai (No BF)	AZT	300mg PO 2X/d from 36wks	300mg PO 3 hourly	No	No	50% at age 6 months
Ivory Coast (BF)	AZT	300mg PO 2X/d from 36wks	300mg PO 3 hourly	No	No	37% at age 3 months
Ivory Coast/ Burkina Faso (BF)	AZT	300mg PO 2X/d from 36-38wks	600mg PO at onset of labour	300mg 2x / wk for 1 wk	No	38% at age 3 months
Africa: Petra arm 1 (BF)	AZT	300mg PO 2X/d from 36wks	300mg PO 3 hrly	300mg 2x/d/ wk for 1 wk	4mg/kg PO 2X/d for 1 wk	50% at age 6 wks
	3TC	150mg PO 2X/d from 36wks	150mg PO 12 hrly	150mg 2x/d/ wk for 1 wk	2mg/kg PO 2X/d for 1 wk	
Petra arm 2	AZT	No	300mg PO 3 hourly	300mg 2x/d/ wk for 1 wk	2 mg/kg PO 2X/d for 1 wk	37% at age 6 wks
	3TC	No	150mg PO 12 hrly	150mg 2x/d/ wk for 1 wk	2mg/kg PO 2X/d for 1 wk	
HIVNET 012	Nivera-pine	No	labour200mg at onset of labour	No	2mg/kg within 72 hrs of delivery	47% at 12-14 wks

Caesarian Section

Caesarian section (C/S) has been shown to reduce MTCT transmission of HIV. In March 1998, a European randomized trial was terminated because preliminary results indicated a reduction in transmission from 10.7% to 1.7% among infants born by elective C/S.[17] Two thirds of the women in this trial also received anti-retroviral therapy. No deaths or major morbidity as a result of C/S were reported.

No similar study has been conducted in Africa. However, C/S as an intervention for the reduction of MTCT of HIV has to be weighed against the risk of the operation itself, expertise available, prevalence of HIV infection

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among the pregnant women, and overall maternal mortality in the setting. In Africa where fertility rates are high, these results have to be interpreted with caution.

Breast feeding

In Africa, breast-feeding is still the norm in most communities. The current WHO-UNAIDS-UNICEF guidelines recommend that HIV positive pregnant women should be counselled with regard to feeding options so that they are able to make informed choices.[34] The results from the only randomized study of breast-feeding vs formula were announced in September 1999.[35] Intention to treat analysis showed a 44 % reduction in infection at 24 months in the formula fed babies. The findings also demonstrated no difference in mortality rate between the formula fed and breast fed babies. Risk factors for transmission were mastitis and breast abscess. Despite these findings, implementation of formula feeding as an option in MTCT control programmes is a complex issue requiring community solutions. Formula feeding is expensive and, if preparation and storage instructions are not adequately followed, feeding with formula may lead to severe malnutrition and life threatening infectious diseases. HIV positive women also run the risk of being ostracized by the community if they opt not to breast-feed. Therefore, support systems for HIV positive women should be strengthened, especially at the community level.

Of interest are the new findings from Durban, South Africa.[36] In a retrospective analysis of infant feeding patterns in an MTCT study which was designed to determine the efficacy of vitamin A supplementation, the HIV transmission rates were 28.5% in mixed feeders, 21.8% in exclusive breast feeders (up to four months) and 19.4 % in the formula feeders at 15 months. These results, though the first to be reported in literature, have important implications on MTCT prevention programs in situations where woman have no choice but to breast feed.

Vaginal cleansing during labour

The intervention using an antiseptic, chlorhexidine, to cleanse the vagina and inactivate the virus has potential for low-income countries. It is cheap enough to be provided to all women. A quasi-randomized study in Malawi by Biggar et al, did not show any overall reduction in MTCT of HIV except for women who had prolonged rupture of membranes (over four hours).[37] The important finding in this study was the significant reductions in neonatal and puerperal sepsis. In Africa, where sepsis is a major contributor to maternal and child morbidity and mortality, MTCT interventions should include vaginal cleansing.

Micro-nutrient supplementation

Though studies using micro-nutrient supplementation (including vitamin A) have shown no effect on reduction of MTCT of HIV, there are benefits of micro-nutrient supplementation in HIV positive pregnant women.[38-39] These include:

- Improved maternal CD4 counts;
- Reduced morbidity and mortality in the infants; and
- Reduced low birth weight rate (severe prematurity and intrauterine growth retardation).

These outcomes are still important and should form the basis for promoting multivitamin supplementation in the minimum package of care for HIV positive pregnant women in Africa.

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Conclusion

Prophylatic ARV therapy is effective in both breast-feeding and non-breast feeding populations. There are now a number of MTCT interventions that African countries can evaluate for national programmes depending on the available infrastructure and resources. UNAIDS is currently working in 10 countries in Africa to assess the feasibility of integrating some of these MTCT interventions in reproductive care services.

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STRATEGIES FOR PREVENTION OF MOTHER TO CHILD TRANSMISSION OF HIV

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Introduction

HIV infection in children threatens to reverse steady progress achieved with wide scale implementation of child survival programmes such as immunisation and breast-feeding promotion. The infection in children is directly linked to that in mothers. Over 90% of paediatric HIV infections are maternally acquired. Despite major advances in scientific understanding of effective interventions that reduce mother to child transmission (MTCT) of HIV over the last 6 years, global estimates for newly acquired HIV infections in children remain high.

Of the 2.4 million HIV infected women delivering each year[1] about 600,000 transmit the infection to their babies either in utero, at delivery or during lactation[2]. Over 90% of these children are born in Sub-

Saharan Africa, in countries where Ministries of Health can only afford a few dollars per person per year on health and health services are already overburdened by other disease[2,3].

Impact of HIV on infant and under 5 mortality

Infant and child mortality has increased in most sub-Saharan countries as a result of HIV infection. The relative impact of HIV on mortality is probably through two main mechanisms, both of which are extremely important. Firstly, maternal infant HIV transmission directly increases child morbidity and mortality, in areas where antenatal HIV prevalence rates are high. Secondly, increased child mortality may be as a consequence of the impact of HIV related morbidity on service delivery. In most highly affected areas the health services are dwindling due to the high HIV disease burden.

In Zambia and Zimbabwe, where the prevalence in pregnant women is over 20% in urban centres, the infant mortality rate is 25%[4] higher than it would have been without AIDS[4]. Under-five mortality has increased by over 70% in Botswana and Zimbabwe. The mortality trends underscore the humanitarian and ethical obligation for urgent global action, to protect children from the scourge of HIV infection.

Rate of mother to child transmission and associated risk factors

In industrialised countries, without maternal anti-retroviral therapy, 13-33% of non breast-feeding HIV infected women transmitted HIV infection to their infants compared 20 to 43% in breast-feeding women in low resource settings[5,6,7,8]. With the adoption of anti-retroviral therapy in pregnancy MTCT rates have declined to 4-6% in the US and other industrialised countries[9]. Sadly, in low resource settings, where most of the children are continuously being exposed to HIV, the cost of anti-retroviral therapy is prohibitive for most HIV infected women. Very few countries, such as Botswana, Thailand and Brazil have national policies for integration of preventive anti-retroviral therapy in antenatal clinics.

Interventions for mother to child transmission of HIV

Anti-retroviral therapy: Where are we?

PACTG 076 Regimen

In 1994, the French-American Paediatric AIDS Clinical Trials Study Group 076 (PACTG 076), randomised trial results demonstrated that zidovudine preventive therapy could reduce MTCT of HIV by as much as two thirds, in non-breast-feeding women[10]. After the release of the 076 results, many industrialised countries adopted this regimen as the standard of care and impressive reductions in MTCT of HIV have been achieved in these countries. However, the 076 regimen is not only expensive, it's administration is complex. In areas with poor basic reproductive and child health services, the practical feasibility of this regimen is questionable.

CDC Thai Trial

The picture became brighter 2 years ago (1998) when a clinical trial in Thailand demonstrated that a shorter (from 36 weeks with no post-natal treatment arm) and less complex regimen, at 10% the cost of the 076 regimen, could reduce HIV transmission by as much as 50%[12] (table1). Soon after the release of Thai results, the UNAIDS/UNICEF/WHO working group announced a series of pilot projects to assess feasibility of integration of MTCT interventions in reproductive and child-care services, including

counselling mothers on feeding choices[18]. Thirty sites in 11 countries were selected for the UNAIDS initiative, 8 in Sub-Saharan Africa, 2 in Asia and 1 in Latin America.

Table 1 Anti-retroviral randomised controlled trials for prevention of MTCT

Study	Drug	Anti-retroviral therapy				% reduction in MTCT (placebo vs treatment)
		Antepartum	Intrapartum	Postpartum		
				Woman	Infant	
No Breastfeeding						
USA/ FRANCE PACTG 076[10]Connor *et al* 1994	AZT	100mg PO 5X/d from 14 to 34 weeks	IV infusion 2mg/kg, then PO 6 hrly	None	2mg/kg 6hrly for 6 wks	68 % at age 18 months (25.5 vs 8.3%)
USA PACTG 185[11] Mofenson *et al* 1999	AZT	076/HIV Ig monthly	076 regimen	None	076 regimen	33% (6.0 vs 4.1%)
Thailand[12] Shaffer *et al* 1999	AZT	300mg PO from 36 wks	300mg PO 3hrly	None	None	50% (18.9 vs 9.4%)
Thailand[13] *Lallemant et al 2000*						
Long-long		300mg/kg PO BD from 28 wks	300mg/hrly	None	2mg/kg 6 hrly for 6 wks	7.8%
Long short		300mg/kg PO BD from 28 wks	300mg/hrly	None	2mg/kg 6 hrly for 3 days	4.8%
Short-long		300mg/kg PO BD from 35 wks	300mg/hrly	None	2mg/kg 6 hrly for 6 wks	8.6%
Short-short		300mg/kg PO BD from 35 wks	300mg/hrly	None	2mg/kg 6 hrly for 3 days	10.5% at interim analysis (arm dropped)
Breastfeeding **Cote d'Ivoire**[14] Witkor *et al* 1999	AZT	Thai regimen	Thai regimen	None	None	44% at 1 month (21.7 vs 12.2%) 37% at 3 months (24.9 vs 15.7%)
Cote d'Ivoire /Burkina Faso[15] Dabis *et al* 1999	AZT	300mg PO 2X/d at 36-38 wks	600mg PO at onset of labour	300mg PO 2X/d for 1 wk	None	37% at 3 months (25.1 vs 16.8%) 38% at 6 months (27.5 vs 18%)
Petra[16]						
Saba *et al* 1999 A	AZT	300mg PO 2X/d from 36 wks	300mg PO 3hrly	300mg PO 2X/d for 1 wk	4mg/kg PO 2X daily for 1 wk	50% at 6 wks (16.5 vs 7.8%)
	3TC	150mg PO 2X/d from 36 wks	150mg PO 12hrly	150mg PO 2X/d for 1 wk	2mg/kg PO 2X daily for 1 wk	
B	As A	None	A regimen	A regimen	A regimen	38% at 6 wks (16.5 vs 10.8%)
C	As A	None	A regimen	None	None	None
Uganda HIVNET 012[17] Guay *et al* 1999	NVP	None	200mg PO of NVP at onset of labour	None	Single dose 2mg/kg at day 2 to 3	44% at 2 months (21.3 vs 11.9%) 47% at 4 months (25.1 vs 13.1%)

Questions have been raised as to the public health benefit of the UNAIDS initiative in low resource settings, in view of limited capacity of antenatal and delivery services and the practical dilemma of implementing HIV counselling services[19].

