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PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV-1 USING ANTIRETROVIRAL DRUGS IN DAR ES SALAAM, TANZANIA

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av
Charles Kilewo

Huvudhandledare:
Professor Gunnel Biberfeld
Institutionen för Mikrobiologi, Tumör och Cellbiologi
Karolinska Institutet

Bihandledare:
Professor Fred Mhalu
Department of Microbiology and Immunology
Muhimbili University of Health and Allied Sciences
Dar es Salaam, Tanzania

Professor Andrew Swai
Muhimbili National Hospital
Dar es Salaam, Tanzania

Fakultetsopponent:
Professor Thorkild Tylleskär
Centre for International Health
Bergen, Norway

Betygsnämnd:
Docent Ann-Britt Bohlin
Institutionen för Klinisk Vetenskap, Intervention och Teknik
Enheten för Pediatrik
Karolinska Universitetssjukhuset

Professor Lars-Åke Persson
International Maternal and Child Health
Uppsala University

Docent Gabriella Scarlatti
Viral Evolution and Transmission Unit
San Raffaele Scientific Institute
Milan, Italy

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ABSTRACT

This thesis describes studies of prevention of mother-to-child transmission (MTCT) of HIV-1 in breast-feeding populations in sub-Saharan Africa by perinatal and postnatal prophylactic antiretroviral therapy (ART) of mothers and infants. The acceptability of HIV counseling and testing in pregnant women and the mortality among HIV-1-infected women during 2 years after delivery was also studied.

Three short course regimens of zidovudine (ZDV)+lamivudine (3TC) prophylaxis were evaluated for their efficacy to prevent MTCT of HIV in HIV-1-infected pregnant women in Uganda, Tanzania, and South Africa (the Petra trial). In group A treatment was given antepartum for 2 weeks, intrapartum and postpartum for 7 days to mother and infant. In group B the treatment started intrapartum and continued as in group A. In group C the treatment was given intrapartum only. At 6 weeks after birth the HIV transmission rates were 5.7%, 8.9%, 14.2% and 15.3% in groups A, B, C, and the placebo group, respectively and the relative risks (RR) for transmission in the treatment groups compared to placebo were 0.37, 0.58 and 0.93 respectively. The combined HIV-1 infection or death rates at 6 weeks were 7.0%, 11.6%, 17.5% and 18.1% in groups A, B, C, and the placebo group, respectively and the RR for HIV infection or death compared to placebo were 0.39, 0.64 and 0.97, respectively. After 18 months of follow up the transmission rates were 14.9% (95%CI 9.4-22.8), 18.1% (12.1-26.2), 20.0% (12.9-30.1) and 22.2% (15.9-30.2) for groups A, B, C and the placebo group respectively (Turnbull analysis). Arm A and B were effective in preventing HIV transmission at 6 weeks after birth but the preventive effect had diminished considerably at 18 months because of continued breastfeeding. The implication of this finding is that extended prophylaxis is necessary to reduce postnatal transmission in breast-feeding populations.

Two studies evaluated prevention of postnatal MTCT of HIV-1 through breast milk by extended antiretroviral (ARV) prophylaxis to the infant (the Mitra study) or the mother (the Mitra Plus study) during breast-feeding in Dar es Salaam, Tanzania. In the Mitra study mothers received the Petra arm A regimen and infants 3TC for 6 months. In the Mitra Plus study mothers received highly active ART (HAART) (ZDV+3TC+nevirapine or nelfinavir) irrespective of their stage of infection starting at 34 weeks gestation. Treatment was stopped at 6 months after delivery except in women who needed HAART for their own health. There were 398 infants included in the transmission analysis in the Mitra study and 441 infants in the Mitra Plus study. Cumulative HIV transmission rates determined by Kaplan-Meier survival analysis were 3.8% (95% CI 2.0-5.6) and 4.1% (95% CI 2.2-6.0) at 6 weeks and 4.9% (95% CI 2.7-7.1) and 5.0% (95% CI 2.9-7.1) at 6 months in the Mitra and the Mitra Plus studies, respectively. The cumulative risk of HIV infection between 6 weeks and 6 months was 1.2% and 1% in the Mitra and the Mitra Plus studies, respectively. At 18 months the cumulative transmission was 6.0% (95% CI 3.7-8.3) in the Mitra Plus study. Cox regression analysis showed that the transmission of HIV-1 up to 6 months was about 50% lower in the Mitra and Mitra Plus studies than in the breast-feeding women in group A of the Petra trial ($p \leq 0.001$). These results showed that infant and maternal ARV prophylaxis for 6 months during breast-feeding resulted in similar low transmission rates at 6 months.

A study to determine the acceptability of HIV counseling and testing and participation in a PMTCT intervention was performed at the Dar es Salaam site of the Petra trial. HIV counseling and testing was offered to 10 010 pregnant women of whom 7647 (76.4%) agreed to be tested and 1050 (13.7%) were HIV-1 seropositive. Sixty-eight percent returned to receive results. Only 16.7% of the 288 enrolled HIV-1 seropositive women disclosed their positive HIV serostatus to their sexual partners. Reasons for not disclosing the HIV status were fear of stigma, divorce and violence. Sixty percent (29 of 48) of the informed sexual male partners agreed to HIV testing and 69% (20 of 29) were HIV seropositive. In the Mitra study, out of 10 179 pregnant women counseled 92% accepted rapid HIV testing and 93.6% received their results. In the Mitra Plus study, out of 14 255 pregnant women counseled 95.7% accepted rapid HIV testing and 98.9% received their results. The Petra, Mitra and Mitra Plus studies showed that the proportion of women accepting HIV testing and receiving their test results was high and higher in the last two studies in which the rapid HIV testing strategy was used.

A secondary analysis of the Petra cohort at the Dar es Salaam site was performed to determine the mortality of the HIV-1-infected women during 24 months of follow up after delivery in relation to their CD4 cell count and viral load at enrolment. About 14.5% of the women had CD4 cell counts <200/μL at enrolment and would have benefited from ART for their own health. However, ART was not available in Tanzania at the time of the study. The mortality was 29.9% in women with CD4 cell counts <200/μL and 15% in women with viral load >100 000 copies/mL. The mortality in women with 200-499 CD4 cells/μL was similar to that in women with >500 CD4 cells/μL (3.3% and 2.9%, respectively). In the multivariate analysis low CD4 cell counts and high viral load were both independent risk factors for mortality ($p<0.001$ and $p=0.004$, respectively).

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1 GENERAL BACKGROUND

1.1 Introduction

Since the beginning of the 1980s the world has witnessed a pandemic of an unimaginable proportion due to the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome (AIDS), which has impacted severely on resource-limited countries particularly in sub-Saharan Africa. HIV infects people in the most economically productive and reproductive age groups and the infection is potentially 100% fatal without a life long antiretroviral therapy (ART). AIDS is among the leading causes of death globally and is the major cause of death of adults in sub-Saharan Africa. The HIV pandemic is unlikely to be controlled unless medical science develops a treatment accessible to all and a successful vaccine to prevent infection.

AIDS is characterized by depletion of CD4+ T lymphocytes and clinically by opportunistic infections and certain malignancies, mainly Kaposi´s sarcoma and B cell lymphomas (Gottlieb et al. 1981, CDC 1981). The syndrome is caused by two retroviruses, HIV types 1 (HIV-1) and 2 (HIV-2), first described during the early 1980s (Barre-Sinoussi et al. 1983, Gallo et al. 1984, Clavel et al. 1986, Albert et al. 1987). Most of the HIV infections in the world are due to HIV-1. HIV infection has proved difficult to control because the virus integrates in the genome of the cells it attacks leading to persistent infection, which has a silent period of many years from initial infection to the onset of serious symptoms.

HIV is transmitted through sexual intercourse, exposure to infected blood and blood products, injection drug use and through transmission from mother to child. The subject of HIV and AIDS is usually associated with stigma, discrimination and denial, which have frequently prevented the adoption and application of rational measures of prevention and control. The HIV epidemic experienced by women and children in the African region threatens to slow down the progress of achieving the Millennium Development Goals (MDGs number 4, 5, 6) of reducing child mortality, improving maternal health and combating HIV/AIDS, malaria and other diseases (UN 2009). Although strategies for HIV prevention and care for people living with HIV are now better understood, the burden of disease and suffering continues especially among women and children in resource-constrained populations world wide.

1.2 The epidemiology of HIV infection

1.2.1 Global situation

The UNAIDS/WHO 2008 report on the global AIDS epidemic showed that, in 2007, 33 million (30-36 million) people were estimated to be living with HIV including 2.0 million (1.9-2.3 million) children <15 years. It was estimated that in 2007, 2.7 million (2.2-3.2 million) people became newly infected, including 370 000 (330 000-410 000) children <15 years, a decline from 3 million, estimated in 2001. In 2007, more than 7400 new HIV infections occurred per day of which 1000 were attributed to children <15 years. In the same period deaths due to AIDS were estimated to be 2.0 million (1.8-2.3 million) people, including 270 000 (250 000-290 000) children <15 years. Since 2001, when the United Nations Declaration of Commitment on HIV and AIDS was signed by the world national leaders, the rate of HIV infections (adults and children) in sub-Saharan Africa and in industrialised countries has declined. However, in Eastern Europe and Central Asia it increased from 630 000 (490 000-1.1 million) in 2000/2001 to 1.5 million (1.1-1.9 million) in 2007. In East Asia the estimated number of people living with HIV in 2007 was 740 000 (480 000-1.1 million) and in South East Asia 4.2 million (3.5-5.3 million). Indonesia had the fastest growing epidemic of HIV (UNAIDS/WHO 2008).

Overtime since the description of the epidemic the number of people infected with HIV and those who progressed to AIDS increased steadily because there were no robust interventions to prevent and control spread of the virus especially in developing countries which have carried the heaviest burden of the epidemic. Many developing countries have received massive financial support to fight the epidemic and in collaboration with development partners and donors considerable progress has been made since 2001, in addressing the national epidemics. Developing countries have also committed more funds from their annual budgets to HIV prevention and control programs and adopted recommended HIV policies. In the past 8 years there has been improved and expanded HIV surveillance systems in low-resource countries and new, population-based studies have been conducted which provided more accurate information about the HIV prevalence than in the past. This has resulted in better and more accurate estimation of people infected with HIV. As a result this has led to substantial revisions of the past global and in country estimates of the HIV and AIDS epidemic. The UNAIDS/WHO

report showed that the global HIV prevalence has levelled off and that the number of new infections has fallen, in part as a result of the impact of HIV prevention programmes but also due to deaths of people infected in the previous years.

1.2.2 HIV infection in sub-Saharan Africa

In most of sub-Saharan Africa the HIV epidemic has stabilized or begun to decline. However, the region still remained the most severely affected in the world. In 2007, an estimate of 22 million (20.5-23.6 million) people (adults and children) were living with HIV, representing 67% of the global total and 75% of deaths due to AIDS occurred in the same region. Women represent 58% of adult people living with HIV in the region (UNAIDS/WHO 2008). An estimated 1.9 million (1.6-2.1 million) new HIV infections occurred in sub-Saharan Africa in 2007. Seven countries in this region (Botswana, Lesotho, Namibia, South Africa, Swaziland, Zambia and Zimbabwe) had an adult national HIV prevalence exceeding 15%.

Following various interventions implemented over a number of years a declining trend of the magnitude of HIV infection especially among young women has been reported in several African countries. Significant reductions in both HIV prevalence and incidence were demonstrated already in the 1990s in Uganda (Mbulaiteye et al. 2002) and in the Kagera region of Tanzania (Kwesigabo et al. 2000, Kwesigabo et al. 2005). Declines in HIV prevalence have also been reported from other areas, e.g. Ethiopia (Tsegaye et al. 2002), Kenya (Cheluget et al. 2006), various parts of Tanzania (Kwesigabo et al. 2005, Urassa et al. 2006, Msuya et al. 2007), Zambia (Fylkesnes et al. 2001, Stringer et al. 2008), Zimbabwe, Botswana and Côte d´Ivoire (UNAIDS/WHO 2008).

1.2.3 HIV and AIDS in Tanzania

The United Republic of Tanzania currently has an estimated population of about 40 million people. Tanzania is ranked as one of the poorest countries in the world with gross domestic product per capita of USD 660 (2005) and with 48% of the population living in absolute poverty with less than USD 2 a day. Annual government expenditure is about USD 16 per capita on health. Though the country has benefited from a long period of political stability since independence and the economy has been improving steadily, these improvements are being

threatened by the growing impact of HIV and AIDS which is a considerable roadblock to development, to improvement of the health of the people and a threat to child survival.

Infection with HIV in Tanzania is due to HIV-1 and to-date no HIV-2 infections have been reported. The HIV-1 clades circulating in the country are mainly A, C, D and their recombinants (Renjifo et al. 1998, Lyamuya et al. 2000, Koulinska et al. 2001, Hoelscher et al. 2001, Kiwelu et al. 2003). The heterosexual route is by far the most important mode of transmission accounting for 78% of HIV infection transmission in 2004 followed by mother-to-child transmission (5%). Transmissions through blood and contaminated needles accounted for 0.5%. In about 17.0% of the HIV infections, the mode of transmission was not known [National AIDS Control Programme (NACP) 2005]. The first cases of AIDS in Tanzania were reported in 1983 in the Kagera region of northwest Tanzania and were serologically confirmed two years later (Mhalu et al. 1987). This was followed by a rapid spread of the epidemic to other parts of the country (Mhalu et al. 1987, Killewo et al. 1990, Kigadye et al. 1993, Killewo et al. 1993, Mnyika et al. 1994, Kwesigabo et al. 1996, Petry et al. 1996, Kwesigabo et al. 1998, Kapiga et al. 2002, the NACP annual reports). The rate of spread of HIV-1 in Tanzania can also be seen by examining the trend of the prevalence among women attending antenatal clinics (ANCs) in Dar es Salaam, the commercial capital city of Tanzania where the prevalence was 1.3% in 1984/85 (Haukenes et al. 1992), 3.6% in 1986 (Mhalu et al. 1987), 15.2% in 1993 (Mwakagile et al. 1996), 13.7% in 1996 (Kilewo et al. 2001) and 11.0% in 2001-2003 (Kilewo et al. 2008). There have been several reports of declining prevalence and incidence rates of HIV infection from different parts of the country. In population-based studies conducted in Bukoba urban, Kagera region, the age adjusted prevalence of HIV infection declined from 24.2 % in 1987 to 18.2% in 1993 and down to 13.3% in 1996 (Kwesigabo et al 2005). The HIV prevalence among ANC attendees in the Kagera region has also shown a decline (Kwesigabo et al. 2000). The HIV-1 incidence in Bukoba urban declined from 47.5 per 1000 person-years of observation in 1989 to 9.1 per 1000 person-years in 1996 (Kwesigabo et al. 2005).

The national estimates for 2007 showed that the average national HIV prevalence for adults aged 15-49 years was 6.2% (5.8-7.2%) and the number of people living with HIV was on average 1.4 million (1.3-1.6 million), including 760 000 women (58%) and 140 000 children 0-14 years. There were regional variations in HIV prevalence rates. The highest estimated prevalence rates in 2007 were found in the three regions of Iringa (13.5%), Mbeya (13.4%) and

Dar es Salaam (10.9%). The estimated number of deaths due to AIDS during 2007 were 140 000 (110 000-180 000) cases (UNAIDS 2008).

1.3 Virology of HIV

HIV is a retrovirus belonging to the family Lentivirinae, which is characterized by the presence of an RNA genome and a reverse transcriptase (RT) enzyme necessary in viral replication. HIV is characterized by extremely high genomic variability and high production of viral particles (Levy 1993).

HIV-1 occurs worldwide whereas HIV-2 is primarily found in West Africa. HIV-1 is divided into three groups: M (main), O (outlier) and N (non-M non-O). Group M which represents the main group of HIV-1 strains circulating worldwide has been subdivided into nine different subtypes or clades (A-D; F-H; J-K) which have different geographical distribution (Essex 1999, Alaeus 2000, Thomson et al. 2002). Subtype B is mainly found in Europe and North America, while the other subtypes are found in Africa, Asia and South America. Subtype C is the most widely spread globally (Essex 1999, Alaeus et al. 2000, Thomson et al. 2002). Studies in Africa have shown wide distribution of various HIV-1 subtypes including recombinant viruses (Essex 1999, Alaeus et al. 2000, Holm-Hansen et al. 2000, Koulinska et al. 2001, Blackard et al. 2002). The original subtypes E and I have been shown to be recombinant viruses (Levy 2009). Recombination of genes from different subtypes can occur if a cell is infected with two different viruses. If an inter-subtype recombinant virus is transmitted from one person to others and becomes one of the circulating strains in the epidemic it can be classified as a circulating recombinant form (CRF). In Tanzania subtypes A, C and D and recombinant forms have been reported to be circulating (UNAIDS/WHO 1997a, Lyamuya et al. 2000, Holm-Hansen et al. 2000, Hoelscher et al. 2001, Renjifo et al. 2001, Kiwelu et al. 2003). It has been reported that subtype A virus infections have a slower disease progression than infections with other subtypes (Kanki et al. 1999, Kaleebu et al. 2002). HIV-1 can also be divided in two groups depending on the use of the co-receptors CCR5 or CXCR4 (Berger et al. 1999). Viruses which utilize the CCR5 co-receptor are called R5 viruses and viruses which utilize the CXCR4 receptor are called X4 viruses. R5 viruses are also known as macrophage-tropic and non-syncytium-inducing viruses and X4 viruses as T cell-tropic and syncytium-inducing viruses (Levy 2009). Dual-tropic R5/X4 viruses also exist. R5 viruses predominate during early

infection whereas the more cytopathic X4 viruses are more frequent during later symptomatic stages of infection (Levy 2009).

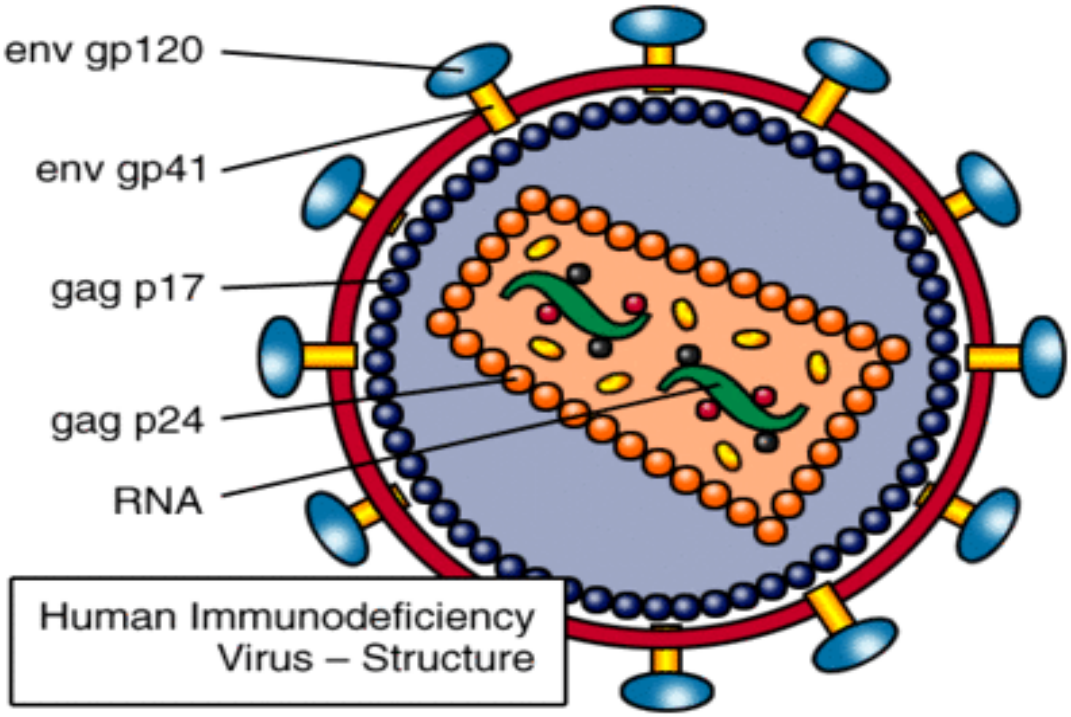

Figure 1.

The HIV particle is spherical with a diameter of 100 nm. It has an outer lipid bilayer which is of host cell origin. The lipid bilayer surrounds an inner nucleocapsid and a central core (Figure 1). The outer membrane contains glycoprotein (gp) complexes each composed of trimers of external gp120 responsible for virus attachment to cells and a transmembrane spanning protein (gp41) responsible for membrane fusion. The matrix protein (gp17) is anchored to the inside of the viral lipoprotein membrane. A protein membrane (gp24) with a molecular weight of 24 000 daltons surrounds the core. The HIV-1 proviral genome is 9.7 kilobytes in length consisting of three major genes, *gag*, *env* and *pol*. The *gag* gene codes for the capsid proteins where the precursor protein p55 is processed to gp17 (matrix), gp24 (capsid), p7 (nucleocapsid) and p6 by viral protease. The *env* gene codes for gp120 and gp41, which are cleaved from the precursor gp160. The *pol* gene codes for the viral enzymes, RT, protease and intergrase. Six genes *vif*, *vpr*, *tat*, *rev*, *vpu* and *nef* code for regulatory and accessory proteins which are essential in the viral life cycle including viral infectivity (Vif), transport of envelope protein to the cell surface

(Vpu), down regulation of viral replication (Nef) or amplification of viral gene expression (Tat). The genome is flanked at its 5´ and 3' end by long terminal repeats (LTRs) (Rubbert et al. 2007).

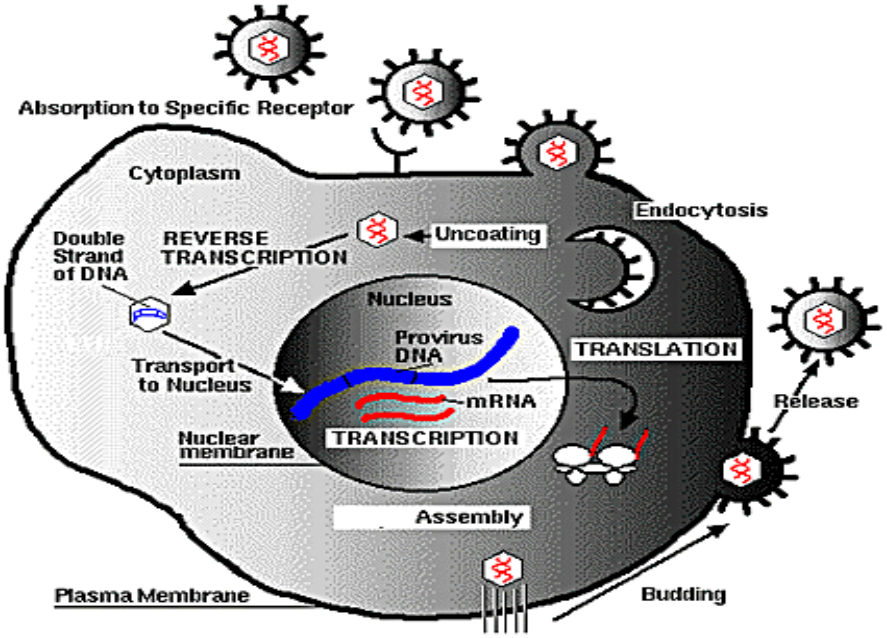

Figure 2. HIV replication cycle

HIV targets cells expressing the CD4 surface antigen. The CD4+ host cells are T-helper lymphocytes, monocytes/macrophages and dendritic cells. The first step in the viral life cycle is binding of the viral envelope to the CD4 receptor which leads to conformational changes in gp120 and subsequent binding to one of the main co-receptors CCR5 or CXCR4 (Figure 2). The transmembrane gp41 mediates fusion between the viral and cellular membranes which results in the release of the viral core into the cytoplasm. After uncoating, reverse transcription of the viral RNA to double-stranded DNA takes place and the viral DNA is transported to the cell nucleus and integrated into the host chromosomal DNA. Cellular activation is necessary for integration of the proviral DNA. The integrated provirus serves as the template for viral transcription to RNA which is translated to viral proteins. Viral poly-proteins are cleaved by HIV protease. The virion is assembled at the cell membrane. Budding through the cell membrane leads to the formation of mature infectious virus (Rubbert et al. 2007).

1.4 Modes of transmission of HIV infection

The main modes of transmission of HIV include sexual intercourse, mother-to-child transmission (MTCT), exposure to infected blood and blood products and use of contaminated medical devices including un-sterile syringes. In North America, Europe and parts of Asia sexual intercourse between men and injection drug use are the predominant modes of HIV transmission. In sub-Saharan Africa, including Tanzania, HIV is primarily transmitted through heterosexual intercourse (UNAIDS/WHO 2002, NACP 2001]. A number of risk factors facilitate HIV transmission. These include high viral load (Quinn et al. 2000), having multiple sexual partners and presence of ulcerative and non-ulcerative sexually transmitted diseases (STD) which have been shown to increase the risk of HIV-1 transmission due to breaches in the genital epithelium (Plummer et al. 1991, Lamptey 2002, Kapiga 1998, Tengia-Kessy 1998). Male circumcision has been shown to be protective against HIV acquisition for males (Weiss et al. 2008).

1.5 Natural history of HIV-1 infection

The clinical course of HIV infection can be divided into different phases including primary infection, a chronic asymptomatic phase and a symptomatic phase with ultimate AIDS. The WHO clinical staging system which is usually used for clinical classification of HIV infection and disease in developing countries includes 4 main stages (WHO 1990). Studies from developed countries on the natural history of HIV-1 infection have established that in the absence of ART the median time for an infected person to develop AIDS is about 10 years (Rutherford et al. 1990, Veugelers et al. 1997, Vergis et al. 2000). However, the course of infection varies among HIV-1-infected individuals and is influenced by viral factors such as viral load and viral phenotype and host factors including age, immune response, CCR5 gene defects and human leucocyte antigen (HLA) type. Individuals who are homozygous for a 32 base pair deletion in CCR5 are resistant to infection with R5 viruses (Liu et al. 1996). Heterozygotes for the deletion show a slow progression to AIDS (Dean et al. 1996). Certain HLA types, including HLA-B27 and -B57 are also associated with slow disease progression (Levy 2009). A small population of HIV-1-infected individuals have a rapid decline in CD4+ T cells within 2-3 years and develop full blown AIDS within 3-5 years after primary infection, so-called rapid progressors (Hogan et al. 2001). They usually have high plasma HIV-1 RNA

levels and harbour virulent syncytium-inducing X4 viruses (Fauci 1996). Another minority (approximately 5%) of HIV-1-infected individuals have normal CD4+ cell counts, low plasma HIV-1 RNA levels and show no signs of disease for more than 10 years, so-called long-term non-progressors (LTNP). A subgroup of LTNPs called elite controllers do not have detectable viral load (<50 RNA copies/mL) (Saez-Cirion et al. 2007). A few studies have been conducted to determine the natural history of HIV infection in Africa (Anzala et al. 1995, Leroy et al. 1995, Jaffar et al. 1997, Morgan et al. 2002, Salamon et al. 2002, Urassa et al. 2004, Bakari et al. 2004). In a study among commercial sex workers in Kenya the median time to AIDS was only 4.4 years (Anzala et al. 1995). However, recent studies in Africa showed no significant difference in the rate of HIV-1 disease progression when compared to that in Europe and North America (Morgan et al. 2002, Urassa et al. 2004, Bakari et al. 2004).