Although antenatal attendance rates in low resource settings countries are generally high, the quality of care is questionable[20] and most women do not deliver in health facilities. Globally, 68% of women receive antenatal care but only 42% have assisted delivery[10]. These shortcomings are important programmatic challenges and should not be visualised as obstacles. The threat that HIV is posing on children, requires urgent action and should draw attention to strengthening systems for delivering interventions.

CDC and Ditrame trial in Cote d'Ivoire and Burkina Faso

Both the 076 and Thai regimen were evaluated in non-breast-feeding populations. In March 1999, results of 2 studies of short course AZT regimens in breast–feeding women (CDC Cote d'Ivoire & Ditrame ANRS 049a Burkina Faso and Cote d'Ivoire studies) were published (table 1)[13,14]. Both these studies demonstrated that short course zidovudine reduced HIV MTCT by over one third in early infancy (37% at 3 months for the CDC and 38% at 6 months for the Ditrame).

UNAIDS Petra Multicentre Trial in Uganda, South Africa and Tanzania

Development of drug resistance with single drug anti-retroviral therapy is a concern in prevention of HIV MTCT, especially with long term use. The Petra study conducted in Uganda, South Africa and Tanzania evaluated combination drug therapy in pregnancy[15]. This study compared placebo treatment to 3 different regimens of zidovudine/lamivudine (3TC) combination therapy (table 1). The reduction in HIV MTCT in early infancy was 50% and 38% in the longest arm (ante-natal, intra-partum and post-natal treatment) and the shorter arm (intra-partum and post-natal treatment). No reduction was observed with intra-partum treatment only.

HIVNET 012 nevirapine regimen

The nevirapine results provoked an outcry for action amongst scientists at the global conference for prevention of transmission of HIV from mothers to infants in Montreal, Canada in September 1999, for wider implementation of HIV preventative therapy in HIV infected pregnant women in low resource settings. Nevirapine has a prolonged half life and rapidly crosses the placenta when taken orally[21,22]. Early intra-partum and baby (within 48 hours of delivery) treatment given singly and orally showed a 47 % reduction in HIV MTCT (table 1) when compared zidovudine administered intra-partum and post-natally to babyfor 7 days after birth.

Apart from nevirapine regimen being the cheapest preventive drug regimen (4 US$), it is the most practical and feasible option for most low resource settings with limited health delivery services. The woman can be empowered to independently take the drug at the onset of labour. Moreover, the baby's dose could easily be linked to BCG immunisation. HIV is contributing substantially to the rising child mortality. So while it might take a long time to strengthen health systems, adoption of nevirapine in the interim will help save a lot of children.

Cost benefit analysis of nevirapine preventive therapy as a strategy has revealed that giving the drug to all women without screening is a cost effective strategy than when the intervention is targeted to HIV infected women only[23]. Without undermining the importance of evaluating the cost in any prevention strategy and ensuring that as many women benefit from an intervention, current outcries for universal nevirapine therapy without screening should be treated with caution. The benefits of VCT and other intervention strategies such as family planning, condom promotion, preventive therapy for tuberculosis and counselling on feeding options cannot be overlooked in countries with high HIV prevalence. Prevention of HIV MTCT interventions in these countries should be a component of the broader response to the HIV epidemic.

Duration of zidovudine preventive therapy for prevention of MTCT

A recent study in Thailand evaluated the optimal duration of zidovudine treatment administration for prevention of HIV MTCT[13]. Four treatment regimens were compared: long-long (starting at 28 weeks gestation with 6 weeks treatment to the baby); short-short (starting at 35 weeks with 3 day treatment to the baby), long-short and short-long. The babies were formula-fed. The transmission rates were 6.5% for the long-long, 4.7% for the long short and 8.6% for the short long. The short-short arm was dropped after interim analysis showed 10.5% transmission. These results appear to suggest that short antenatal treatment results in more HIV MTCT but not the duration of treatment in the baby.

Does breast feeding affect the long term effect of anti-retroviral therapy (12-24 months)

HIVNET 012, Petra and the Cote d'Ivoire/ Burkina Faso studies provided information at the International AIDS Conference in Durban in July 2000,on whether the short term benefits of the different ARV regimens is maintained in infants exposed to HIV through the breast-feeding period. The pooled analysis from the studies in Cote d'Ivoire and Burkina Faso showed that the observed difference was still present up to 24 months, although the reduction in transmission was about 26%[24]. Similar results were observed for HIV 012[25].

Beyond the 076 regimen in industrialised countries

In industrialised countries, the focus for MTCT interventions is currently adding on other drugs/interventions to the standard 076, to determine whether greater reduction in the rate of MTCT can be achieved. The PACTG 185 trial[11] combined infusions of hyperimmune HIV immunoglobulin with zidovudine in women with advanced HIV disease. Although the study was not able to answer whether passive immunisation further reduced the rate of MTCT, it confirmed the efficacy of zidovudine preventive therapy in women with CD4 cell counts less than 200 cells /mm^3. The

PACTG 316 is evaluating nevirapine given as a single dose intra-partum in women exposed to a minimal dose of zidovudine preventive therapy but who may get more anti-retroviral therapy[26]. Moreover, women already on combination therapy before conception are now encouraged to carry on with their therapy and are monitored for adverse effects.

Lessons learnt from anti-retroviral trials

What can be drawn from the above studies? Anti-retroviral therapy, whether long or short course, reduces HIV MTCT, although the long course PACTG 076 is the most effective. The effect is lower in breast-feeding than non-breastfeeding populations. In the first few months of life, in breast-feeding women short course combination therapy (Petra A) or the nevirapine regimen women have similar effect to the Thai regimen. Petra B (intra-partum and post-partum treatment to both mother and the baby) is a feasible option for women who miss antenatal care but deliver in a health facility. The new data from Thailand suggests that long antenatal treatment (from 28 weeks) is important in reducing in utero transmission.

Non anti-retroviral interventions

Elective caesarean section

A significant proportion of MTCT of HIV occurs during delivery as a result of infant exposure to infectious maternal cervical and vagina secretions. A meta-analysis from 15 international prospective cohort studies of non-breast-feeding women estimated that caesarean section reduced MTCT by 50% whereas when combined with zidovudine, transmission was reduced by up to 85%[27,28]. These estimates are similar to the findings from mode of delivery intervention trial conducted in centres in Europe[29] . In this study, 3% of children born by caesarean were infected compared to 10% in those delivered vaginally. Although the evidence for the protective effect of elective caesarean section is convincing, in making policy decisions, countries need to consider a number of issues (Box1).

Box 1 Policy concerns for implementation of caesarean section as an intervention for HIV MTCT

- Prevalence of HIV in pregnant women
- Existing infrastructure
- Available expertise
- Post-partum morbidity and mortality
- Cost relative to other interventions

In low resource settings, with high HIV prevalence, elective caesarean section will not be a feasible intervention as deliveries are mostly attended to by midwives and many of the facilities have no operative theatres. In addition, HIV infected women might be at increased risk of post-operative complications[30].

Feeding options

Breast-feeding appears to almost double HIV-1 transmission risk[31]. In a clinical trial in Nairobi, Kenya, formula feeding by cup reduced post-natal HIV transmission by 44%[32]. About 75% of the breast-feeding transmission occurred during the first 6 months of life, although transmission continued throughout the duration of exposure. The study, however lacked of information on feeding patterns. Two studies have suggested that early mixed infant feeding (breast milk plus other foods or juices) is associated with higher

transmission than exclusive breast-feeding[33,34]. Coutsoudis *et al* in Durban showed that, at 3 months, MTCT in children exclusively breastfed (14.6%) was significantly lower than in those given mixed feeding (24.1%)[33]. Moreover, exclusive breast-feeding did not appear to increase the risk of MTCT over formula feeding. This effect appears to be sustained to 18 months despite continued breastfeeding. The mechanism for this reduction in risk is not clear but may be associated with early feeding causing gut mucosa inflammation, facilitating transmission.

Whereas HIV infected mothers in industrialised countries are advised not to breast-feed their baby, feeding options in low resource settings are limited. Recognising the high infectious morbidity and mortality associated with formula feeding in low resource settings, WHO and UNICEF in 1992 issued a statement recommending that breast feeding be protected in these settings[35]. With new understanding of the rate of HIV MTCT associated with breast-feeding, guidelines were produced by UNAIDS, UNICEF and WHO in 1996 advising that HIV infected women be counselled about HIV and feeding choices including replacement feeding, to enable HIV them to make an informed decision[36,37].