1.5.1 Primary HIV infection

Acute primary HIV-1 infection, which lasts from a few weeks to a few months, is defined as the period from infection until the immune response to HIV gains some control over viral replication. This stage of the disease process can be symptomatic in about 40-90% of individuals (Altfeld and Walker 2007). The most common symptoms which in general appear 2 weeks after exposure include fever, maculopapular non-pruritic rash, oral ulcers, lymphadenopathy, arthralgia, pharyngitis, malaise and myalgia. During this period patients have high levels of HIV-1 replication and experience a rapid decline of CD4+ T cells (Gaines et al. 1990, Vergis et al. 2000, Lindbäck et al. 2000, Altfeld and Walker 2007). There is a severe destruction of memory CD4+ lymphocytes particularly in the lymphoid tissue of the gut (Guadalupe et al. 2003). At this stage these patients are very infectious. After the initial peak of viraemia the plasma viral RNA concentration decreases drastically reaching an equilibrium level referred to as the ´´viral set point" 2 to 6 months after initial infection. It is well established that the viral load set point after acute infection is a strong predictor of the rate of subsequent fall of CD4+ T lymphocytes and of clinical course of the disease (Mellors 1995, Lyles et al. 2000, Giorgi et al. 2002). Once infection is established viral replication persists and is associated with continuing destruction of infected CD4+ T cells. A reservoir of latently infected cells is also established in the body, where the virus remains viable indefinitely as integrated proviral DNA (Levy et al. 1993).

1.5.2 Chronic asymptomatic infection

In most individuals primary HIV infection is followed by an asymptomatic period which is characterized by relative stabilization of the level of viraemia at viral set point, chronic immune activation and persistent viral replication despite lack of signs and symptoms of disease (Vergis et al. 2000). During this clinical latency period, the number of circulating CD4+ T cells falls less rapidly than during the primary acute infection. However, eventually the effective cytotoxic T lymphocyte (CTL) responses decline and plasma viraemia escalates. The declining CTL activity is considered to be due to the development of viral escape mutants that evade recognition by CTL (Vergis et al. 2000).

1.5.3 Symptomatic disease and AIDS

Advanced disease is characterized by an AIDS-defining illness and a decline in the levels of circulating CD4+ T cells to below 200 cells/µL and high levels of plasma viraemia (Levy 1993). The severe immunosuppression results in susceptibility to various opportunistic infections and development of certain tumors. In sub-Saharan Africa tuberculosis and other bacterial infections, fungal infections including candidiasis and cryptococcal infections and infections with herpes simplex virus types 1 and 2 and varicella-zoster virus are among the most common infections in HIV-infected patients (Onen 2002). The most common tumors are Kaposi´s sarcoma and B cell lymphoma (Thomas 2001). Several studies have shown that the principal cause of Kaposi´s sarcoma is infection with human herpesvirus 8 (Biberfeld et al. 2008).

1.6 Immunopathogenesis of HIV infection

The central immunologic feature of HIV-1 disease is the loss of CD4+ T lymphocytes and several potential mechanisms of destruction of CD4+ T lymphocytes have been suggested (Fauci 1996, Levy 2009). They include mainly a) direct cytopathic effects of HIV and its proteins, b) apoptosis induced by immune activation, c) CD8+ cytotoxic T lymphocyte activity, d) antibody-dependent cellular cytotoxicity (ADCC) which can occur when non-infected CD4+ T cells are coated by antibodies to gp120 and become susceptible to destruction by natural

killer (NK) cells, e) destruction of bone marrow and lymphoid tissue (e.g. thymus) with reduced production of new cells and f) anti-CD4+ cell autoantibodies.

Peripheral blood monocytes and tissue macrophages are important in the immunopathogenesis because they are relatively resistant to the cytopathic effects of HIV and thus may serve as tissue reservoirs of infection (Fauci 1996). Most of the HIV within infected monocytes and macrophages are sequestrated in intracellular vacuoles, thus they escape detection by the immune system. Moreover, monocyte/macrophages release proinflammatory cytokines some of which are potent inducers of HIV expression in CD4+ T cells and other infected monocytes and macrophages.

1.7 HIV-1 specific cellular immune responses

CD8+ T cells are able to kill virus-infected cells. A number of studies have demonstrated that HIV-1 specific CD8+ cytotoxic T cells play an important role in control of viral replication. The appearance of HIV-1 specific CD8+ T cells coincides in time with the initial decline of HIV viral load in primary infection (Koup et al. 1994) and precedes the appearance of neutralizing antibodies. It has been demonstrated that HIV-infected non-progressors preferentially maintain highly functional HIV-specific CD8+ T cells compared to progressors (Betts et al. 2006). A large study of HIV-1-infected individuals in South Africa has shown that CD8+ T cell responses to Gag and broad Gag-specific responses were associated with low viremia (Kiepiela et al. 2007). Furthermore, the development of CTL escape mutants in HIV patients with chronic infection has been shown to be associated with a rapid decline in CD4+ T cells (Goulder et al. 1997). CD8+ T cell activity is dependent on CD4+ T cell help. Strong HIV specific CD4+ T cell responses have been shown to be associated with control of viremia (Rosenberg et al. 1997). Specific CD8+ T cells are also detectable in patients with AIDS but may not have the capacity to kill (Levy 2009). HIV-1-specific CD4+ and CD8+ cell responses have been demonstrated in HIV-exposed uninfected individuals (Rowland-Jones and McMichael 1995, Hirbod and Broliden 2007). Furthermore, HIV-specific proliferative T cell responses have been shown to be associated with reduced HIV acquisition in Kenyan sex workers (Hirbod et al. 2008).

In addition to the adaptive HIV specific cytotoxic activity, CD8+ T cells from HIV-infected patients also display an innate noncytotoxic CD8+ cell activity mediated by a CD8+ cell antiviral factor (CAF) (Levy 2009). Chemokines, including MiP-1α, MiP-1β and Rantes may account for a part of this antiviral activity (Cocchi et al. 1995). High CAF activity has been found in LTNPs (Levy 2009).

1.8 HIV-1 specific antibody responses

HIV-1 specific binding antibodies are usually demonstrable earlier after initial infection than neutralizing antibodies. Neutralizing antibodies to autologous virus develop in HIV-1-infected individuals. However, viral mutants resistant to neutralization evolve and antibodies in chronically infected individuals can usually neutralize earlier virus isolates but not contemporaneous viral isolates (Albert et al. 1990, Richman et al. 2003). A small number of broadly neutralizing human monoclonal antibodies that react with gp120 or gp41 has been identified (Burton et al. 2005). Experiments in macaque models have shown that passive immunisation of macaques with a combination of human neutralizing monoclonal antibodies prevented infection after simian-human immunodeficiency virus (SHIV) challenge (Mascola et al. 2003, Ruprecht et al. 2003). Neutralizing antibodies to HIV-1 have been demonstrated in the cervical fluid but not in blood of HIV-exposed non-infected women (Hirbod and Broliden 2007). Genital neutralizing IgA has been shown to be associated with reduced acquisition of HIV infection in Kenyan sex workers (Hirbod et al. 2008). The presence of HIV-1-specific antibodies active in ADCC in HIV-1-infected individuals has been shown to correlate with slow clinical progression (Ahmad et al. 2001).

1.9 Treatment of HIV-infected individuals

The use of highly active antiretroviral therapy (HAART) which usually includes a combination of three antiretroviral (ARV) drugs was introduced in 1995/96. HAART has dramatically reduced HIV-related morbidity and mortality resulting in longer survival of people living with HIV. There are five classes of licensed ARV drugs, nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), e.g. zidovudine (ZDV) and lamivudine (3TC), non-nucleoside reverse transcriptase inhibitors (NNRTIs), e.g. nevirapine (NVP) and efavirenz, protease inhibitors, entry inhibitors and integrase inhibitors (Hoffman and Mulcahy 2007, Hammer et al.

2008). In resource-rich countries first line HAART regimens usually include a protease inhibitor while in developing countries first line HAART regimens usually contain two NRTIs and one NNRTI which is a cheaper combination than use of protease inhibitor-containing combinations. Major problems associated with the use of ARV drugs include poor adherence, development of toxic effects and emergence of HIV resistance to the ARV drugs. In developing countries, especially sub-Saharan Africa, an initiative of access to treatment for eligible individuals was established in 2003 by the UNAIDS/WHO, following the availability of safe and effective generic preparations of ARV drugs and the reduction of prices of ARV drugs. This programme had successfully been able to enroll nearly 3 million people on ART in low and middle-income countries by the end of 2007 (WHO/UNAIDS/UNICEF 2008).

1.10 Laboratory diagnosis of HIV infection

Routine laboratory diagnosis of HIV infection is based on the demonstration of HIV antibodies (Read 2007). The most commonly used methods for detection of HIV antibodies are enzyme-linked immunosorbent assays (ELISAs) and various types of rapid simple assays. New generation ELISAs combine antibody and p24 antigen tests. In resource-rich countries screening for HIV antibodies is usually done by ELISA and reactive samples are then tested by a supplementary or so-called confirmatory assay, most commonly a Western blot assay or other types of immunoblot assays. In resource-limited areas the Western blot assay is seldom used because of the high cost. Instead, cheaper alternative testing strategies are used, including a combination of two or three rapid simple antibody assays or ELISAs. HIV antibody assays are not suitable for the diagnosis of HIV infections in infants younger than 18 months because of the presence of passively transferred maternal antibodies. In infants HIV infection is diagnosed by gene amplification techniques, usually by a qualitative HIV-1 DNA polymerase chain reaction (PCR) assay using peripheral blood mononuclear cells. An alternative is to use HIV-1 RNA PCR assays which detect plasma viral RNA (Read 2007). Detection of p24 antigen for diagnosis of HIV-1 infection in infants has also been used, especially in resource-limited settings (Read 2007, Lyamuya et al. 1996).

1.11 Mother-to-child transmission of HIV infection and its prevention and control

MTCT of HIV-1 is the most common route of transmission to children. Transmission of HIV-1 from an infected mother to her child can occur during pregnancy, birth and during breast-feeding. In the absence of prevention of MTCT (PMTCT) interventions, the risk of MTCT of HIV-1 ranges from 25 to 48% in developing countries where breast-feeding is commonly practiced and from 14 to 32% in populations where HIV-infected mothers do not breast-feed (De Cock et al. 2000, Edgeworth and Ugen 2000). The majority of transmissions occur at the end of pregnancy, during labour and delivery and during breast-feeding. The risk of in utero transmission is 5-10% and of intrapartum transmission 10-20% (De Cock et al. 2000). Most of the intrauterine infections occur during the last months of pregnancy (Ehrnst et al. 1991, Lallemant et al. 2000). Estimates of the risk of transmission through breast-feeding vary from 10-20% (Dabis and Ekpini 2002). In a study of children born to HIV-1-infected mothers in Dar es Salaam, there was a late postnatal HIV transmission rate of 6.2 per 100 child-years between 6 and 27 months of age (Karlsson et al. 1997). A randomized clinical trial in Nairobi, Kenya which compared HIV-1 transmission in breast-fed and formula-fed children showed that the breast milk HIV-1 transmission risk was 16.2% during a 2-year follow up period (Nduati et al. 2000). The total MTCT attributable to breast-feeding was 44% (Nduati et al. 2000). In a meta-analysis of data from 9 studies of late postnatal HIV transmission (after 4 weeks of age) in breast-fed infants, the overall risk of late postnatal transmission was 8.9 transmissions per 100 child-years of breast-feeding and the cumulative probability of postnatal transmission at 18 months was 9.3% [The Breastfeeding and HIV International Transmission Study (BHITS 2004)]. Maternal, obstetrical, viral and child factors have been associated with increased risks for MTCT of HIV globally (European Collaborative Study 1992, Pitt et al. 1997, Edgeworth and Ugen 2000, Renjifo et al. 2001, Mwanyumba et al. 2002, Lehman and Farquhar 2007). The main maternal risk factors are high levels of HIV-1 RNA in plasma and breast milk and low CD4+ T cell count. High viral load is an independent risk factor which is more strongly correlated with transmission than the CD4+ cell level (Garcia et al. 1999, Mofenson et al. 1999, Kilewo et al. 2008, Thomas et al. 2008). High levels of both cell-free and cell-associated virus in breast milk correlate with risk of transmission (Lehman and Farquhar 2007). Primary infection during pregnancy or breast-feeding increases the risk of MTCT of HIV. Obstetrical and fetal risk factors for HIV-1 transmission include chorioamnionitis, prolonged rupture of

membranes, premature delivery and low birth weight (Edgeworth and Ugen 2000, Lehman and Farquhar 2007). Caesarean section reduces the risk of HIV transmission compared to vaginal delivery (The international perinatal HIV group 1999). Mastitis increases the risk for transmission through breast-feeding (Lehman and Farquhar 2007). The possible influence of HIV-1 subtypes in MTCT of HIV-1 has been studied (Murray et al. 2000, Renjifo et al. 2001). A study in Tanzania demonstrated that subtype C was preferentially transmitted in utero compared to subtype A and D (Renjifo et al. 2004). Chemokine receptor usage of maternal viruses is not a predictor of MTCT of HIV-1. Most HIV-1 infected newborns and their mothers carry CCR5-using viruses (Scarlatti 2004). In utero infection probably occurs mainly through the placenta either through placental lesions or by infection of placental cells (Scarlatti 2004). Intrapartum and postpartum infections occur through the oral route when the infant is exposed to infected maternal blood, genital secretions and breast milk. HIV can bind to galactosyl ceramide receptors on intestinal mucosal cells. Intestinal dendritic cells express DC-SIGN which also can serve as a receptor for HIV (Scarlatti 2004, Lehman and Farquhar 2007, Levy 2009).