Apart from the expense and limited availability, replacement feeding in low resource settings raises a number of issues and concerns (Box 2) which should be addressed as part of the programme and community response. In communities where breast-feeding is the norm, replacement feeding will indirectly result in disclosure of the mother's HIV status in setting. Unless the family and community understand the issues and the importance of the programme, replacement feeding is likely to result in stigma, blame and violence. Community mobilisation is, therefore, a key component of HIV MTCT prevention programmes.

Box 2 Replacement feeding concerns in low resource settings

- Cost and limited availability of replacement feeds
- Lack of basic needs such as running water
- Loss of confidentiality or indirect disclosure with replacement feeding
- Low education status of most mothers
- Women not empowered to make independent decisions
- Stigma and blame for mothers after disclosure
- Spill-over of information on replacement feeding to negative women and those of unknown HIV status
- Poor support systems at both at health centres and community level
- Existing high mortality from infectious diseases and malnutrition
- Possibility of breaking of the code of marketing for breast milk substitutes
- Early subsequent pregnancy due to low family planning uptake

Women in these settings are not the key decision-makers on what is best for the baby. Partner or family involvement is critical to the success of the programme. Midwives need adequate training in infant feeding in relation to HIV to better provide appropriate counselling support.

There is an ongoing debate regarding reinforcing promotion of exclusive breast-feeding for women with limited resources, based on the findings in the Durban study[38]. With the current understanding, exclusive breast-feeding might be the only practical option for many women with limited feeding alternatives until more information becomes available.

Supportive interventions

Voluntary counselling and testing

The identification of HIV infected pregnant women through confidential counselling and testing (VCT) is the initial step to provision of MTCT interventions. Although difficult to implement, available data indicates most women opt for testing[39]. Testing algorithms for HIV should be designed within the context of existing infrastructure. Rapid tests algorithms are useful in that women can get results on the same day[40]. Group pre-test discussion is one way of dealing with congestion and limited personnel if it is locally acceptable[41]

The inputs for provision of counselling and testing are shown in box 4.

Box 4: Requirements for provision of VCT in antenatal services

Trained staff with counselling skills or volunteers from non-governmental organisations

Staff time which might require creation of counselling posts in health facilities

Laboratory capacity for testing (skilled staff and test kits)

Counselling space in antenatal clinics

Antenatal facilities that are partner friendly for provision of couple or family counselling

Flexible counselling services to provide continuous support

Establishment and strengthening of referral networks

Cleansing of the birth canal

Cleansing of the birth canal with a virucidal antiseptic solution, chlorhexidine, to avoid HIV exposure during vaginal delivery was studied in a clinical trial in Malawi[42]. Although the study did not show a reduction in HIV MTCT, there was a decrease in neonatal infections and mortality[43]. In areas where neonatal sepsis is a major cause of mortality in infancy, chlorhexidine vaginal cleansing should be included in the comprehensive package of care in MTCT programmes.

Micro-nutrient supplementation

Vitamin A deficiency and other micro-nutrient deficiencies have been shown to increase the risk of MTCT and outcomes such as prematurity, intra-uterine growth retardation[44,45,46]. In addition anaemia has been associated with HIV in pregnancy[47].

Two randomised trials conducted in Africa, involving vitamin A supplementation, however, failed to demonstrate a reduction in the rate of HIV MTCT[48,49]. On the other hand, multivitamin supplementation was shown to significantly reduce foetal death, low birth weight, severe premature birth and intra-uterine growth retardation[48,49]. These birth outcomes are major causes of morbidity and mortality in infancy. Women in low resource settings are likely to be micro-nutrient deficient and should be supplemented

with multivitamins. Ensuring adequate provision of iron and folate supplements is also essential to reduce anaemia-related morbidity.

Malaria treatment

Malaria in pregnancy is an important cause of morbidity and mortality in HIV affected areas[50,51,52]. Studies from Malawi and Kenya demonstrated that placental malaria in HIV infected women increased neonatal mortality[50,51]. Moreover, while a two-dose regimen of sulfadoxine-pyrimethamine (fansidar) malaria treatment in pregnancy may be effective in HIV negative women, this regimen does not seem to control placental malaria in HIV positive women[53]. More frequent dosing of fansidar in pregnancy, probably, is probably necessary in HIV infected women.

Economic considerations of prevention

Wood *et al* (2000) in their analysis reported that in South Africa, without anti-retroviral therapy for HIV infected pregnant women, between the year 2000 and 2005, there will be 276,000 cumulative HIV positive births[54]. By contrast 110,000 HIV positive births could be prevented short course treatment. The direct cost of the universal coverage of this intervention for 5 years would be US $ 54 million. Although this cost appears astronomical, the authors concluded that this was less than 0.001% of the per person health care expenditure during the same period.

Is preventive therapy for MTCT simply generating orphans?

The issue of anti-retroviral treatment for the mothers and perhaps the fathers has been raised[19]. Anti-retroviral therapy for protection of children without treatment for the parents can only yield short-lived gains. If they become orphans at a young age, their future is bleak. French and Broklehurst (1998) in their systematic review of studies, with maternal outcome as an end point, concluded that HIV progression in pregnancy was significantly more common in low resource settings (OR 3.71, 95% CI 1.82-7.75) than industrialised countries (OR 0.55, 95% CI 0.27-1.11)[55]. Even without MTCT interventions the number of HIV related orphans is on the increase in a number of countries[56]. The issue of orphans becomes more significant if prevention of MTCT is visualised only as provision of anti-retroviral therapy in pregnancy and not a comprehensive package to include community mobilisation, education, counselling of infected couples, family planning promotion and early referral and access to care for the mothers and father.

Access to health care is a basic right for children. Child survival strategies for HIV infected children, such as immunisation, could also be questioned for fear of generating orphans. Concern over promotion of formula feeding for children born of HIV infected mothers increasing infant mortality remains a major concern and yet provision of anti-retrovirals for prevention of HIV MTCT is visualised as an intervention for generating orphans and not a child survival strategy.

MTCT interventions are an integral part not only of maternal and child health programmes but a response to HIV prevention in populations. With a well-planned programme, women will have access to better care, family planning as well as counselling services. Positive women will be afforded the opportunity to plan for their children and future pregnancies and negative women will be counselled on HIV prevention.

Building and strengthening of community support systems will help strengthen responses for the mitigation of HIV at the community level.

Safety of anti-retroviral therapy

Anti-retroviral drugs given to pregnant women may induce severe health hazards to both mothers and their children although to date the data on preventative therapy in pregnancy does not show any need for concern. Rare but severe toxic effects have been observed with long term treatment with zidovudine, 3TC and nevirapine. Prolonged exposure is more of a concern in industrialised countries where more women are on combination therapy even prior to the pregnancy.

Data on short-term therapy is encouraging although in 1999 a rare neurological disease (mitochondrial disease) was queried in two HIV negative babies who died and had been exposed to antenatal zidovudine treatment by French researchers. A subsequent careful retrospective search led to the discovery of 6 additional cases of uninfected children exposed to 3TC and zidovudine[57]. However, no similar cases were found amongst more than 15,000 exposed children in the US[58].

Mucocutaneous rash is the most frequent complication observed with long-term use of nevirapine although some patients may develop more severe reactions such as Steven Johnson syndrome. No severe reactions have been noted amongst the pregnant women who have been exposed to one dose of nevirapine[17].

Implementation of programmes for prevention of MTCT

Strategy development

Preventing mother to child transmission is a child survival strategy. It is a critical component of maternal and child health services as well as HIV control programmes. The first step to programme implementation in a given country is the development of a country-specific strategy based on the prevailing situation. The strategy development process should involve representatives from maternal child health services, policy makers, researchers, nutritionists, tutors, HIV counsellors and people living with HIV, underscoring the complexity of issues that should be addressed.

Elements of the programme strategy

Key elements to be addressed should include:

- VCT models;
- the comprehensive package of care in pregnancy and post-natally such as access to antenatal and delivery services; anti-retroviral preventive therapy; malaria treatment; family planning; multivitamin, iron and folate supplementation; counselling on feeding options and post-natal care for the child and mother);
- and the strategy for advocacy, programme communication and community mobilisation.

Decisions should be based on scientific evidence as well as what is achievable within available resources and infrastructure. Some of the interventions, such as provision of VCT and partner involvement, might require piloting before implementation. The counselling must be adapted to the local situation without overwhelming the staff and compromising other services. Additional requirements and training needs

should be identified. A number of anti-retroviral regimens are now known to be effective and countries can chose what option is most practical and cost effective.

Integration into existing services

The package of care should be fully integrated within a continuum of care in the overall antenatal, delivery, maternal child-care with an overall objective of reducing maternal and infant morbidity and mortality. Apart from administration of drugs, antenatal and delivery care and follow up services may require re-organisation and strengthening.

Referral networks both for the mother and the child should be in place or if available should also be strengthened. Such services include preventative therapy for tuberculosis, screening and treatment for opportunistic infection, support counselling services and child-care services.

UNAIDS/UNICEF/WHO[59] implementation pilot projects

The comprehensive care package in the pilot projects are shown in Box 4.

Box 4 UNAIDS/ UNICEF/ WHO package of interventions to be implemented in pilot countries[59]

- **Expansion and strengthening of family planning services**
- **Early access to quality antenatal care**
- **Voluntary counselling and testing for women and their partners**
- **Provision of anti-retroviral therapy for prevention of MTCT**
- **Improved care during pregnancy, labour and post-natally**
- **Counselling of HIV positive women on infant feeding choices, making replacement feeding available and offering support to the women on their feeding choice**

These pilot projects will furnish practical information on how to scale up programmes, particularly VCT, replacement feeding and monitoring and evaluation

Conclusion

Prevention of MTCT is possible with available intervention options. In industrialised countries, with widespread use of anti-retroviral therapy in HIV infected pregnant women, paediatric HIV has become an insignificant problem. The challenge today remains to realise the same gains in resource poor setting, where achievements observed in the 1980s with child survival programmes are being eroded. No doubt there are many challenges in the implementation process but the call for action should be today. We simply can not to wait for tomorrow, when systems are established or strengthened.