Maternal and infant innate immunity as well as HIV-1-specific immune responses can affect MTCT of HIV-1 (Lehman and Farquhar 2007). Innate factors include chemokines and secretory leucocyte protease inhibitors in breast milk, genital secretions and saliva. Studies of maternal HIV-specific neutralizing antibody responses as possible prognostic markers of MTCT of HIV-1 reported during the 1990s gave inconsistent results (Scarlatti 2004). However, more recent studies indicate that maternal neutralizing antibodies play a role in preventing MTCT of HIV-1 (Lehman and Farquhar 2007). Furthermore, studies in monkey models have shown protection against oral AIDS virus challenge in newborn macaques passively immunized with a combination of human neutralizing monoclonal antibodies (Ruprecht et al. 2003). HIV-specific CD4+ T-helper cell responses as well as CTL responses have been demonstrated in HIV-1-exposed uninfected infants which appeared to be associated with protection against MTCT of HIV-1 during delivery and breast-feeding (Kuhn et al. 2002, Lehman and Farquhar 2007). In a recently reported study, maternal and infant NK cell responses to HIV-1 were found to be associated with protection against MTCT of HIV-1 (Tiemessen et al. 2009).

1.11.1 Prevention of MTCT of HIV-1

Interventions to reduce MTCT of HIV have focused on prophylactic ART, mode of delivery and counselling on infant feeding options including practising exclusive breast-feeding. In 1994 the ACTG 076 PMTCT trial conducted in the US and France demonstrated that it was possible to reduce the HIV-1 transmission rate from 25% to 8% with ZDV prophylactic treatment given orally to the mother from the second or early third trimester of pregnancy, intravenously during labour and delivery and orally to the child during the first 6 weeks (Connor et al. 1994). Prophylactic HAART of the mother during pregnancy followed by caesarean section for delivery and ZDV to the infant during the first six weeks of life has further demonstrated reduced transmission rates to less than 2% in non-breast-feeding populations in industrialised countries (The international perinatal HIV group 1999, Dorenbaum et al. 2002, Cooper et al. 2002, Cooper et al. 2005). Caesarean delivery is expensive and can be associated with high postoperative complications. It is not an intervention that can easily be implemented in many developing countries.

Following the success of the ACTG 076 trial, several studies of short-course regimens of ARV prophylactic treatment were conducted in resource-limited countries which demonstrated a reduction of MTCT of HIV by 35-67% (Dabis et al. 1999, Wiktor et al. 1999, Guay et al. 1999, the Petra study team 2002, Leroy et al. 2005, Fowler et al. 2007). Trials in Côte d'Ivoire and Burkina Faso using short-course ZDV treatment from 36 weeks gestation to the peripartum period showed a 37% reduction of MTCT at age 3 months (Dabis et al. 1999, Wiktor et al. 1999). The use of NVP intrapartum in the HIVNET 012 trial in Uganda led to a reduction of the risk of MTCT of HIV during the first 14-16 weeks of life by nearly 50% in breast-feeding women (Guay et al. 1999). The Petra trial conducted in three sub-Saharan African countries, including Tanzania showed that a combination of ZDV and 3TC given from 36 weeks of gestation to the end of the first week after delivery had an efficacy of 63% by 6 weeks after delivery (The Petra study team 2002, Paper 1). A trial in Thailand in non-breast-feeding women revealed that initiating ZDV from 28 weeks of gestation and continuing with extended infant prophylaxis for 3 days (long short arm) or 6 weeks of life (long long arm) led to a substantial reduction of MTCT to 4.7% and 6.5% respectively, at 6 months after birth compared to a transmission rate of 10.5% in the short arm in which prophylaxis was given from 35 weeks gestation to delivery and the infant received ZDV prophylaxis for 3 days only postnatally

(Lallemant et al. 2000). In a subsequent study conducted in Thailand further reduction in MTCT to as low as 2% at 6 months was observed when ZDV was initiated at 28 weeks followed by adding a single dose (sd) of NVP during labour/delivery in a non-breast-feeding population (Lallemant et al. 2004). Interventions that attempted to prevent infant exposure to infectious blood and secretions in the birth canal by use of antiseptics during labour were not found to be effective in preventing transmission (Biggar et al. 1996, Gaillard et al. 2001). Studies on the use of prophylactic antibiotics in prevention of chorioamnionitis did not result in prevention of MTCT of HIV (Andiman 2002, Taha et al. 2006a). Similarly trials using vitamin A to prevent MTCT of HIV did not show effectiveness (Semba 1997, Coutsoudis et al. 1999a, Fawzi et al. 2000).

Short-course ARV prophylaxis for PMTCT, especially if a single ARV drug is used carries the risk of development of viral resistance to ARV drugs and this may compromise use of these drugs for future prophylaxis or treatment (Lockman 2008). Studies in Africa have demonstrated viral resistance to NVP in 25-75% of women 2-8 weeks postpartum and in 33-87% of infants exposed to sdNVP (Lockman 2008). However, adding a tail of treatment with ZDV and 3TC for a week from delivery for all women who received intrapartum sdNVP can minimize this risk (McIntyre et al. 2005). The alternative is to use triple ART for prophylaxis.

Prevention of postnatal HIV-1 transmission due to breast milk exposure remains one of the major challenges in developing countries where more than 90% of women breast-feed their infants (Fowler et al. 2002) because they do not have alternative acceptable, feasible, affordable, sustainable and safe infant feeding options. In a study performed in Nairobi, Kenya comparing breast-fed and formula-fed infants, the probability of HIV-1 infection was significantly lower in the formula-fed children compared to that of breast-fed children and also the HIV-1 free survival at two years was significantly higher in the formula arm of the study (Nduati et al. 2000). However, more recent studies indicate that even though formula-feeding reduces HIV transmission there may be no benefit for HIV-free survival because of increased mortality in formula-fed infants (Kuhn et al. 2009). Studies from South Africa, Zimbabwe and Zambia have demonstrated that exclusive breast-feeding is associated with lower risk of HIV transmission compared to mixed feeding (Coutsoudis et al. 1999b, Iliff et al. 2005, Coovadia et al. 2007, Kuhn et al. 2007). In most developing countries HIV seropositive mothers are counselled to breast-feed exclusively for six months if other feeding options are not acceptable, feasible, affordable, sustainable and safe (WHO 2001). Recent studies, including the Mitra and

Mitra Plus studies carried out in Dar es Salaam have shown that extended infant ARV prophylaxis or maternal prophylaxis with HAART during breast-feeding can reduce breast milk HIV transmission (Kuhn et al. 2009). These studies are discussed in the section Results and Discussion.

1.11.2 PMTCT implementation

Following the encouraging results from perinatal studies many countries with limited resources including Tanzania introduced and integrated PMTCT of HIV in the reproductive and child health (RCH) service clinics, where pregnant women are routinely counselled and tested for HIV and those found HIV seropositive are provided with interventions including ART and counselling on infant feeding options including exclusive breast-feeding. Due to its simplicity to administer and the low costs that are involved, the sdNVP given to the mother during labour and delivery and to the neonate soon after birth became an attractive public health strategy to policy makers and programme managers implementing PMTCT programs in resource-poor settings. This eventually became a recommended standard regimen in national PMTCT programs in resource-limited countries before 2006. It was anticipated that this regimen would motivate women to participate and that there would be a rapid roll out of the programme. Unfortunately this did not happen in many countries as it is estimated that only 18% of pregnant women had accessed PMTCT services by 2006 (UNAIDS/WHO 2008). However, this regimen is currently in the process of being phased out because access to more efficacious regimens is now possible in resource-limited countries. Another reason for opting for more efficacious regimens is to avoid the threat of possible development of resistance to NVP, because this can complicate future prophylaxis or treatment with NVP-based combinations. The current recommendation by WHO is, whenever possible, to implement a more efficacious regimen in the PMTCT programs. Women eligible for ART, including all women with a history of AIDS defining illness or a CD4+ cell count <200/µL, should be treated with HAART for their own health which will also prevent MTCT of HIV. None-HAART eligible women should be treated prophylactically with ZDV given from 28 weeks gestation or thereafter, and continued during labour and delivery. 3TC and sdNVP should be administered to the mother during labour and delivery. The mother should also receive a tail of ZDV+3TC for one week. Postnatal sdNVP should be administered to the new born within 24 hrs after delivery and followed by ZDV for 7 days. However, the sdNVP interventions for PMTCT of HIV is still

recommended in many areas where comprehensive HIV and AIDS care and treatment is not yet available (WHO 2006).

As of 2007, 33% of all pregnant women living with HIV in 109 countries received any ARV regimen for the prevention of MTCT of HIV. From the 60 countries where data was available on type of prophylactic regimens, 49% used sdNVP, 26% a combination of two ARV drugs and 7% HAART for MTCT prevention and for the mother's own health (UNAIDS/WHO 2008). The reduced transmission risk gained from the use of ARV drugs during the antenatal and intrapartum periods is not fully sustained in infants exposed to breast milk postnatally. Furthermore, the HIV-infected pregnant women who are eligible for treatment but are not receiving combination ART is of major concern.

1.11.3 PMTCT in Tanzania

The latest report on surveillance of HIV infections among antenatal attendees in Tanzania showed an HIV prevalence of 8.2% among antenatal attendees (NACP 2006). Annually approximately 1.4 million women become pregnant, of whom 122 000 per year are expected to be HIV-infected. It is estimated that in Tanzania each year approximately 48 800 children <5 years are infected through MTCT (16 000 of them through breast-feeding). The PMTCT activities in Tanzania started in 1996 in the form of research by participating in the Petra multicentre PMTCT trial conducted in three countries in sub-Saharan Africa (Paper 1). Based on experience and knowledge acquired from this trial and from others, the Ministry of Health in Tanzania in collaboration with UNICEF initiated a two-year PMTCT pilot programme beginning year 2000. The pilot programme was conducted in the four consultant hospitals and one regional hospital, Muhimbili National Hospital, Bugando Medical Centre, Kilimanjaro Christian Medical Centre, Mbeya Referral Hospital and Kagera Regional Hospital. The aim of the pilot project was to determine the feasibility of integrating PMTCT services in the routine RCH services in Tanzania. The programme was evaluated in year 2002 and the report showed that the acceptance rate for HIV testing among pregnant women was high (85%) and that it was feasible to integrate PMTCT of HIV in routine RCH services. However, it was also observed that there was inadequate knowledge about PMTCT among health care providers. Male involvement was low and there was inadequate community participation in the programme. The recommendation from that evaluation was to integrate PMTCT in RCH services and

expand it to other health facilities. PMTCT interventions have now been integrated within RCH services in the country. The following care is provided: counselling and testing during pregnancy, modified obstetric care, provision of ARV prophylaxis to HIV seropositive pregnant women and counselling on safer infant feeding options. To increase access to PMTCT services, efforts and mobilization activities to involve communities, families and men have been given priority, including training of service providers on effective counselling, care and linkage to other services. Also PMTCT activities have been streamlined and coordinated by the PMTCT unit in the Ministry of Health and Social Welfare (MoHSW). Development partners (financial supporters) and other stakeholders are deeply involved in scaling up PMTCT services throughout the country.

2 RATIONALE OF THE STUDY

MTCT of HIV is a big public health problem in Tanzania where it accounts for about 5% of all new HIV infections (NACP 2005) and for about 90% of HIV infections in infants and young children below 5 years. The continued existence of children infected with HIV from their mothers calls for research on interventions that will minimize MTCT of HIV not only during the perinatal period but also postnatally during breast-feeding. Prevention of MTCT of HIV is one of the major priority areas of HIV and AIDS interventions in Tanzania as stipulated in the National Multisectoral Strategic Framework on HIV/AIDS 2003-2007 and 2008-2012 and Health Sector Strategy for HIV/AIDS 2003-2006 and 2007-2012 policy documents.

The studies in this thesis address the prevention of MTCT of HIV through a package of interventions which include voluntary counselling and testing and the use of ARV prophylaxis perinatally and postnatally during breast-feeding in Dar es Salaam, Tanzania. The thesis also includes a study on the mortality during 2 years after delivery in HIV-1-infected mothers before ART became available in Tanzania.

3 OBJECTIVES

3.1 Broad objective

To reduce MTCT of HIV-1 by prophylactic treatment of mother-child pairs using ARV drugs perinatally and postnatally during breast-feeding in Dar es Salaam, Tanzania.

3.2 Specific objectives

1. To evaluate the efficacy of three short-course drug regimens using ZDV and 3TC for the prevention of MTCT of HIV-1 (the Petra trial) - **Paper I**.

2. To reduce MTCT of HIV-1 in breast-fed infants by prophylactic treatment of mother-child pairs with ZDV and 3TC during late pregnancy, intrapartum and one week postpartum followed by extended infant prophylaxis with 3TC during breast-feeding (the Mitra study) - **Paper II**.

3. To reduce MTCT of HIV-1 through breast milk by maternal prophylaxis/treatment with triple ARV drugs during late pregnancy and breast-feeding in Dar es Salaam, Tanzania (the Mitra Plus study) - **Paper III**.

4. To determine the acceptability of HIV counselling and testing and participation in a HIV-1 MTCT intervention study using short-course ART among women enrolled in the Petra trial at the Dar es Salaam site - **Paper IV**.

5. To determine the mortality during 24 months following delivery in relation to CD4+ T lymphocyte levels and viral load in breast-feeding HIV-1-infected women participating in the Petra trial at the Dar es Salaam site. - **Paper V**.