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Infant feeding and HIV-1 infection

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Introduction

It has been estimated that, in 1999, over 600 000 children worldwide became infected with HIV through mother-to-child transmission (MTCT), with between one-third and one-half of these infections acquired through breastfeeding (Fig. 1) [1,2]. Over 90% of paediatric infections occur in the resource-poor world, where a median survival of 4–5 years is found for HIV-infected children [3]. Over 95% of children are also initially breastfed in those countries (Fig. 2), and concern over MTCT has caused confusion for policy-makers promoting universal breastfeeding [4–7].

In this paper, we re-examine the current data relating to infant feeding and HIV infection [8]. We review the possible mechanisms of HIV transmission through breastfeeding, consider approaches to prevention (including feeding options) in different settings and identify research needs. Information was obtained from diverse sources and a systematic review, using computerized literature searches on breastfeeding and HIV, supplemented by consultation with relevant specialists.

Infant feeding practices: breastfeeding prevalence, patterns and duration

Trends and patterns of breastfeeding in developing countries were obtained primarily from the Demographic and Health Surveys (DHS) and other studies that employed standardized methods and definitions (Table 1). The DHS represent an ongoing (since 1984) programme of national surveys in developing countries based on household interviews by trained interviewers with women aged 15–49 years [4]. In particular, we consulted a review of data from over 250 000 births over the period 1990–1996 in 37 developing countries in six regions; sub-Saharan Africa, Asia, the Middle Eastern Crescent (which includes North Africa), South America, Central (Latin) America, and the Caribbean. This included trend analyses for 27 countries where two or more DHS or World Fertility Surveys were carried out between 1975 and 1996 [4]. Not all other studies used the necessary standard definitions (Table 1), and data are more rare and less comparable for industrialized countries [9].

Over 95% of babies in developing countries are initially breastfed, and most children continue to receive some breastfeeding until 6 months of age [4]. After 6 months, large differences between countries emerge with prolonged breastfeeding common in sub-Saharan Africa and Asia, where artificial (formula) feeding is rare, while fewer than 50% of children in Latin America, the Caribbean, North Africa and the Near East are breastfed after 16 months of age (Fig. 2). The median duration of breastfeeding varies from an average of 21 months in sub-Saharan Africa and Asia to 14–15 months in Latin America and the Caribbean. However, national statistics conceal important social differences [4]. Breastfeeding is less common in urban and higher socioeconomic groups compared with rural areas and poor communities [4,10]. The high cost of formula feeds and the diffi-

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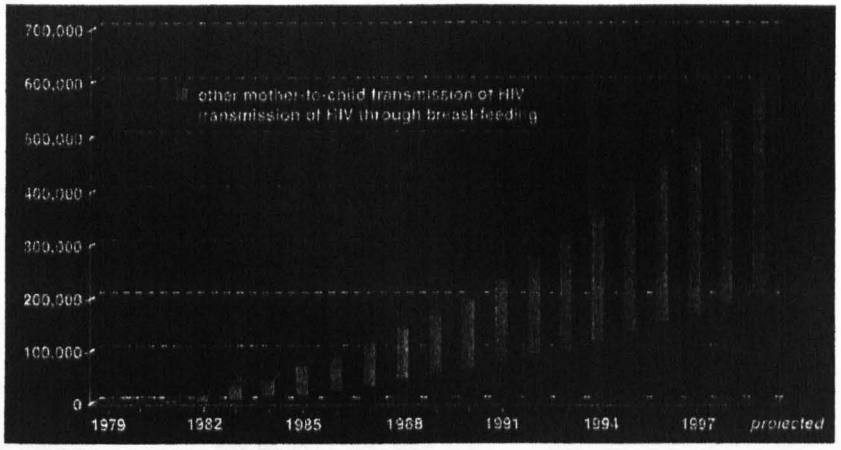

Fig. 1. Mother-to-child transmission of HIV. UNAIDS/WHO figure (1998) of global trends in children newly infected in the world with HIV through mother-to-child transmission subdivided by modes of transmission (breast versus other).

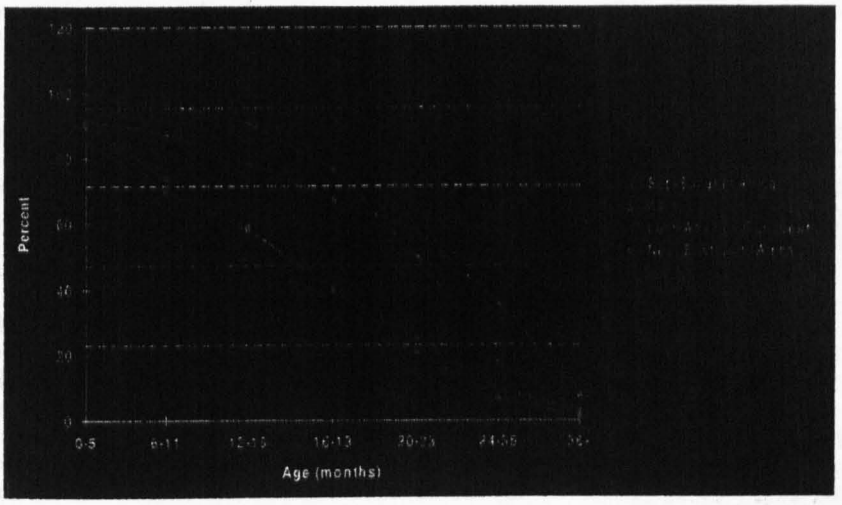

Fig. 2. Percentage of children breastfeeding (any form) by age and region [4].

culties of preparation and storage make artificial feeding unfeasible in many poorer settings, even with subsidized formula [11]. Breastfeeding in these settings tends to be less common among more educated women, a factor that seems independent of level of income [4,10]. This contrasts with western industrialized countries where breastfeeding rates are highest among more educated women, possibly because of awareness of the health advantages [12–14].

The proportion of breastfed infants remained stable or increased in the 1990s. Increases were notable in Latin American countries, when breastfeeding was promoted, with the greatest increases occurring in towns and among more educated women. Similarly, the prevalence of prolonged breastfeeding increased, with some countries, notably Kenya, Peru and Senegal, doubling the percentage of children still being breastfed at age 20–23 months [4].

Exclusive breastfeeding

Exclusive breastfeeding is considered the best form of feeding for the child during the early months of life, and helps protects the mother against another pregnancy [15,16]. Recently, one study has suggested that exclusive breastfeeding may also be associated with a lower risk of HIV transmission than mixed feeding (Table 1) [17,18]. Although it is recommended that babies receive exclusive breastfeeding for at least 4 months, and if possible 6 months [19], prevalences are variable, and by 4 months exclusive breastfeeding is uncommon, especially in Africa where some cultural beliefs may mediate against it (Fig. 3) [4,20]. National breastfeeding programmes, such as the Baby Friendly Hospital Initiative of the World Health Organization and UNICEF, appear able to raise the prevalence of exclusive feeding. Within countries having repeat DHS surveys in the 1990s, increases in

Table 1. Feeding options with definitions.

Category of infant feeding	Requires that infant receive	Allows the infant to receive	Does not allow the infant to receive
Exclusive breastfeeding	Breastmilk (including milk expressed or from wet-nurse)	Drops, syrups (vitamins, minerals, medicines)	Anything else
Predominant breastfeeding	Breastmilk (including milk expressed or from wet-nurse) as the predominant source of nourishment	Liquids (water, and water-based drinks, fruit juice, ORS), ritual fluids and drops or syrups (vitamins, minerals, medicines)	Anything else (in particular, non-human milk, food-based fluids)
Complementary feeding	Breastmilk and solid or semi-solid foods	Any food or liquid including non-human milk	No restriction
Breastfeeding	Breastmilk	Any food or liquid including non-human milk	No restriction
Bottle feeding	Any liquid or semi-solid food from a bottle with nipple teat	Any food or liquid including non-human milk. Also allows breastmilk by bottle*	No restriction

* However, for surveys and studies, it is essential to distinguish between artificial feeding using formula feeds versus expressed breastmilk. Source: [55]. ORS, oral rehydration solution.

exclusive breastfeeding prevalences were most often seen for countries with national breastfeeding promotion campaigns (M. Labbok, personal communication, 1999) [4,21].

Optimal breastfeeding requires antenatal preparation and support for the mother postnatally to ensure that she has a good understanding and technique. Lack of support, poor technique, the need of mothers to work (with a lack of adequate maternity leave or of workplace childcare) and the availability and promotion of artificial feeds are all associated with failure to establish breastfeeding, early cessation, a poor experience for the mother and increased risk of mastitis and breast abscesses [15,22–25].

Mastitis, inflammation of the maternal breast, may increase shedding of HIV in breastmilk [24]. Recently, a subclinical form has been described, detectable only biochemically [25–28] through the presence of raised breastmilk sodium and an inflammatory chemokine, interleukin-8. Both forms probably result from stasis of milk in the breast tissue (Fig. 4), possibly due to poor breastfeeding technique and use of supplementary feeding [26,29,30]. Most clinical studies report a cumulative incidence (per birth) of mastitis of under 10% but studies based on biochemical detection have reported prevalences of 13–25% in the first month and 10–12% at 3 months [26,27,31]. It may be possible to reduce the incidence by supporting mothers to improve their breastfeeding technique and to breastfeed exclusively or by mothers taking micronutrients, but the effects of such interventions on either breast health, infant health or HIV shedding remains to be fully investigated [25,31,32].

Breastfeeding transmission of HIV

In 1992, it was estimated that if a woman with established HIV infection breastfed, the additional risk of MTCT of HIV was 14% [95% confidence interval (CI), 7–22%] while, if a woman became infected while breastfeeding, the risk was 29% (95% CI, 16–42%) [33]. More recent data support these estimates [34–36]. A randomized controlled trial in which mothers were allocated to breast or artificial feeding found an additional risk of MTCT from breastfeeding of 16% at 2 years follow-up, with breastfeeding contributing 44% of all transmission [2]. Observational studies of later postnatal MTCT give compatible estimates taking into account that they cannot distinguish intrapartum transmission from breastfeeding transmission occurring soon after birth [37]. In a meta-analysis of breastfeeding transmission beyond age 2.5 months, the annual risk was estimated to be about 3% [37], while in a study in Malawi the annual risk of transmission from breastfeeding after 1 month was estimated to be 10%, which is not significantly different [38]. Generally, increased breastfeeding duration is associated with greater risk and all these data suggest an overall estimate of risk of MTCT for breastfeeding mothers of 25–45%, with one-third to one-half of MTCT attributed to breastfeeding (Fig. 1) [35].

The mechanism and routes of breastfeeding transmission remain unclear (Fig. 4). HIV is expressed as either free virus or within cells in milk, and it is unknown which is most associated with transmission. Virus is also shed by epithelial cells in the mammary alveoli and ducts [35,39]. Cellular and cell-free virus

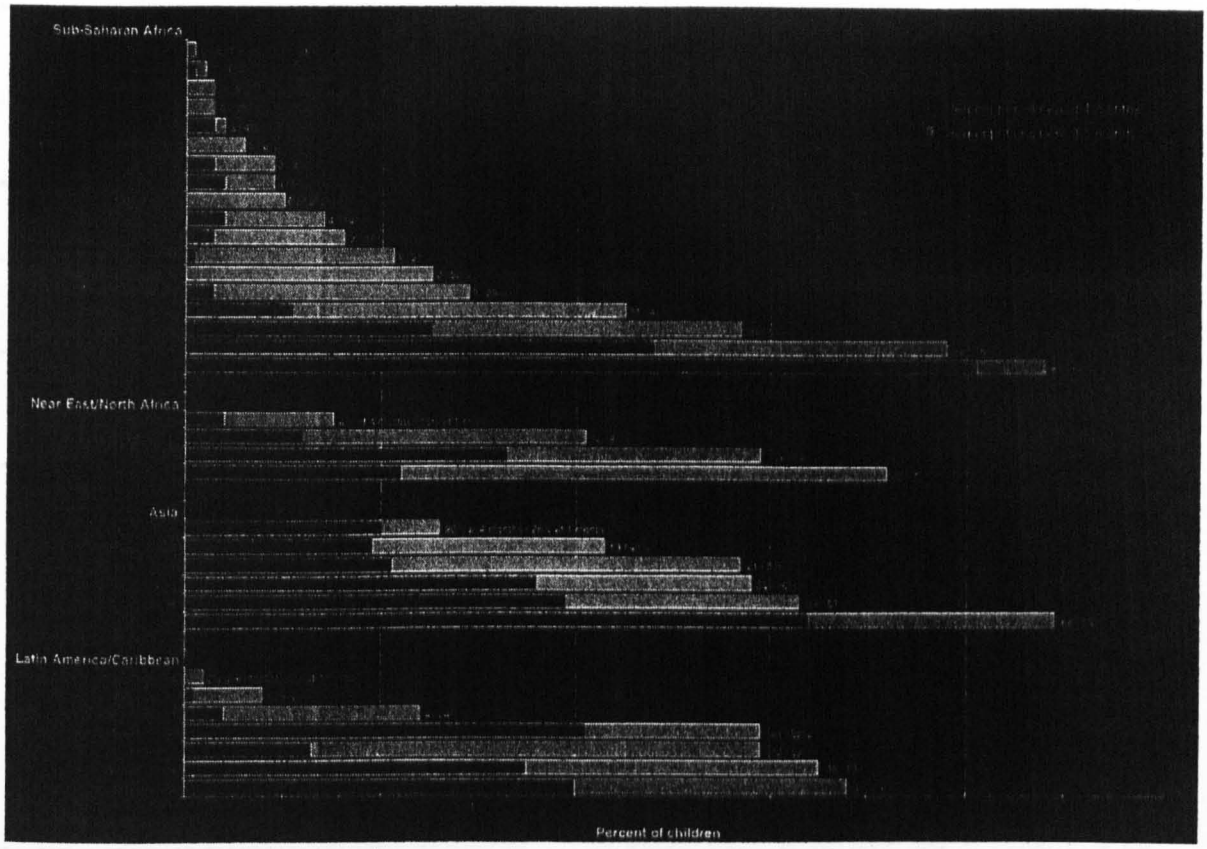

Fig. 3. Prevalence of exclusive breastfeeding at 1 and 4 months by country (DHS Surveys [4]).

are both detectable in colostrum and breastmilk, and the relative importance of colostrum and mature breastmilk in transmission is unclear [39]. The route of entry into the child is also unknown. Virus could be absorbed either in the infants mouth, throat or intestine, although passage of the virus through the acid environment of the stomach (after neonatal achlorhydria) will reduce intestinal absorption (Fig. 4) [39]. Cell-free virus could penetrate the mucosal lining of the gastro-intestinal tract of infants by infecting cells, or pass directly into the blood stream via mucosal breaches. Equally, lymphatic areas lining the tract may capture and transport HIV [35].

The risk of breastmilk transmission of HIV may depend on the size of the viral inoculum, the amount of cell-free and cell-associated HIV, the infant's individual susceptibility and local specific immune responses to HIV. There are several substances in breastmilk that may be protective, notably antiretroviral substances and polyanionic milk proteins, HIV antibodies, lactoferrin, secretory leucocyte protease inhibitor and vitamin A [35,39]. HIV can elicit local humoral immune response in breastmilk. Both colostrum and mature breastmilk of HIV-infected women contain secretory IgA and secretory IgM to HIV, which are some protection [39]. Maternal factors are important, notably viraemia and acquisition of infection while breastfeeding [35]. Vitamin A deficiency in plasma has been associated with increased amounts of breastmilk HIV [40]. However, attempts to reduce MTCT by vitamin supplementation have been unsuccessful [35]. The amount of HIV in breastmilk may be increased in women with mastitis or breast abscesses [24,41–44]. It has also been suggested that a damaged mouth in the infant, mouth ulcers or even just oral candida might be associated with increased risk [35].

Lack of early breastfeeding and premature introduction of non-human milk may facilitate breastfeeding HIV transmission. Epidermal growth factor and other factors in colostrum and milk may have a special role in developing and 'closing' the mucosa lining of the neonatal gut to foreign agents, including HIV [45,46]. Later damage to the infant's lower intestinal tract, perhaps through the early introduction of foods apart from maternal milk, could facilitate permeability to

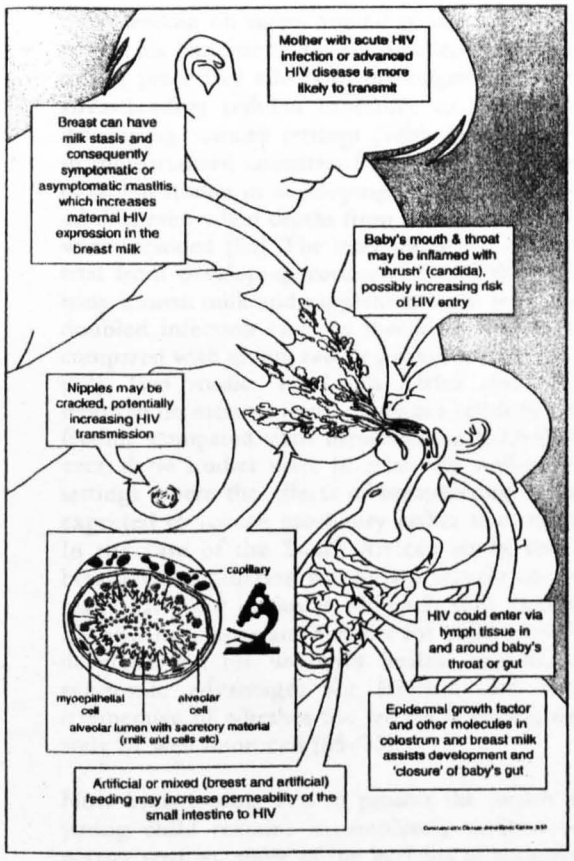

Fig. 4. Possible mechanisms for HIV transmission through breastfeeding.

HIV so that mixed feeding could actually increase risk of MTCT [8,17,47]. It has long been recognized that early introduction of foreign protein into infant feeds damages the newborns immature gastrointestinal tract, leading to occult bleeding and iron-deficiency anaemia [48–50]. Exposure to foreign dietary antigens and enteric pathogens may cause an inflammatory process facilitating HIV transmission across the gut mucosa. A model for this is the premature baby where non-human milk feeds increase the risk of necrotizing enterocolitis and other infections [51,52].

These facts suggest possible interventions. Shortening duration of breastfeeding by HIV-infected or all women would reduce exposure to HIV and the risk of MTCT, although mathematical modelling suggests that the effect would be small and there could be adverse effects from loss of the benefits of breastfeeding [37,53]. Improving the breastfeeding technique and promoting exclusive breastfeeding is attractive, as this might reduce breastfeeding MTCT, either by reduced mastitis and therefore maternal shedding or excretion of HIV virus, or decreased permeability of the infant's gastrointestinal tract, or both. However, only limited epidemiological evidence is available to support this strategy as most MTCT studies (for example, those that estimate the risk of postnatal transmission [37,38]) have neither recorded data on the mode of feeding nor used standardized definitions. They have usually recorded only 'breastfeeding', which usually means that an unknown number of children received mixed feeding, i.e. breastfeeding plus other milks, or other drinks such as waters or teas or dilute cereals or other foods (Table 1) [2,17,54–56].

Intervention studies

Short-term antiretroviral therapy for HIV-infected women late in pregnancy, during labour and to the newborn has been shown in randomized controlled trials to reduce the risk of MTCT, even in breastfeeding populations [18]. Nevirapine is particularly attractive because of the simplicity and cheapness of the drug regime (only one dose each for the mother and newborn) [18,57]. Fears that prevention of MTCT during pregnancy and childbirth could be undone through later breastfeeding transmission, through rebound rises in infectivity when therapy stops or simply prolonged exposure to infected breastmilk [58], are so far unsupported. Rates of postnatal MTCT in infants of treated and untreated mothers are similar while, if 'rebound' was of importance for postnatal MTCT, it would be expected that rates would be higher to babies of treated mothers [39,59,60]. Use of longer acting antiretrovirals such as nevirapine given to infants while breastfeeding might prevent postnatal infections through breastfeeding, and safety and dosing studies are underway. However, there are concerns with this approach, or of giving nevirapine to all mothers irrespective of HIV status, because of difficulties in controlling medicines in some settings and the rapidity with which nevirapine resistance can develop [61].