4 PATIENTS AND METHODS

4.1 Study population and setting (Papers I, II, III)

The Petra trial was a UNAIDS-coordinated multicentre clinical trial that evaluated the efficacy of three regimens of antiretroviral drugs using ZDV in combination with 3TC versus placebo to prevent MTCT of HIV-1. The study was conducted from 1996 to January 2000 in five sites in three African countries: King Edward VII Hospital in Durban and Chris Hani Baragwanath Hospital in Johannesburg, both in South Africa, Muhimbili National Hospital in Dar es Salaam, Tanzania and two hospitals in Kampala, Uganda: Mulago and Nsambya. The study in Dar es Salaam was a collaborative project between the former Muhimbili Medical Centre (MMC) now Muhimbili University of Health and Allied Sciences, (MUHAS) and the Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania and the Swedish Institute for Infectious Disease Control (SMI) and Karolinska Institutet (KI) in Stockholm, Sweden.

The Mitra and Mitra Plus studies were observational prospective studies conducted from 2001-2004 and 2004-2007, respectively in the former Petra site in Dar es Salaam, Tanzania. The aim was to evaluate the efficacy of ARV drugs to prevent breast milk transmission by extended infant prophylaxis using 3TC during breast-feeding or alternatively by treating mothers with HAART in late pregnancy, intrapartum and postnatally during the first 6 months of breast-feeding for women who were not eligible for HAART and to continue HAART for eligible mothers. The studies were collaboration projects between the same institutions in Tanzania and Sweden as for the Petra trial.

4.2 Recruitment procedures for study participants (Papers I, II, III, IV)

In the Petra trial (Paper I), pregnant women attending prenatal clinics in the five study sites were offered pre-test counselling and voluntary HIV testing using HIV antibody ELISAs. Seropositivity was confirmed by testing another sample by two sequential HIV antibody ELISAs. HIV seropositive women received post-test counselling. For participants in the Mitra and Mitra Plus studies (Papers II, III) screening for HIV antibodies in the pregnant women was done at the recruitment sites using the Capillus rapid simple assay (Trinity Biotech, Ireland) for initial testing followed by testing of reactive samples by the Determine rapid simple assay

(Abbott Laboratories). For seropositive women agreeing to participate in the studies a second sample was collected for confirmation of reactivity at the research laboratory in the Department of Microbiology/Immunology, MUHAS before enrolment. The second sample was tested for HIV antibodies by two consecutive anti-HIV ELISAs, Enzygnost anti-HIV 1+2 Plus ELISA (Behring, Marburg, Germany) and Wellcozyme HIV-1 recombinant ELISA (Murex, Dartford, UK). Sera reactive on both ELISAs were considered HIV-1 antibody positive. Those with repeatedly discordant results on ELISA were tested by a Western blot assay, and if positive on Western blot they were considered HIV-1 antibody positive. The eligibility criteria for the studies included evidence of HIV-infection, intention to breast-feed, being 18 years or older, ability to give informed consent, estimated gestation of less than 36 weeks (34 weeks for the Mitra Plus study) at enrolment, absence of severe fetal anomalies, absence of life-threatening disease, haemoglobin over 7 g/dL at enrolment and availability for 18 months of follow up. Women interested in participating in any of the studies were given a patient information sheet describing the detailed requirements of the respective study. Women who met all eligibility criteria and signed an informed consent were enrolled in the studies. For the Mitra plus study, enrolment was at 34 weeks gestation or earlier for women with WHO clinical disease stage 3 or 4 or CD4+ cell count <200/µL.

4.3 Study procedures (Papers I, II, III)

The Petra trial (Paper I) was a multicentre, double-blind, placebo-controlled trial performed to compare three regimens using oral ZDV plus 3TC to prevent MTCT of HIV. The longest drug regimen (arm A) started during week 36 of pregnancy with ZDV 300 mg plus 3TC 150 mg twice daily until the onset of labour. At the onset of labour, women received ZDV 300 mg and 3TC 150 mg and during labour they received ZDV 300 mg every 3 hours and 3TC 150 mg every 12 hours until delivery. For the first 7 days postpartum, mothers received ZDV 300 mg plus 3TC 150 mg twice daily and the newborns received ZDV 4 mg/kg plus 3TC 2 mg/kg twice daily. The intermediate regimen (arm B) treatment started at the onset of labour with ZDV 600 mg and 3TC 150 mg followed by ZDV 300 mg every 3 hours and 3TC 150 mg every 12 hours until delivery, followed by postnatal treatment similar to arm A for the mother and the neonate. In arm C which was an intrapartum regimen only women received ZDV 600 mg and 3TC 150 mg at the onset of labour, then ZDV 300 mg every 3 hours and 3TC 150 mg every 12 hours until delivery. There was no postnatal treatment. In the control group (arm D) no active

treatment was given (placebo arm). This placebo arm was stopped midway during the study when other studies published results showing that short-course prophylactic ART was effective in reducing the rate of MTCT. A block randomisation list by site was prepared before the trial. The study medication was packaged by Glaxo Wellcome (the manufacturer of ZDV and 3TC), according to the randomisation list. Each site received the pre-randomised packs labelled by patients' numbers.

For the Mitra study (Paper II) all participants in the study received from week 36 gestation, the same regimen as in the Petra trial arm A. From day 7 after birth the newborns then continued with 3TC alone (week 2-4, 2 mg/kg twice daily and after week 4, 4 mg/kg) during breast-feeding (maximum 6 months) and two weeks after stopping breast-feeding.

In the Mitra Plus study (Paper III) ARV treatment started at 34 weeks gestation or earlier in women with symptomatic HIV infection (WHO clinical stage 3 or 4) or CD4+ T cell counts ≤ 200/μL. The treatment regimen consisted of ZDV 300 mg twice daily plus 3TC 150 mg twice daily plus NVP 200 mg lead dose once daily for 14 days then stepped up to 200 mg twice daily during the rest of the treatment period. The same combined regimen was continued intrapartum and postnatally for 6 months during breast-feeding and was then stopped except in women who were eligible for HAART for their own health. At 6 months treatment with NVP was stopped but treatment with ZDV plus 3TC continued for one more week for non-eligible women. For women who showed adverse reaction to NVP, the drug was replaced with nelfinavir (NFV). Towards the end of enrolment women with CD4+ cell counts >200/μL received a regimen containing NFV instead of NVP because of new information regarding NVP-related side effects (WHO 2004, Dao et al. 2007, Hittii et al. 2004). Infants were treated with ZDV (4 mg/kg bid) and 3TC (2 mg/kg bid) from birth to 1 week of age.

With exception of the women in the Petra trial, mothers were advised to exclusively breast-feed for 6 months and wean rapidly. Mothers and infants received free medical care within the study clinic. Follow up was done at the study clinic sites according to appointment schedules which were at weeks 1, 3 and 6 and at months 3, 4, 5, 6, then every 3 months up to 24 months post delivery. At each follow up visit clinical examinations of the mothers and children were done including recording growth measurements of the child, adverse events since the last visit, and detailed information on feeding practices. Baseline socio-demographic information, medical and pregnancy history were recorded. Comprehensive labour and delivery forms were

completed for each woman. Full blood-cell count and measurements of haemoglobin, leucocytes, lymphocytes, thrombocytes, creatinine and liver enzymes (transaminases) were done at enrolment, delivery and one week postpartum for both mothers and children, then at 3 months interval until when ARV treatment was stopped. Haemoglobin was measured in children at 6 weeks of age. CD4+ cell counts were done in mothers at enrolment and month 3, 9, and 18. Serious adverse events were recorded on special forms.

4.4 HIV laboratory diagnosis in infants (Paper I, II, III)

Cell pellets were prepared by the Amplicor whole blood PCR sample preparation method (Roche Diagnostic Systems, Alameda, CA, USA). For the early-efficacy analysis in the Petra trial, all blood samples available at week 6 either plasma or cell pellets or both were tested. Cell pellets were tested for HIV-1 DNA by a prototype Roche Amplicor v1.5 qualitative PCR assay (Roche Diagnostic Systems, USA). For a sample to be considered positive for HIV-1 DNA, two consecutive test results were required. PCR testing for HIV-1 DNA was done at SMI in Stockholm, Sweden, and the Georg-Speyer-Haus in Frankfurt, Germany. For the early and the late efficacy analysis plasma was tested for the presence of HIV-1 RNA by nucleic acid sequence-based amplification (NASBA) test. Children who tested positive for PCR, NASBA or both were considered HIV-1 positive. If the results from the PCR test and the NASBA test were different for a child, the results from a repeated NASBA test or from later follow-up visits were conclusive. At months 15 and 18 ELISA testing determined the HIV-1 serostatus of the children. Positive results were confirmed, using a second ELISA or a Western blot assay.

In the Mitra and Mitra plus studies, children were tested for HIV-1 infection at week 6 and month 3 and 6 by the Amplicor HIV-1 DNA v1.5 qualitative PCR assay (Roche Diagnostics, USA or Randburg, South Africa) at MUHAS in Tanzania and/or at SMI in Sweden. In the Mitra Plus study month 9 samples were also tested by the HIV-1 DNA PCR assay. Children with a positive PCR test were retested at the next scheduled visit. Children with two positive HIV tests were diagnosed as being HIV-1-infected. However, children who died or were lost to follow up after a single positive PCR test were considered HIV-1 positive in the transmission analyses. In the Mitra Plus study children with a positive HIV test at 6 weeks were tested at 1 week after birth by the Amplicor HIV-1 RNA Monitor v1.5 assay (Roche Diagnostics, Randburg, South Africa). The RNA PCR test at birth or 1 week was considered positive if the viral load was more than 1000 copies/mL in the Mitra Plus study. ELISA antibody tests were

done at 12, 15 and 18 months. Month 12 and 15 reactive samples were tested by the HIV-1 RNA PCR assay. The diagnostic threshold of the RNA PCR assay at 12 or 15 months was 10 000 copies/mL (Read 2007).

4.5 Paper IV

The aim of this study was to determine the acceptability of HIV counselling and testing and participation in a PMTCT study using ART in Dar es Salaam, Tanzania, one of the Petra trial sites. Details of the study design and methods in the Petra trial have been described in Paper I. Briefly HIV testing was offered to all pregnant women who visited three prenatal clinics in Dar es Salaam before 34 weeks gestation. Trained nurse midwives performed group or individual pre-test counselling. Laboratory diagnosis of HIV infection was based on two sequential HIV antibody ELISAs. Post-test counselling was given two weeks later to women who wished to know their HIV status. Those who came for results after the second test and were HIV seropositive were then invited to participate in the trial if they met the eligibility criteria which are described in Paper 1.

4.6 Paper V

The objective of this study was to analyse the mortality during 24 months after delivery in relation to CD4+ T lymphocyte levels and HIV-I viral load at enrolment (36 weeks gestation) in the cohort of HIV-1 seropositive breast-feeding women enrolled at the Dar es Salaam site of the Petra trial. The clinical status of the women at enrolment was determined using the WHO clinical staging system for HIV infection and disease. The enrolled women received prophylactic ARV or placebo treatment according to the trial randomization for PMTCT of HIV-1. Follow up of mother-child pairs are described in detail in Paper I. Among laboratory monitoring parameters were determination of T lymphocyte subsets, which was done using the SimulSET flow cytometry method (Becton Dickinson, Immunocytometry System, San Jose, CA) and plasma HIV-1 RNA which was quantified by the Amplicor HIV-1 RNA Monitor v1.5 assay (donated by Roche Diagnostic Systems, USA).

4.7 Statistical analysis (Papers I, II, III)

In the Petra trial comparison was done between each of the three drug regimens used and the placebo group. An early-efficacy analysis was done on the basis of samples collected at week 6. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated for HIV-1 infection and/or death, whichever came first. Transmission rates were tested for significance compared to the placebo arm using the x^2 test. To adjust for differences between sites, this analysis was followed up with a stratified analysis, looking at the weighted average of site-specific estimates, with inverse variance weights. A late-efficacy analysis was done using Turnbull technique (PROG RELIABILITY, SAS version 8.0) to estimate HIV-1 infection rates and HIV-1 infection or death rates after 18 months of follow-up. In this analysis the age at which HIV-1 infection occurred in the infants was interval-censored (infection occurred between the last negative test and the first positive test). Cumulative incidences were derived from the Turnbull analysis. Survival curves were plotted for the probability of remaining free from HIV-1 infection and free from HIV-1 infection or death (HIV-1-free survival). Separate curves were obtained for treatment groups within subgroups with or without ever being breast-fed. In all analyses, only the first-born infant was considered in multiple infant pregnancies.

In the Mitra and Mitra Plus studies, comparison was made with historical data from the breast-feeding population in arm A of the Petra trial. Data analysis was done using the SPSS software system 14.0 and 15.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago Illinois, USA), respectively. HIV-1 transmission, mortality, HIV-free survival and breast-feeding were analysed using the Kaplan-Meier survival technique. In the transmission analyses time for HIV-1 infection was considered to be the midpoint between the date for the last negative sample and the date for the first positive sample. Univariate and multivariate analyses with continuous background factors were performed with Cox regression. The analysis of transmission of HIV-1 in the MITRA study in relation to mothers´ viral load at enrolment was performed as a case/control study in which all transmitting mothers and 3 non-transmitting controls for each transmitting mother were included (viral load at enrolment was not determined in the other mothers). To enable a direct comparison of HIV-1 transmission in the MITRA study and the breast-feeding population of arm A in the Petra trial individual data on the breast-feeding population in Petra arm A were provided by the data management centre at the International Antiviral Therapy Evaluation Centre, Amsterdam. Cox regression was used to

study the relative effectiveness of the preventive measures taken in the Mitra study compared to the Petra trial (pooled data). In the analysis of transmission, uninfected children were regarded as being at risk only as long as they were breast-fed (censoring was done at the day breast-feeding stopped or at the date of the last visit to the clinic if still breast-fed at that time). All HIV infections up to 6 months after delivery were considered as events. The analysis of the Mitra Plus study was done in the same way as the analysis of the Mitra study using data on the breast-feeding population in the Petra trial arm A as historical controls (pooled analysis). However, in the Mitra Plus study, censoring at stop of breast-feeding was not done in the transmission analysis since the proportion of breast-feeding mothers over time was not lower than that in the Petra trial arm A until 24 weeks after delivery. The analysis of the Mitra Plus study covered a follow up of 18 months instead of the 6 months follow up in the Mitra study. In all analysis of the Mitra and the Mitra Plus studies only the firstborn baby was included in case of twins.