Benefits and risks of modes of feeding

An earlier review noted a lack of empirical data on the overall risk to child survival from artificial feeding [8]. Such data are particularly lacking from sub-Saharan Africa where breastfeeding is nearly universal [4,54]. However, analyses of data from recent DHS surveys have replicated earlier findings of strong protective effects of longer breastfeeding on child survival and longer intervals between births [62]. This evidence of protective effects of breastfeeding is strongly supported by various observational studies published in the 1990s on the risks of artificial versus

breastfeeding on infant mortality and morbidity due to specific conditions. These consistently demonstrate strong protective effects of prolonged breastfeeding. Breastfeeding reduces mortality and morbidity in developing country settings (Table 2) and morbidity in industrialized countries (Table 3). A meta-analysis of recent studies in developing countries demonstrated this, even when deaths from non-infectious causes were included [54]. The only published randomized trial from developing countries found that pasteurizing human milk and supplementing it with formula doubled infection rates in low birth weight babies compared with giving raw or pasteurized human milk [52]. Two studies in South Africa and Malaysia reported no increase in mortality as a result of artificial feeding compared with breastfeeding [63,64]. However, these studies were in relatively well-resourced settings where the effects of breastfeeding would be expected to reduce morbidity rather than mortality. In the case of the South African study, the mean breastfeeding duration was only 3 months and was in many cases not exclusive (Table 3) [63]. Breastfeeding also has important benefits for child growth and development, for women's health, and has many economic advantages for families and societies irrespective of whether the setting is poorly, moderately or well resourced [65–73].

How breastfeeding acts to protect the health of the young child remains incompletely understood. In poorer settings, some of the benefits of breastfeeding results from its physiological contraceptive effect, which helps to maintain an advantageous birth interval [16,23,69,74–77]. In addition, in some cultures, there is a reduction in coitus associated with breastfeeding, which may enhance the effect [20]. Hence, the issue of replacement contraception must be addressed whenever interventions for the prevention of MTCT include replacement feeding as an option.

How feeding modality and transmission of HIV through breastfeeding may interact to affect child mortality

For the HIV-infected mother, the protective effects of breastfeeding for child survival have to be set against the risk of a child acquiring HIV infection. Where the risks of artificial feeding are small, it is appropriate for an HIV-infected mother to decide to avoid breastfeeding [78,83]. Such decisions cannot be made at a national level as risks associated with artificial feeding may vary considerably between individuals within countries [54,79]. Modelling studies of the competing risks of infection and death from MTCT versus the risk associated with artificial feeding have concluded that programmes that promote artificial feeding could have unintended adverse outcomes. In poorly resourced settings, children born to HIV-infected mothers who use artificial feeding may escape MTCT through breastmilk but succumb to the heightened risks of artificial feeding [8,53,54,79]. A randomized trial of breast versus artificial feeding in Nairobi demonstrated that artificial feeding resulted in significantly less HIV infection (21% versus 37%) compared with breastfeeding. However, there was no significant difference in overall all-cause child mortality at 2 years of age (20% of children in the artificially fed, and 24% in the breastfed children) despite the fact that the mothers randomized to use artificial feeds had access to treated water and infant formula, and careful instruction on its use was provided [2]. Equally, it is suggested that the use of artificial feeds by HIV-infected women may encourage their use by the uninfected majority, a so-called 'spill-over' effect [8,80]. A recent modeling study re-iterated the potential adverse effects on mortality that could occur from spill-over. The model also suggested that early weaning (at 3 months) by all mothers would be beneficial if the risk of later postnatal transmission was above 7%, although, at maternal HIV prevalences of 15% or less, the number of infections that would be saved would be small if the background infant mortality was high [53]. Feeding a baby completely on artificial feeds is costly, even with home-prepared formula, and is uncommon in resource-poor settings, particularly in sub-Saharan Africa [4,11]. Yet that is what it would be necessary for a mother to do in order to avoid the risk of transmission of HIV through breastfeeding. Exclusive artificial feeding by all women would only be cost-effective in countries with substantial HIV seroprevalence if background levels of child mortality were low, and dangerous where infant mortality was higher than 70/1000. Modelling analyses suggest that early breastfeeding replaced by formula feeding at 4 or 7 months (early weaning) would save few lives and would offer poor value for money as an intervention [81].

Options for reducing MTCT of HIV

Governments and policy-makers are under pressure to reduce MTCT through policy changes such as prenatal screening, use of antiretrovirals perinatally, and adapting infant feeding policy [6,18,83]. Policy-making is difficult in this area. Much of the information required to determine local policy and inform women is as yet unavailable, such as whether artificial feeding is feasible or acceptable locally, and whether feeding can be delivered safely locally. Difficulties arise when there are no specific infant feeding policies or when they are part of nutrition policies and amount only to a general recommenda-

tion to promote breastfeeding. What is needed is clear overall infant feeding policies that strengthen implementation of the International Code of Marketing of Breast-milk Substitutes, interventions such as the Baby Friendly Hospital Initiative, breastfeeding counselling, and improved complementary feeding [22,35,83]. These all remain relevant even in HIV high-prevalence areas. Within these policies, allowance should be made for the needs of HIV-positive women to make an informed choice and to be supported in their choice.

Options for interventions to reduce MTCT include general HIV prevention and/or promotion of modified feeding practices in the whole population (Table 4), and those based on antenatal HIV testing with interventions, including use of antiretrovirals by infected mothers only (Table 5) [11].

General population approaches, i.e. potentially involving all women (Table 4), have the advantage of avoiding the stigma of HIV-infected women having to 'advertize' their infection status by adopting visible practices different from other mothers [84,85]. General HIV prevention, especially targeting adolescents and young adults, is a highly defensible policy option, irrespective of feeding practices [1,6,18,35]. The prevention of HIV infection in young women, and men is prioitized by WHO, UNICEF, UNAIDS and other agencies, and is an International Development Target in its own right, although with the prime aim of protecting adult health [1,21,83,86]. This approach has been most successfully applied in Uganda where prevalence of HIV infection among younger pregnant women declined substantially in urban areas in the 1990s [87,88]. Since fewer women giving birth are HIV infected, it also has the effect of reducing the number of childhood infections occurring in young children. Avoidance of breastfeeding by all women is not an acceptable option. In a resource-poor setting, it would increase infant mortality and compromise lactational contraception, birth spacing and maternal health [5,53,69]. Promotion of exclusive breastfeeding and helping mothers to use a good breastfeeding technique is attractive and feasible (Fig. 3) [4]. It is in line with general policy on breastfeeding and would have general benefits for the health of the child and probably the mother (Tables 2 and 3) [15,21,89]. Although it is uncommon at present, there is evidence that national breastfeeding programmes increase exclusive breastfeeding rates (Fig. 3) [4]. However, it can pose difficulties for women who have to work without adequate maternity protection or child-care facilities. It might result in a lower risk of MTCT than mixed feeding, but this is yet to be proven and its acceptability is only now being tested in situations of high HIV prevalence where there is awareness of breastfeeding MTCT among women. Equally, until exclusive breastfeeding is trialled, adverse effects cannot be excluded, as the volume of breastmilk consumed will be larger than for mixed feeding. Reduction of the duration of breastfeeding has been suggested but is also untested. The consequent reductions in MTCT may be small, and there are substantial economic costs for the family as well as loss of health benefits for the child [11,18,81]. Treatment of all pregnant women with nevirapine in high-prevalence areas has been proposed but must be approached cautiously because of concerns of safety, control of medication, the development of antiviral resistance and cost [61,90].

Options involving only HIV-infected women (Table 5) require large-scale voluntary confidential counselling and HIV testing with support for those found to be HIV-positive. Such services are usually considered an integral part of HIV prevention, care and control. However, they require substantial investment in both training and deployment of staff, and so are unlikely to be available universally. Testing has to be of high quality as false positives and false negatives are highly undesirable. Testing can also have adverse effects for women if performed insensitively and with insufficient skill, or if a woman is pressured to accept an option without being given a genuine choice [6,79,85]. Perinatal antiretrovirals for mother and child have proven effectiveness but pose challenges in drug administration and deciding on optimal regimens; for example, whether to give further nevirapine to breastfeeding babies [18,91]. Promotion of exclusive breastfeeding in the first 4–6 months by HIV-infected women alone is theoretically possible but would best be promoted among all women [11]. Modified use of breastmilk, expressing and pasteurizing human breastmilk, has been suggested [22]. A feasibility study among literate women with primary school education in Zambia concluded they might accept this approach, but that recommendations of heating at set temperatures for 30 min were not feasible [11]. Breastfeeding by another HIV-negative woman has also been suggested, where this is culturally acceptable, but also remains unevaluated. Artificial feeding, or as it is now known in this context, replacement feeding [83], is usually the most appropriate approach in well-resourced settings where the risk associated with use of artificial milks can be minimized [78]. However, there are many potential disadvantages and major feasibility issues in poor and moderate resource settings. Child nutrition needs to be supported up to age 2 years, as an essential part of replacement feeding and family planning must be provided for the mother [22]. The Zambian feasibility study concluded that replacement feeding was not an acceptable option [11]. The costs to the family or society (if feeds are subsidized) of artificial feeds are considerable. In Zambia, 6 months of infant formula

Table 2. Recently demonstrated effects of breastfeeding on child mortality and morbidity in developing countries (studies published since 1994).