4.8 Statistical analysis (Paper V)

Data analysis was done using the SPSS software system 11.0. Differences between groups of observations with skewed distributions were tested by the Kruskal-Wallis test. Mortality and breast-feeding were analysed by the life-table technique, taking censored observations into account. Women lost to follow-up were censored at the time for their last visit to the clinic. Wilcoxon-Gehan statistic was used for testing differences between groups. Univariate analyses were also carried out with Cox regression. The forward stepwise Cox regression model was used in the multivariate analyses.

4.9 Ethical clearance

The Petra trial was approved by the WHO Secretariat's Committee on Research Involving Human Subjects and the ethics review boards of the respective trial sites. The Mitra and Mitra Plus studies were approved by ethics review boards of MUHAS, NACP, National Institute for Medical Research, MoHSW, Tanzania and KI, Sweden.

5 RESULTS AND DISCUSSION

This work is based on three clinical intervention studies conducted in resource-limited settings in sub-Saharan Africa. The first study (the Petra trial) was performed at five sites in three African countries including a site in Dar es Salaam, Tanzania. The two subsequent studies (the Mitra and the Mitra Plus studies) were performed at the Dar es Salaam site only. The primary aim of these studies was to prevent early and late MTCT of HIV-1 using perinatal and postnatal ARV prophylaxis. Papers I, II and III report the outcomes of these interventions and the other two papers (IV and V) report on related studies: acceptance of counselling and HIV testing by antenatal women (paper IV) and maternal mortality 24 months after delivery in breast-feeding HIV-1-infected women at the Dar es Salaam site of the Petra trial (Paper V). Paper I reports the results of a study of short-course ART for PMTCT. Papers II and III report results of studies of postnatal PMTCT of HIV by extended ARV prophylaxis either to the infant (the Mitra study, paper II) or to the mother during 6 months of breast-feeding (the Mitra Plus study, paper III).

5.1 Prevention of MTCT of HIV-1 by short-course ARV prophylactic treatment (Paper I)

The Petra trial, conducted in South Africa, Tanzania and Uganda assessed the efficacy of short-course regimens of ZDV and 3TC in the prevention of MTCT of HIV-1. There were 1457 HIV-1-infected women randomised in four arms. In the whole study population 74% of the women breast-fed during a median period of 28 weeks [interquartile range (IQR) 7-59 weeks]. In South Africa 53% of the women breast-fed during a median of 7 weeks (IQR 2-21 weeks), whereas in East Africa 97% breast-fed during a median of 45 weeks (IQR 26-69 weeks). In South Africa there was a better perinatal care and therefore a better infant survival and the infants were less exposed to the risk of breast milk transmission than in the East African sites. In East Africa children were breast-fed for a longer period as a means of ensuring child survival at the expense of being exposed for a longer period to the risk of breast milk transmission. The HIV transmission analysis included PCR results from 1093 infants. The 6 weeks HIV-1 transmission rates and RR and the HIV-free survival and RR (early efficacy) between the treatment arms are shown in Table 1.

TABLE 1. HIV-1 infection and HIV-1 infection or death at 6 weeks in the Petra trial

HIV-1 infection				**HIV-1 infection or death**		
Treatment arms	**HIV infection %**	**Early efficacy RR (95% CI*)**	**p-value**	**HIV infection or death %**	**Early efficacy RR (95% CI)**	**p-value**
Regimen A	5.7	0.37 (0.21-0.65)	0.001	7.0	0.39 (0.24-0.64)	0.001
Regimen B	8.9	0.58 (0.36-0.94)	0.025	11.6	0.64 (0.42-0.97)	0.003
Regimen C	14.2	0.93 (0.62-1.40)	0.74	17.5	0.97 (0.68-1.38)	0.85
Placebo	15.3	1.00	-	18.1	1.00	-

*confidence interval (CI)

The longest regimen of ZDV and 3TC given antepartum (2 weeks), intrapartum and post partum (7 days) (regimen A) reduced early transmission at 6 weeks by 63% and transmission or mortality by 61% compared to the placebo arm. Regimen B which started intrapartum and continued for one week postpartum was able to reduce early HIV transmission by 42% and infection and death by 36%. When treatment was given intrapartum only (regimen C), it was not effective.

At the time when the Petra trial was completed it showed the highest reported early relative efficacy of all short-course regimen studies conducted in resource-limited countries (Shaffer et al. 1999, Wiktor et al. 1999, Dabis et al. 1999, Guay et al. 1999), except for the long ZDV regimen study conducted in Thai non-breast-feeding women, where prepartum dosing started at 28 weeks´ gestation (Lallemant et al. 2000).

In the Petra study baseline factors significantly associated with low risk of HIV transmission at week 6 were high maternal CD4+ cell count and delivery through caesarean section. Both elective and emergency caesarean sections were found to have a protective effect independently of the drug regimens. Maternal viral load was not determined in the Petra study. After week 6, continued HIV-1 transmission was predominantly found in the breast-fed infants. Cumulative incidence of HIV infection obtained with interval censored analysis and HIV-free survival after 18 months of follow up are shown in Table 2.

TABLE 2. HIV-1 infection and HIV-1 infection or death at 18 months in the Petra trial

Treatment arms	HIV infection (18 months) % (95% CI)	HIV infection or death (18 months) % (95% CI)
Regimen A	14.9 (9.4-22.8)	18.9 (12.7-27.3)
Regimen B	18.1 (12.1-26.2)	23.8 (15.6-34.5)
Regimen C	20.0 (12.9-30.1)	25.2 (16.9-35.8)
Placebo	22.2 (15.9-30.2)	25.6 (19.2-33.2)

Considering only breast-fed children the cumulative incidences of HIV-1 infection or death at 18 months were 21.6%, 24%, 27.8% and 28.2% in arm A, B, C and the placebo arm, respectively. At month 18 a high maternal CD4+ cell count was significantly associated with a low risk but breast-feeding was associated with high risk of HIV-1 transmission. After 18 months of follow up of infants in the Petra study very little prevention effect acquired early remained. The diminished effect on reducing HIV infection or death was most likely due to continued breast-feeding that resulted in HIV-1 transmission and to the high infant mortality rates, which are common in resource-limited countries. Another study has also reported diminished effectiveness of short-course ART after 18 months of follow up of breast-fed children in resource-limited countries (Guay et al. 1999). The implication of this finding is that to prevent MTCT of HIV-1 in breast-feeding populations short-course ARV regimens should be accompanied by interventions to minimise the risk of subsequent breast milk transmission in the postnatal period. It is clear from the results of the Petra trial and other PMTCT studies that HIV-1 transmission continued to occur over the whole period of follow up in the breast-fed children. Shortening breast-feeding to 6 months, as recommended by WHO (WHO 2001) and administering postnatal ARV prophylaxis to mothers or to uninfected infants during breast-feeding are strategies that have been studied in papers II and III and are discussed below.

5.2 Prevention of postnatal MTCT of HIV-1 through breast-feeding (Papers II, III)

In resource-limited countries breast-feeding is vital for infant survival because a majority of women cannot implement other safe infant feeding options. Refraining from breast-feeding altogether or stopping breast-feeding at a very early age is a threat to child survival in resource-constrained populations (Kuhn et al. 1997, WHO collaborative study team on the role of breastfeeding on the prevention of infant mortality 2000, Kuhn et al. 2008). Analysis of infant feeding methods has shown that infants who are exclusively breast-fed for up to 6 months have lower HIV-1 infection rates than children who receive mixed feeding (Coutsoudis 1999b, Coutsoudis et al. 2001a, Iliff et al. 2005, Coovadia et al. 2007, Kuhn et al. 2007). However, the risk of breast milk HIV transmission cannot be eliminated by exclusive breast-feeding alone.

The Mitra and Mitra Plus studies addressed the possibility of preventing breast milk transmission of HIV-1 by using extended infant (the Mitra study) or maternal (the Mitra Plus study) ARV prophylaxis during breast-feeding. In the Mitra study mother-child pairs were treated with ZDV and 3TC according to the Petra trial arm A regimen and infants were then treated prophylactically with 3TC alone during breast-feeding for a maximum of 6 months. In the Mitra Plus study, all women were given HAART irrespective of their stage of HIV infection from week 34 of gestation (or earlier) and for 6 months during breast-feeding. Continued HAART was given to mothers who needed treatment for their own health. These two studies were performed at the same site but at different time periods. There were 398 children included in the transmission analysis in the Mitra study and 441 children in the Mitra Plus study. The MTCT transmission rates in these two studies were analysed using historical controls from the breast-feeding population in arm A of the Petra trial (all 5 Petra sites, n=264). The baseline characteristics of mothers at enrolment and infants at birth in these three studies are shown in Table 3.

TABLE 3. Baseline characteristics for mothers and children in the Mitra and Mitra Plus studies and the breast-feeding population in the Petra trial arm A

	Mitra study	**Mitra Plus study**	**Breast-feeding mothers in Petra arm A**	**P** $_{\text{Mitra}}$	**P** $_{\text{Mitra Plus}}$
Mother/child pairs in transmission analysis	n=398	n=441	n=264		
At enrolment:					
Age, y, median (IQR)	26 (23-30)	26 (24-30)	26 (23-30)	0.13	0.700
Hb, g/dL, median (IQR)	9.6 (8.6-10.6)	10.0 (8.9-11)	10.7 (9.8-11.7)	<0.001	<0.001
CD4 count, cells/μL, median (IQR)	411(269-611)	415 (265-577)	459 (295-643)	0.05	0.014
CD4%, median (IQR)	21 (14-27)	21 (15-28)	27 (19.4-35.4)	<0.001	<0.001
CD4 count < 200 cells/μL	15.4%	17.5%	9.4%	0.03	<0.001
Viral load, median (IQR)	-	14621 (2954-59738)	not available		
^{10}log viral load, mean (sd)	-	4.1595 (0.8775)	not available		
WHO stage 1	353 (88.7%)	414 (93.9%)	234 (88.3%)		
2	28 (7%)	20 (4.5%)	18 (6.8%)		
3	15 (3.8%)	2 (0.5%)	8 (3.4%)		
4	2 (0.5%)	5 (1.1%)	2 (0.8%)	0.88*	
At delivery:					
Caesarean section	18.6%	18.3%	31.1%	<0.001	<0.001
Birth weight, kg, median (IQR)	3.0 (2.7-3.3)	2.9 (2.5-3.2)	3.1(2.8-3.3)	0.01	<0.001
Low birth weight (< 2.5 kg)	14.6%	16.8%	5.8%	0.001	<0.001
Female child	53%	49%	51%	0.70	0.578

*x^2 test for trend

The mothers in the Mitra and Mitra Plus studies had a significantly lower level of haemoglobin and of percentage CD4+ cells compared to the mothers in the Petra trial arm A. The proportion of mothers with low CD4+ cell counts (<200 cells/μL) was significantly higher in the mothers in the Mitra and Mitra Plus studies compared to those in the Petra trial arm A. Caesarean section was less frequent in the Mitra and Mitra Plus studies than in the Petra trial. Low birth weight was more frequent in infants in the Mitra and Mitra Plus studies. The comparison of baseline characteristics indicates that the mothers in the Mitra and Mitra Plus studies were at higher risk of transmitting HIV compared to mothers in the Petra trial arm A since a low CD4+ cell level is a well documented risk factor for MTCT and caesarean section is known to reduce

the risk of HIV-1 transmission (Newell et al. 1996, Edgeworth and Ugen 2000, The international perinatal HIV group 1999). In both the Mitra and Mitra Plus studies most of the mothers reported to have practiced exclusive breast-feeding and a short weaning period. There was no information about the type of breast-feeding in the Petra trial. Breast-feeding proportions at different time points are shown in Table 4.

TABLE 4. Breast-feeding proportions at different time points in the Mitra and Mitra Plus studies and in the breast-feeding population in arm A of the Petra trial

Time point	**Mitra study**	**Mitra Plus study**	**Breast-feeding mothers in Petra trial arm A***
	%	**%**	**%**
6 wk	95	97	85
12 wk	86	90	77
16 wk	61	80	72
20 wk	44	74	69
24 wk	30	51	68
26 wk	18	17	64
28 wk	15	13	61

*Breast-feeding in the Petra trial was calculated on the assumption that mothers lacking information on the date of stopping breast-feeding went on breast-feeding to 6 months or longer.

The median duration of breast-feeding was longer in the Mitra Plus study (24 weeks) than in the Mitra study (18 weeks). The proportion of breast-feeding mothers was higher in the Mitra Plus study than in the Petra trial up to 5 months.

HIV transmission and HIV-free survival were analysed by the Kaplan-Meier technique up to 6 months (26 weeks) in the Mitra study and up to 18 months in the Mitra Plus study. The cumulative HIV transmission rates in the Mitra and Mitra Plus studies and in the breast-feeding population of the Petra trial arm A are shown in Table 5.

TABLE 5. Transmission rates of HIV-1 in the Mitra and Mitra Plus studies and in the breast-feeding population in the Petra trial arm A

Point estimates	**Mitra study % (95% CI)**	**Mitra Plus study % (95% CI)**	**Petra arm A breast-feeding population % (95% CI)**
At 6 weeks	3.8 (2.0 to 5.6)	4.1 (2.2 to 6.0)	5.4 (2.7 to 8.1)
At 6 months	4.9 (2.7 to 7.1)	5.0 (2.9 to 7.1)	11.9 (7.9 to15.8)
At 18 months	-	6.0 (3.7 to 8.3)	17.7 (12.8 to 22.6)

The cumulative HIV transmission rates in the Mitra and Mitra Plus studies were very similar at 6 weeks (3.5% and 4.1%, respectively) as well as at 6 months (4.9% and 5%, respectively).
The cumulative risk of acquisition of infection between 6 weeks and 6 months was very low and similar in the Mitra and the Mitra Plus studies, 1.2% (95% CI:0.0 to 2.4) and 1.0% (95% CI: 0.02 to 1.98), respectively.

In a Cox regression analysis of baseline characteristics associated with transmission, the only factor significantly associated with transmission in the Mitra study was CD4 percentage (p=0.046). Viral load at enrolment which was determined in all transmitting mothers and in three matched controls for each case was also significantly associated with transmission (p=0.012) but was not included in the regression analysis. In the Mitra Plus study, maternal and infant factors that were associated with transmission up to 18 months after delivery were high viral load at enrolment (p<0.001), duration of treatment before delivery (p=0.02) and low birth weight (p=0.009) in the univariate analysis. In the multivariate analysis high viral load (p=<0.001), duration of treatment before delivery (p=0.02) and the female sex of child (p=<0.04) were associated with HIV-1 transmission.

HIV transmission in the Mitra study was analysed censoring for breast-feeding time to enable comparison with the HIV transmission in the Petra trial arm A since the proportion of breast-feeding mothers was lower from 16 weeks after delivery in the Mitra study than in the Petra trial arm A (Table 4). However, censoring for breast-feeding time was not done in the Mitra Plus study since the proportion of breast-feeding mothers over time was not lower than that in the Petra trial arm A until 24 weeks after delivery (Table 4). Comparison of the transmission in the Mitra and Mitra Plus studies with that in the breast-feeding women in arm A of the Petra

trial by Cox regression analysis showed that the transmission of HIV-1 up to 6 months during breast-feeding was about 50% lower in the Mitra and Mitra Plus studies ($p \leq 0.001$ for the Mitra and the Mitra Plus studies). The cumulative transmission at 18 months in the Mitra Plus study was about one third of the cumulative transmission at 18 months in the breast-feeding population of the Petra trial arm A ($p<0.001$).

The HIV infection or death rate at 6 months was similar in the Mitra and Mitra Plus studies (8.5% and 8.6%, respectively). Most of the infant deaths reported in the Mitra and Mitra Plus studies occurred in children who were HIV-negative at their last HIV test and the main causes of the deaths were pneumonia, malaria, diarrheal diseases and other febrile illnesses. The HIV infection or death rate at 18 months in the Mitra Plus study was 13.6%. The combined effect of reducing breast milk infection by using ARVs and counselling the women to stop breast-feeding at 6 months probably contributed to the high HIV-free child survival at 18 months in the Mitra Plus study.

An important conclusion from the Mitra and Mitra Plus studies is that infant ARV prophylaxis and maternal prophylaxis with HAART for 6 months during breast-feeding resulted in similar low HIV transmission rates at 6 months and a similar low risk of postnatal acquisition of infection between 6 weeks and 6 months. Other studies in breast-feeding populations in resource-limited countries have also evaluated the reduction of the risk of breast milk transmission of HIV by extended infant ARV prophylaxis (Vyankandondera et al. 2003, Thior et al. 2006, SWEN 2008, Kumwenda et al. 2008) or maternal prophylaxis with HAART during breast-feeding (Palombi et al. 2007, Tonwe-Gold et al. 2007, Arendt et al. 2007, Thomas et al. 2008). In the Mashi trial, where breast-fed children were treated with ZDV for 6 months the transmission rate at 7 months was 9.6% compared to 4.9% in the Mitra study at 6 months. The cumulative risk of acquiring infection between 6 weeks and 6 months in the Mitra study (1.2%) was also lower compared to that between 4 weeks and 7 months in the Mashi trial (4.5%) (Thior et al. 2006). Two randomised trials (PEPI and SWEN) published recently evaluated the reduction of breast milk transmission of HIV through extended infant prophylaxis with NVP. In the PEPI trial in Malawi, infants were treated with NVP or NVP plus ZDV during the first 14 weeks of life and a control group was given single dose maternal/infant NVP plus 1 week of infant ZDV. The cumulative risk of acquisition of infection from birth to 9 months of age was significantly reduced from 10.6% in the control group to 5.2% in the extended NVP group and 6.4% in the extended NVP/ZDV group. The estimated protective efficacy of the extended NVP

regimen was 67% at 14 weeks, 60% at 6 months and 51% at 9 months (Kumwenda et al.2008). In the SWEN trial, conducted in Ethiopia, India and Uganda infants were given extended NVP prophylaxis for 6 weeks and compared with a control group given single dose maternal/infant NVP. There was a significant reduction of the risk of acquisition of infection between birth and 6 weeks in the infants given extended NVP prophylaxis but at 6 months the difference in postnatal transmission between the two groups was no longer significant (SWEN study team 2008). A difference between the Mitra regimen and that of the PEPI and SWEN randomised trials is that there was an antepartum component of ARV prophylaxis which was lacking in the randomised trials.

Observational studies including the Mitra Plus study have demonstrated low postnatal transmission when HIV-infected pregnant women were treated with HAART for their own health or as prophylaxis to reduce MTCT of HIV during breast-feeding (Palombi et al. 2007, Tonwe-Gold et al. 2007, Arendt et al. 2007, Thomas et al. 2008). In the Mitra Plus study, the rate of HIV transmission was similar to that in the KiBS PMTCT study in Kisumu, Kenya in which HIV-infected pregnant women were treated with HAART irrespective of their stage of HIV infection from 34 weeks gestation to 6 months after delivery. The cumulative HIV-1 transmission rates up to 12 months in that study were 3.9% at 6 weeks, 5.0% at 6 months and 5.9% at 12 months (Thomas et al. 2008). In the Mitra Plus and KiBS studies, the risk of acquisition of infection between 6 weeks and 6 months was similar to that between 1 month and 6 months in the DREAM study in Mozambique in which breast-feeding women irrespective of stage of HIV infection were treated with or given HAART prophylaxis from 25 weeks of gestation to 6 months after delivery (Palombi et al. 2007). However, the cumulative HIV-1 transmission rates at 1 month (1.2%) and 6 months (2.2%) were lower in the DREAM study than at 6 weeks and 6 months in the Mitra Plus and KiBS studies. A PMTCT study in Abidjan, Côte d`Ivoire, in which women started HAART as early as 24 weeks gestation resulted in cumulative transmission rates of only 1% at 1 month and 3.3% at 6 months (Tonwe-Gold et al. 2007). It is known that the duration of antepartum ART is important in reducing early HIV transmission (Lallemant et al. 2000). Women in the DREAM study and in the Abidjan study were exposed longer to antepartum ART compared to the women in the Mitra Plus and KiBS studies. A Cox regression analysis of MTCT of HIV in the Mitra Plus study also showed that duration of treatment before delivery was significantly associated with transmission. A learning lesson from these observations is to initiate the antepartum

prophylaxis treatment in early third trimester as emphasized in the current WHO recommendations for ARV prophylaxis for infected pregnant women who are not eligible for HAART.

5.3 Maternal vs infant ARV prophylaxis during breast-feeding to prevent MTCT of HIV-1 (Papers II, III)

The emerging evidence from the studies on extended infant and maternal ARV prophylaxis is that ARV drugs are effective in reducing postnatal transmission of HIV through breast milk. The dilemma now is whether in breast-feeding mothers who do not require HAART for their own health the extended infant ARV prophylaxis strategy or the extended maternal prophylaxis strategy should be adopted. For public health programming this dilemma requires an evidence-based answer for policy formulation in deciding the best approach in preventing postnatal breast milk transmission. More data is required to compare the two approaches. More information about the outcome of maternal HAART prophylaxis will be obtained from two ongoing, randomised, controlled trials (the Ban trial in Malawi and the Kesho Bora multicentre trial in sub-Saharan Africa) which are evaluating the efficacy of 6 months of postnatal maternal HAART prophylaxis in preventing HIV transmission during breast-feeding in mothers who do not need HAART for their own health. There is also an ongoing, randomised, placebo controlled multicentre trial in sub-Saharan Africa (Promise PEP) evaluating the efficacy and safety of extended 3TC prophylactic treatment for 9 months of infants of breast-feeding mothers not eligible for HAART.

Extended maternal ARV prophylaxis appears to have more disadvantages compared with extended infant prophylaxis. The choice of ARV drug combination in mothers who do not need treatment for their own health will be a problem. The regimen used by the majority of women in the Mitra Plus study (ZDV+3TC+NVP) is no longer acceptable because of the risk for NVP toxicity in women with CD4+ cell counts >250/µL (WHO 2004, Dao et al. 2007, Hittii et al. 2004). Another issue that is not in favour of maternal prophylaxis is the cost of the ARV regimen. The cost for a combination of ZDV plus 3TC plus a protease inhibitor, for example Kaletra, for 6 months will be USD 327 compared to USD 11 for 6 months of infant 3TC prophylaxis based on the prices in the public health programme in Tanzania in 2009. Studies performed with infant postnatal prophylaxis are reassuring as far as safety is concerned. On the

basis of currently available data, postnatal infant ARV prophylaxis for 6 months seems to be preferable to postnatal maternal ARV prophylaxis to reduce MTCT of HIV through breast milk among infants of HIV-infected mothers who do not need HAART for their own health.

5.4 When and how to stop breast-feeding (Papers II, III)

In spite of the fact that the mothers in the Mitra Plus study were counselled to stop breast-feeding at 6 months after delivery, 8% of the mothers were still breast-feeding at 12 months and 3% were breast-feeding at 18 months after delivery. Four infants became HIV-infected between 6 and 18 months corresponding to a cumulative rate of acquisition of infection of 1.1%.

WHO has issued a series of guidelines since 1992 for resource-limited settings regarding safe infant feeding options for HIV-infected mothers based on new emerging evidence. Previously HIV-infected mothers in low-resource settings, were counselled and recommended to breast-feed exclusively for 6 months with rapid transition to other feeds when other feeding options were not affordable, acceptable, safe and sustainable (WHO 2001). Field experience has shown that this recommendation was associated with problems both to the mother and the infant. Studies have provided evidence that weaning at six months or earlier is associated with gastrointestinal morbidity including diarrhoea and gastroenteritis (Kafulafula 2007, Kourtis et al. 2007, Onyango et al. 2007, Thomas et al. 2007). A randomised trial among breast-feeding HIV-infected women in Zambia has shown that early, abrupt weaning at 4 months after delivery did not improve the rate of infant HIV-free survival at 24 months (Kuhn et al. 2008). It has also been reported that abrupt weaning was associated with excessive crying and caused maternal problems like mastitis and stress which may force the woman to resume breast-feeding (de Paoli et al. 2008). The current updated WHO guidelines on infant feeding for HIV infected women recommend that at six months, if an assessment finds out that it is not safe to stop breast-feeding, continuation of breast-feeding with additional complementary foods is recommended while the mother and baby continue to be regularly assessed. All breast-feeding should be discontinued once a nutritionally adequate and safe diet without breast milk can be provided (WHO/UNAIDS 2007a). Obviously more research is required to inform policy about when it is appropriate and safe to stop ARV prophylaxis and breast-feeding.

5.5 Safety of the prophylactic ARV treatment and development of ARV resistance (Papers I, II, III)

The fact that no serious adverse events during the follow-up of the infants were considered to be related to the study medication in the Petra trial (ZDV+3TC) (Paper I), Mitra study (3TC) (Paper II), Mitra Plus study (ZDV+3TC+NVP) (Paper III), Simba study (3TC vs NVP) (Vyankandondera et al. 2003), PEPI (Kumwenda et al. 2008) and SWEN studies (NVP) (SWEN study team 2008) is reassuring of the safety of the ARVs used in the prophylactic treatment despite concerns expressed earlier regarding the safety of exposure of uninfected infants to ARV drugs. A French study reported neurological abnormalities and/or mitochondrial dysfunction in eight children (two of whom died) exposed in utero and postnatally to ZDV alone or ZDV and 3TC (Blanche et al. 1999). Other studies, which investigated this adverse event in their cohorts, including the Petra and the ACTG 076 cohorts, did not reveal excess deaths in nucleoside-exposed infants, which might represent toxic effects on the mitochondria, even after extended follow-up (Culnanane et al. 1999, Morris et al. 1999, Bulterys et al. 2000, Lindegren et al. 2000). In the Petra trial, there was no difference in severe adverse events between nucleoside-exposed children and controls. It should be realised, however, that in the Petra trial, exposure to nucleoside analogues was very short at most 4 weeks. In the Mitra study, infants were exposed to prophylactic 3TC treatment for 6 months without developing any serious adverse events considered to be related to the study medication. The Simba study from Rwanda reported similar findings (Vyankandondera et al. 2003).

In the Mitra Plus study, of the 429 women exposed to the NVP containing HAART, 6.5% showed adverse skin reactions and 1.6% had grade 3 or 4 mucocutaneous rash (Steven Johnson syndrome). Only 0.5% showed grade 3 and 4 hepatotoxicity. The incidence of NVP-associated skin rash in the Mitra Plus study was similar to what has been reported in other studies (Thomas et al. 2005, Natarajan et al. 2007, Jamisse et al. 2007, Marazzi et al. 2007). The use of NVP is no longer recommended for women with CD4+ cell counts above 250/µL unless the benefits clearly outweigh the risks for toxicity (Hittii et al. 2004, WHO 2004, Dao et al. 2007).