Study location and year of study (reference)	Outcome measure (mortality and morbidity)	Study method	Measures of breastfeeding	Controlled variables	Results and comments
26 developing countries, 1992–1998 (measure/DHS+ 1999) [4]	Child survival	Demographic surveys	Duration of any and exclusive breastfeeding	20 socioeconomic and behavioural variables	Short duration of exclusive breastfeeding was associated with decreased likelihood of child survival (Fig. 2a). Long birth interval (> 18 months) was associated with increased likelihood of child survival
Pooled analysis of studies from six countries (Brazil, Gambia, Ghana, Pakistan, Philippines, Senegal), various time periods 1983–1991 [54]	Child deaths (all causes and attributed to infectious disease) under 2 years of age	Cohort and case control	Duration of any breastfeeding	Sex of child, maternal education	Risk of all-cause mortality (based on two countries) associated with not breastfeeding ranged from OR = 4.2 (95% CI, 2.8–6.3) at 0–1 months to OR = 1.7 (95% CI, 1.1–2.5) at 9–11 months. Protection against death from any infectious disease (six countries) ranged from OR = 5.8 (95% CI, 3.4–9.8) at 0–1 months to OR = 1.8 (95% CI, 1.2–2.8) at 9–11 months
Brazil, early 1986–1994 [94]	Infant mortality	Ecological analysis of 140 municipalities	Exclusive breastfeeding in the first 4 months of life	Socioeconomic and behavioural variables	The percentage of infants exclusively breastfeeding was strongly, significantly and independently associated with reduced child mortality (RR = 9.3, $P = 0.0005$). A 10% rise in exclusive breastfeeding was associated with a fall of 6/1000 in the infant death rate (baseline IMR ranged from 102/1000 in 1986 to 80/1000 in 1994)
Malaysia, 1996 [64]	Morbidity: upper respiratory tract infection and diarrhoeal disease	Retrospective cohort analysis	Exclusive breastfeeding versus exclusive artificial feeds	Maternal and paternal education, age and paternal income	In a well-resourced setting and with a small sample, there were no deaths and few hospitalizations, and no evidence of lower rates of URTI or diarrhoeal disease among breastfeeding versus artificial feeding mothers

Malawi, 1987–1992 [95]	Child survival	Demographic survey (Demographic Health Surveys) of live births of 4878 women during 1987–1992	Duration of breastfeeding (number of months) as a discontinuous variable	Varied sociodemographic and environmental variables including maternal and paternal education, maternal education, housing	Children who never breastfed or stopped breastfeeding were four times as likely to die in infancy than those that continued to breastfeed. The effect reducing infant mortality seemed to be mediated through breastfeeding prolonging birth intervals
Mexico, 1988–1999 [96]	Morbidity: cryptosporidium infections	Survey of 403 children attending a hospital emergency room with diarrhoea: comparison of cryptosporidium group ($n = 26$) with all other causes of acute diarrhoea ($n = 377$)	Breastfeeding at time of attendance at hospital	Not controlled for other variables	Breastfeeding was significantly less common in those who had cryptosporidium compared with other cases of acute diarrhoea (9% versus 37%; $P < 0.01$)
Mexico, year unstated [97]	Morbidity: acute respiratory infections and acute diarrhoea	Prospective study of 170 infants followed for 6 months	Fully or partially breastfed or artificially fed	Maternal age and education, marital status, housing conditions, socioeconomic status	Longer duration of full breastfeeding as an independent variable significantly reduced the incidence of ARIs ($P = 0.05$), the days ill with ARIs ($P = 0.04$), the incidence of diarrhoea ($P = 0.02$) and the days ill with diarrhoea ($P = 0.008$)
Philippines, 1988–1991 [98]	Deaths from diarrhoea and acute lower respiratory tract infections	Longitudinal study of 9942 children under 2 years of age followed for up to 2 years	Never breastfed versus early cessation versus continuing to breastfeed	Mother's education, socioeconomic status, previous birth interval (may have over-controlled by controlling for the latter)	In the first 6 months, never breastfeeding or ceasing breastfeeding raised the incidence of deaths due to diarrhoeal disease 8- to 10-fold (95% CI for RR, 4–25). There was no effect on deaths from respiratory infection and the effect did not apply beyond 6 months of age
Nigeria, year unstated [99]	Morbidity: dysentery and persistent diarrhoea	Case–control	Breastfeed	Age	Breastfeeding was associated with decreased risk of having dysentery
South Africa, 1993–1995 [100]	Morbidity and deaths: meningococcal disease	Hospital-based case–control study of 70 cases of disease at all ages and 210 hospital controls	Not breastfed, breastfed for < 3 months, and breastfed for > 3 months	Passive smoking, crowding at home, recent respiratory tract infection	Being breastfed for less than 3 months or not being breastfed at all independently increased the risk of meningococcal disease (OR = 2.4; 95% CI, 1.3–4.4)

CI, Confidence interval; OR, odds ratio; RR, relative risk; URTI, urinary tract infection; ARI, acute respiratory infections.

Table 3. Recently demonstrated effects of breastfeeding on child morbidity in industrialized countries (studies published since 1995).

Study location and year of study (reference)	Outcome measure (morbidity)	Study method	Measures of breastfeeding	Controlled variables	Results and comments
Canada, 1982–1983 [101]	Respiratory, diarrhoeal and all illnesses	Retrospective cohort study at 6 months of age for 776 infants with mothers reporting illnesses	Infants still being breastfed at 6 months versus never breastfed	Infant age, birthweight, day care maternal age, smoking and socio-economic status	After adjustment for the controlled variables, rates of respiratory illness were significantly lower for babies breastfed for 6 months (OR = 0.78; 95% CI, 0.61–1.0) but did not reach significance for gastrointestinal illness
Norway, 1992–1993 [102]	Lower respiratory tract infection	Population-based cohort of 3238 children prospectively followed studying the interaction of breastfeeding and maternal smoking	Length of either any breastfeeding or full breastfeeding in months	Birth weight, maternal age and education, parental income, exposure to air pollution, nationality family history of asthma, home crowding	Breastfeeding for < 6 months compared with longer breastfeeding independently increased the risk of respiratory infections whether mothers smoked (OR = 1.9; 95% CI, 1.3–2.7) or not (OR = 1.4; 95% CI, 1.0–1.8)
Sweden, 1987–1992 [103]	Invasive *Haemophilus influenzae* infection	Case–control	Exclusive breastfeeding	Passive smoking, socioeconomic factors	Short period of breastfeeding was associated with increased risk RR = 3.8 (95% CI, 1.6–8.8). Effect persisted beyond the period of breastfeeding
United Kingdom, 1991–1992 [104]	Wheeze and diarrhoeal disease	Prospectively studied cohort (n = 8501) born 1991–1992	Breastfeeding for 3 months or more	Housing conditions, family size, maternal education and smoking	Breastfeeding for 3 months or more was independently protective against experiencing multiple episodes of wheeze (OR = 0.76; 95% CI, 0.58–0.99) and against experiencing diarrhoea (OR = 0.42; 95% CI, 0.37–0.48). If diarrhoea was experienced by a breastfed infant, it was significantly more likely to be of short duration (OR = 1.34; 95% CI, 1.03–1.75)

United Kingdom, 1983–1993 [105]	Respiratory illness	Cohort	Exclusive breastfeeding for at least 15 weeks	Passive smoking, social class, economic factors, family history of atopy	Children exclusively breastfed experience significantly less risk of respiratory illness by age 7 years (OR = 17%; 95% CI, 16–18%) compared with children artificially fed (OR = 32%; 95% CI, 31–34%)
United Kingdom, 1988–1992 [106]	Meningococcal disease	Population based case–control study of 74 children and at all ages	Ever breastfed versus never breastfed		No protective effect found for breastfeeding, which had similar prevalences in cases (49%) and controls (53%)
United States, 1991–1992 [107]	Pneumonia and gastroenteritis	Uncontrolled population-based intervention study (promotion of exclusive breastfeeding)	Exclusive breastfeeding	NA	Exclusive breastfeeding increased from 16 to 55%. Percentages of infants experiencing bronchitis, gastroenteritis, pneumonia and sepsis all declined significantly
United States, 1988–1990 [108]	Lower respiratory tract infection	Cohort of 1202 infants living in homes without smokers	'Full' breastfeeding	Maternal education, income, family size, family history of atopy	Full breastfeeding independently reduced the risk of lower respiratory infection (OR = 0.81; 95% CI, 0.68–0.96) and the duration of all respiratory illness
United States, date unstated [109]	Ear infection, diarrhoea	Cross-sectional survey	Exclusive breastfeeding *versus no breastmilk*	Maternal education, *income, passive smoking,* family size	Ear infection and diarrhoea were more common in children artificially fed compared with those breastfed

OR, Odds ratio; CI, confidence interval, NA, not available.

Table 4. *Approaches for reducing mother-to-child transmission (MTCT) of HIV[a]: general (whole population) approaches.*

Approach	Advantages	Disadvantages	Likelihood of implementation and success	Feasibility and resource implications	Acceptability
1. General HIV prevention for young people and young adults	May be the most cost-effective policy. Protects parents from infection. Stigma of identifying HIV-infected mothers and their children may be avoided	Could be perceived as neglecting MTCT	May be highly effective in reducing numbers of children being infected	Inputs required are considerable but are those that should be provided by all HIV control programmes. Will need to include information and education about MTCT	Likely to be high if combined with information on MTCT
2. Promotion of exclusive breastfeeding[b]	Available to all women even in areas where testing is not available. May improve child spacing. Beneficial for infant's general health and may benefit mother. In line with current recommendations. Stigma avoided if applied to all women	Currently, very limited data on impact on MTCT of HIV. Exclusive breastfeeding rates are low in some countries	Likely in populations where breastfeeding is the norm both for economic and cultural reasons, but effect on MTCT as yet unknown	Already shown to be feasible in a number of countries but requires continued commitment and reinforcement by service providers, including community education. Requires support networks in the community	Acceptable but difficult to implement in most societies that practice mixed feeding. Difficult to implement where mothers have to work and child-care facilities and maternity protection are unavailable
3. Reduction of duration of breastfeeding (with or without exclusive breastfeeding for first 6 months)	Possible reduction in postnatal transmission. Available to all women even in areas where testing is not available. Stigma avoided if applied to all women	Actual benefits for MTCT are small. Later benefits from breastfeeding would be lost; likely to be an increase in general infant morbidity and mortality. Economic costs to the family are considerable	Difficult in poor communities with inadequate basic needs such as water and sanitation. Need for community education and mobilization	Feasible for women with limited options if required inputs are put in place. Requires support networks in the community	May not be culturally acceptable in populations where breastfeeding is the norm

[a] Note approaches are not mutually exclusive, they might be used in combination.

[b] Current policy is that all babies receive exclusive breastfeeding for at least the first 4 months, and if possible for the first 6 months [19].

or modified cow's milk cost $106, two to four times the average family income [11] (in Kenya, the cost of 6 months artificial feeding was $300 [2]). In addition, there may be increased morbidity and mortality in uninfected HIV-exposed children [53,54]. The approach could be damaging to general breastfeeding promotion and babies born to uninfected women if 'spill-over' occurred [80]. In general, the Zambian project concluded that, although this option should be supported for women who wished to use artificial feeds, this would be difficult without strong community advocacy, sensitization, education and mobilization [11]. A study in South Africa found that many HIV-infected women still preferred to breastfeed, seemingly because artificial feeding would reveal their HIV status to partners [92]. In the artificial versus breastfeeding trial in Nairobi, four-fifths of eligible women declined to participate, principally because of the possibility of being randomized to artificial feeding, and 30% of those in the trial and allocated to this option could not comply [2]. Replacement feeding therefore seems unlikely to be acceptable in societies where breastfeeding is nearly universal. What remains uncertain is how desirable replacement feeding by HIV-infected mothers will be in moderate resource settings where infant formula is already used by a substantial proportion of women. Stigma and loss of confidentiality might not follow, and if families can afford to buy formula there may be no need to subsidize its distribution. Hence, the approach of supporting mothers in carefully controlled use of artificial feeding may be feasible, although issues of economics, child survival and supply of alternative contraception for women remain to be resolved. Careful evaluation and monitoring will need to be undertaken in these settings to assess the impact on feeding patterns, child survival, etc., and parameters for this have been suggested for pilot sites established by UNAIDS, UNICEF and the World Health Organization [6,18]. It will be essential that such detailed monitoring is carried out and that standardized feeding definitions be used in all pilot programmes, surveys and studies (Table 1). For example, the term 'mixed feeding' should be avoided as it covers a wide range of options, including both predominant breastfeeding (where the non-human milk content may be minimal) and complementary feeding (where it may be considerable).

Increasing numbers of women will be HIV infected in the countries of South and South East Asia in the next decade [1]. In those regions, artificial feeding is already more common than in sub-Saharan Africa [4] and, although the policy of counselling and informed choice will be the same, it is not easy to predict the outcome in terms of the percentage of women with known HIV infection who use replacement feeding (Fig. 3) [4].

Research needs

A high priority in the next 2 years will be the careful evaluation in the pilot sites of the impact of voluntary confidential HIV testing and interventions to reduce MTCT including use of antiretrovirals and/or avoiding or modifying breastfeeding [18,89]. Either in these or elsewhere, the feasibility of replacement feeding and modified breastmilk options in different settings needs to be evaluated. There have been many warnings of possible 'spill-over' from artificial feeding by HIV-infected mothers to the general population, but this has yet to be adequately monitored and demonstrated as a real effect. The impact of alternatives to breastfeeding or modified breastfeeding practices on infant morbidity and mortality also needs to be carefully monitored. More monitoring is needed in moderate resource settings where use of artificial feeding is already common but the impact of increasing its use is unknown. Surprisingly little is known also about the effect of breastfeeding on the health of HIV-infected women or how to protect uninfected women from acquiring HIV during breastfeeding when the risk of MTCT is high [33,93]. It needs to be determined whether nevirapine given to breastfeeding infants of HIV-infected mothers reduces MTCT. The possibility that improved breastfeeding practices, such as exclusive breastfeeding, reduces MTCT and trials of improved breastfeeding technique versus normal breastfeeding practice should be undertaken as a matter of urgency.

Conclusions

HIV transmission through breastfeeding is a significant cause of HIV infection in children accounting for one-third to one-half of the estimated 600 000 HIV infections occurring annually in children worldwide. In resource-poor settings, most of these children will die prematurely as a result of HIV. In developing countries, over 95% of infants are initially breastfed, though the duration of breastfeeding is shorter in Latin America and the Caribbean than in sub-Saharan Africa and Asia. Rates of exclusive breastfeeding vary widely between countries. In the 1990s, the quantity and quality of breastfeeding generally improved, and rates increased in a number of developing countries often as the result of breastfeeding promotion. There is no evidence that the risks of child morbidity or mortality associated with artificial feeding have diminished and breastfeeding promotion should be supported and strengthened in all settings irrespective of the prevalence of HIV. The mechanisms for HIV transmission through breast-feeding and the site of entry of the virus are unclear, although there is evidence for both maternal factors and infant susceptibility being important. Mastitis may play a role through increasing viral shedding in breastmilk.

Table 5. Approaches for reducing mother-to-child transmission (MTCT) of HIV: approaches confined to HIV-infected women[a].

Approach	Advantages	Disadvantages	Likelihood of implementation success	Feasibility and and resource implications	Acceptability
1. Counselling on feeding options and perinatal antiretroviral treatment offered to all HIV-infected women and babies or counselling on feeding options alone when antiretroviral treatment is not available[b]	Use of antiretrovirals result in proven reduction in MTCT transmission even when combined with breastfeeding. Use of artificial (formula) feeding alone will also somewhat reduce MTCT	Stigma through identifying HIV-infected mothers and their children. Modification of feeding alone [use of artificial (formula) feeding] can increase child mortality. Need for adequate complementary food with all options	Reasonable, but has proven unfeasible in some common settings [11], and many feasibility questions remain to be investigated in pilot sites	Resource requirements are considerable. In addition to testing (which has to be of particularly high quality) and counselling, need to provide distribution and control of the drugs and resources required for counselling and supporting HIV-infected women, and providing support for chosen feeding method and alternative contraception	Reasonable but receiving HIV test results has been avoided by some HIV-infected women [85] and, in poorly resourced settings, many HIV-infected women have chosen to continue breastfeeding [2]
2. Modified breast-feeding; exclusive or shortened breast-feeding. Expression and treatment of breast-milk (includes the option of wet-nursing by HIV-uninfected women)	Possible reduction in transmission. Some benefits from breast-milk will be preserved. Wet-nursing will lower the risk of transmission	Preparation and storage of expressed milk. Need for adequate complementary feeds. Stigma through identifying HIV-infected mothers and their children in some cases (expressing breastmilk). May be some stigma and there is a need for testing and counselling of wet-nurses	Will need community mobilization and education as well as continued support for the mother and baby. Possible if wet-nurse willing to avoid risk of infection	Resource requirements are less than for approach 1. Exclusive breastfeeding requires child-care facilities and maternity protection for women who have to work options. Need for nutrition counsellors for home visits. Few resources needed: support for wet-nurse	May not be culturally acceptable, although early cessation of breastfeeding may be more acceptable. Culturally acceptable only in some communities
3. Use of commercial breastmilk substitutes	No postnatal transmission provided practiced exclusively	Substantial costs for the family or services. Increased morbidity and mortality among women's HIV-uninfected children. Possible 'spill over' effect to non-infected women. Loss of lactational	Difficult without required inputs, community advocacy sensitization, education and mobilization	Not a feasible option for women with limited resources unless substitutes are free or subsidized and adequate fuel, water and time are available to the mother. Need for	Not acceptable in societies where breastfeeding is the norm. Stigma and loss of confidentiality probably major problems where breastfeeding the norm

		amenorrhoea. Stigma and loss of confidentiality		nutrition counsellors for home visits. May require subsidized artificial feeds, and alternative contraception for women is essential. Sustainability is a major issue		
4. Use of home-produced breastmilk substitutes	No postnatal transmission if practiced exclusively	Some additional costs for the family or services. Increased morbidity and mortality among women's HIV-uninfected children. Possible 'spill over' effect to non-infected women. Loss of lactational amenorrhoea. Stigma and loss of confidentiality	Difficult without required inputs, community advocacy sensitization, education and mobilization	Need for nutrition counsellors for home visits. Alternative contraception for women is essential. Sustainability an issue	Not acceptable in societies where breastfeeding is the norm. Stigma and loss of confidentiality probably major problems	

[a] Note these all require good quality voluntary confidential counselling and HIV testing as false-positive or false-negative test results are highly undesirable.
[b] These approaches may be combined with modifying breastfeeding (approach 2).

General HIV prevention, particularly targeting young women and men, is an important way of reducing the numbers of infected children as well as preserving adult lives. However, this alone offers nothing for women already infected with HIV. The risk of MTCT in breastfeeding populations is between 25% and 45%. Peripartum use of antiretrovirals by HIV-infected mothers reduces the risk even in breastfeeding populations, though the lowest absolute risk occurs if breastfeeding is avoided. It has been proposed that voluntary confidential counseling and HIV testing be offered to all pregnant women, and those found to be infected offered antiretrovirals, and informed about infant feeding options. In resource-poor settings, the avoidance of breastfeeding is unlikely to be feasible, acceptable or affordable, and breastfeeding will remain the most appropriate choice for most women. For infected women whose circumstances allow them to minimise the risks associated with artificial feeding, breastfeeding will usually not be advisable. In moderate-resource settings, artificial feeding is sometimes already fairly widespread, but the impact, desirability and cost-sustainability of its increased use by HIV-infected women is uncertain. In these circumstances it is appropriate for HIV-infected women to decide according to their individual situation. In all these situations women should be supported in their choice of infant feeding method. Careful evaluations are needed to examine the acceptability, impact and sustainability of antenatal HIV testing and interventions in a broad range of settings. Providing personal HIV testing to normally acceptable levels of accuracy in resource-poor settings is difficult and its feasibility on a mass scale has yet to be demonstrated. Pilot programmes, studies and surveys need to employ standardised breastfeeding definitions to allow comparisons to be made. Interventions in which HIV-infected women choose alternatives to breastfeeding must be accompanied by family planning services to preserve birth-spacing. Different modalities of breastfeeding (whether breastfeeding is exclusive or in combination with other fluids, artificial milks, etc) may be important in moderating the risk of MTCT and exclusive breastfeeding in the first 3 months of life might be associated with lower risks of MTCT than mixed feeding. This hypothesis remains unproven; however, exclusive breastfeeding has other benefits and its duration can be increased through breastfeeding support. Its impact on MTCT and child health deserves to be rigorously tested through trials.

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