Another concern when using maternal and infant ARV prophylaxis for a long period is the development of drug resistance. In a small pilot study, HIV resistance testing was performed in 4 infants in the Mitra study who became infected in spite of prophylactic 3TC treatment during

breast-feeding. Mutations (M184V or M184I) associated with 3TC resistance were demonstrated in all of them at 3 months when they were still exposed to 3TC but the mutations had reverted in two of them at 9 months after birth after cessation of 3TC prophylaxis. It has been shown that combination ARV therapy which includes 3TC can still be effective after the appearance of the M184V mutation (Wainberg et al. 2004, Miller et al. 2002). Increased risk of NVP resistance has been demonstrated in infants receiving extended NVP prophylaxis during breast-feeding in the SWEN study in Uganda (Church et al. 2008). Early infant diagnosis at 4 weeks that is now being promoted in PMTCT programmes will identify early HIV-infected infants and avoid exposing them to monodrug prophylaxis. Maternal HAART also entails a risk of drug resistance in HIV-infected infants. In the KiBS breast-feeding study, drug resistant virus was demonstrated in a high proportion of infants who became HIV-1-infected postnatally despite maternal prophylactic HAART (Zeh et al. 2008). HIV resistance testing is ongoing in HIV-infected infants and in mothers in the Mitra Plus study. Furthermore, the question remains whether there are long-term consequences in healthy women of receiving HAART during pregnancy and breast-feeding for prophylaxis of MTCT of HIV and then stopping HAART at the end of breast-feeding. The SMART randomised trial which included 5472 non-pregnant HIV-1-infected adults with a CD4+ cell count >350/μL at entry (most of whom had been on ART for several years) showed that episodic ART significantly increased the risk of opportunistic disease or death as compared with continuous ART (SMART Study Group 2006). In the WITS II study among ART-naive women entering pregnancy with a CD4+ cell count >350/μL and initiating ART for PMTCT, changes in CD4+ cell and HIV RNA levels were similar over the first year postpartum among women stopping or continuing therapy after delivery. No women in either group progressed to AIDS or death during the first year postpartum (Watts et al. 2003).

5.6 Acceptance of HIV testing in antenatal mothers (Paper IV)

HIV counselling and testing are pre-requisites for prevention, care and treatment programmes including prevention and control of MTCT of HIV. Over time different approaches have been developed aiming to increase acceptance rates of HIV counselling and testing. In the late 1980s accurate, reliable and affordable HIV testing became available widely including in developing countries. The WHO issued global guidelines emphasizing the need for testing to be voluntary and therefore respecting human rights (UNAIDS 1997, UNAIDS 1998).

In the late 1990s HIV voluntary counselling and testing (VCT) was introduced in ANCs to identify HIV-infected pregnant women so that they could be provided with prophylactic ART to prevent MTCT of HIV and be counselled on safe infant feeding options. Individual or group pre-test counselling followed by the consenting women being offered HIV testing was established. Individual post-test counselling was given to all consenting women before they received their HIV test results. Pregnant women were free therefore to accept or refuse HIV testing and the choice did not prejudice the services they received henceforth even if they turned down the offer for VCT. This was the method used in the Petra trial at the Dar es Salaam site (Paper IV) where HIV counselling and testing was offered to 10 010 pregnant women from June 1996 to May 1998, of whom 7647 (76.4%) agreed to be tested. Of those tested, 1050 (13.7%) were HIV-1 seropositive. Sixty-eight percent of the HIV-1 seropositive as well as HIV-seronegative women came back for their test results. Forty percent (n=288) of the HIV-1 seropositive women who returned for their test results corresponding to 50% of the women who agreed to a second HIV confirmatory test and 27.4% (288 of 1050) of all the HIV-1 seropositive women were enrolled in the study after fulfilling the eligibility criteria. The mean age of the enrolled women was 27.7 years, 63.5% (183) were married, 88.5% (255) had only primary school education or less and 88.5% (255) were unemployed. Only 22.2% (64) of the enrolled women disclosed their positive HIV serostatus to someone else, 16.7% (48) to their sexual partners and 5.6% to another close relative. The reasons for not disclosing the HIV status included fear of stigma, divorce and violence. Moreover, a substantial proportion, 39.6% (19 of 48), of the informed male sex partners refused to be tested. Of the 29 tested male sex partners, 20 (69%) were HIV seropositive. Thus, there was a high rate of discordance of the HIV serostatus (31%) among the tested sex partners.

In the Mitra study (Paper II), out of 10 179 pregnant women counselled from August 2001 to August 2003 92% (9378) accepted rapid HIV testing and 8779 (93.6%) received their results. The prevalence of HIV-1 infection was found to be 10.9%. The median age for those tested was 26 years (IQR 23-30). In the Mitra Plus study (Paper III), out of 14 255 pregnant women counselled and offered rapid HIV voluntary testing from April 2004 to June 2006, 95.7% (13 637) accepted testing, 98.9% (13 488) received the results and 11,1% were found to be HIV-1 seropositive. The median age was 26 years IQR (23-30).

The Petra, Mitra and Mitra Plus studies (Papers II, III, IV) showed that when pregnant women in Dar es Salaam were properly counselled about the benefits of HIV testing, the proportion of women accepting HIV testing and receiving their test results was high and higher in the last two studies in which the rapid HIV testing strategy was used. A similar rate of acceptability of HIV testing (77.2%) to that found in the Petra study (76%) was observed in another study of acceptance of HIV testing among pregnant women (2001-2002) in Dar es Salaam participating in a study of antibiotic treatment to prevent chorioamnionitis-related perinatal transmission of HIV (Westheimer et al. 2004). Similar findings were also reported from a study in Abidjan, Côte d´Ivoire (Cartoux et al. 1998a), whereas even higher acceptability and return rates were observed among pregnant women in Burkina Faso and South Africa (Cartoux et al. 1998b). In the Petra trial the HIV testing strategy used was a barrier because women had to return to the clinic for test results after two weeks. However, other studies identified additional factors that influenced acceptance of HIV testing and receipt of test results which included the fear of discrimination and domestic violence or divorce (Temmerman et al. 1990), counselling technique used (Cartoux et al. 1999), suspicion of being already infected (Ladner et al. 1996, Cartoux et al. 1999, Maman et al. 2001), possible fear of breach of confidentiality (Lie and Biswalo 1996, Kipp et al. 2001, Pool et al. 2001), fear of having to cope with the result should it be positive, fear of partner involvement and the woman's perceived benefits of taking the test (Antelman et al. 2001, de Paoli et al. 2004).

HIV counselling and testing strategies have changed over the last ten years. Arising from the urgency of enabling more people to learn their HIV status, new innovative strategies had to be developed to improve VCT particularly in countries with high HIV prevalence and especially in ANCs. These new approaches included the use of rapid HIV antibody testing assays with same day results (Downing et al. 1998, Malonza et al. 2003) and provider-initiated routine (opt-out) testing (CDC 2004, WHO/UNAIDS 2007b). As a result of these new innovative approaches, acceptance of HIV counselling and testing has increased (Bolu et al. 2007). The availability of ART for eligible patients, political leadership commitment and mobilization of the people towards fight against HIV and strategies to mitigate stigma, have also contributed to having more people accepting HIV counselling and testing.

The strategy of rapid HIV testing and same day availability of results probably contributed to the high acceptance of counselling and testing in the Mitra and Mitra Plus studies. A meta-analysis of the effectiveness of alternative HIV counselling and testing in the US showed that

rapid testing led to increases in receipt of test results (Hutchinson et al. 2006). In contrast a recently published study from rural South Africa reported that introduction of rapid HIV testing did not improve uptake of HIV testing and receipt of results in pregnant women (Mkwanazi et al. 2008).

Provider-initiated, routine HIV testing in ANCs is initiated routinely by the health worker to all pregnant women seeking antenatal care. All women are HIV tested routinely unless they explicitly refuse to do so (opt out). Currently, routine (opt-out) VCT for HIV of pregnant women is widely practiced in developing countries, including Tanzania where PMTCT services have been integrated in the RCH care services. The aim of this strategy is to enable all pregnant women to access PMTCT services and to identify those eligible for HIV care and treatment including family members. Studies in Botswana have shown that the shift to routine opt-out HIV testing resulted in substantial increases in the uptake of HIV testing and PMTCT interventions (CDC 2004, Creek et al. 2007, Bolu et al. 2007).

In the Petra study few mothers (16.7%) disclosed their HIV serostatus to their sex partners and only 60% of the informed partners accepted HIV testing. However, the disclosure rate was higher (49% among 200 women) in the Mitra Plus study investigated 6 months after delivery (unpublished data). In studies of pregnant women in sub-Saharan Africa rates of disclosure to sex partners have been reported to range from 16.7% to 65% (Paper IV, Medley et al. 2004). A study of HIV-infected pregnant women in Dar es Salaam performed during 1995-2000 showed a low rate of HIV disclosure (22% within 2 months) (Antelman et al. 2001) similar to that in the Petra study in Dar es Salaam. In a study in Moshi in Tanzania of partner participation in antenatal HIV VCT including seronegative as well as HIV seropositive women only 12.6% of male sexual partners agreed to come for HIV VCT (Msuya 2008). Studies of HIV positive pregnant women in Ivory Coast (Brou et al. 2007) and South Africa (Makin et al. 2008) showed disclosure rates of 46% and 59%, respectively. The findings of low rates of HIV disclosure from females to males have several implications for a comprehensive PMTCT programme. One of such is repeated unplanned pregnancies among HIV-infected women and inability to implement a comprehensive HIV prevention intervention when the sexual partner is not involved. The frequency of recurrent pregnancies as reported in Paper IV (4.4 per 100 women years) and the fact that 56% (10 out of 18) of the HIV seropositive pregnant women did not want to remain pregnant show that family planning which is an important intervention in a

comprehensive PMTCT programme cannot be implemented easily without both sex partners getting involved.

5.7 Mortality 24 months after delivery in relation to CD4 lymphocyte levels and viral load in HIV-1-infected breast-feeding women (Paper V)

This mortality study included the cohort of HIV-1-infected women enrolled at the Dar es Salaam site of the Petra trial. Of the 266 mothers included in the analysis, 14.5% had CD4+ cell counts <200 cells/μL, 53.2% had 200-499 CD4+ cells/μL and 32.3% had >500 CD4+ cells/μL at enrolment. The median CD4+ T lymphocyte absolute count at enrolment was 410 cells/μL and the median CD4+ T lymphocyte percentage was 25%. The HIV-1 viral load at enrolment was >100 000 copies/mL in 33.6 % of the mothers. The overall maternal mortality 24 months after delivery was 6.7 %. The mortality 24 months after delivery was 29.9 % for mothers with <200 CD4+ cells/μL at enrolment, 3.3 % for mothers with 200-499 CD4+ cells/μL and 2.9 % for mothers with >500 CD4+ cells/μL (p=< 0.001). The mortality was 15.0 % for mothers with viral load >100 000 copies/mL at enrolment and 2.8 % for mothers with viral load <100 000 copies/mL (p=<0.001). In the multivariate analysis CD4+ cell counts and viral load were both independent risk factors for mortality (p<0.001 and p=0.009, respectively).

The overall mortality in the Petra cohort of HIV-1-infected women in Dar es Salaam was similar to that found previously in two studies on mortality in HIV-1-infected women in Rwanda (Lindan et al. 1992, Leroy et al. 1995). A study from Nairobi published in 2001, reported that mortality 24 months after delivery was higher among HIV-1-infected breast-feeding mothers than among non-breast-feeding HIV-1-infected women, 11% and 4%, respectively (Nduati et al. 2001). The overall mortality in the Petra study at the Dar es Salaam site was not significantly different from either of these mortality rates. The median length of breast-feeding in the Petra study was 11.2 months, compared to 17 months in the breast-feeding group in the Nairobi study. The median plasma viral load and the median CD4+ cell count at enrolment were similar in the women in the Petra cohort in Dar es Salaam and in the women in the Nairobi study. The study of HIV-1-infected women in Nairobi also showed a significant association between mortality and CD4+ cell counts and viral load at enrolment before delivery. An individual patient data meta-analysis conducted using data regarding HIV-1-infected breast-feeding women from eligible clinical trials found that the risk of mortality in

mothers during 18 months of follow up after delivery did not differ significantly by children's feeding modality (BHITS group 2005). Other studies in sub-Saharan Africa have also reported that the risk of mortality in HIV-1-infected women was not increased by breast-feeding (Coutsoudis et al. 2001b, Kuhn et al. 2005, Taha et al. 2006b, Otieno et al. 2007, Coovadia et al. 2008).

In the Petra cohort, 80% of the women who died had clinical AIDS at death. Tuberculosis was the cause of death in one third of the women. Tuberculosis was also reported to be a major cause of death in a study of HIV-1-infected women in Rwanda (Leroy et al. 1995). In the Petra study the haemoglobin level was a significant predictor of mortality in the univariate analysis but not in the multivariate analysis. However, as severe anaemia was an exclusion criterion at enrolment, the possibility of evaluating haemoglobin as a predictor of mortality in our cohort was limited. Anaemia is known to be frequent among women in sub-Saharan Africa, 44% in Malawi [Malawi Demographic and Health Survey (MDHS) 2004] and 48% in Tanzania [Tanzania Demographic and Health Survey (TDHS) 2004-2005] and is among the leading causes of maternal deaths where the underlying cause could be various infections including HIV infection and nutritional deficiencies during pregnancy. In a clinical trial of vitamin supplementation among HIV-1 seropositive pregnant women in Tanzania, anaemia was a predictor of mortality (O'Brien et al. 2005). In a study of use of antibiotics to prevent MTCT of HIV performed in Malawi, Zambia and Tanzania the cause of death 12 months after delivery was anaemia in 19% and tuberculosis in 10% of the HIV-1-infected women (Chilongozi et al. 2008).

This study was conducted in Tanzania between 1996 and 1999 at a time when there was no access to ART. ART became available and started being scaled up as part of the National HIV and AIDS Care and Treatment Plan in October 2004. Fifteen percent of the women in the Petra trial in Dar es Salaam would have been eligible for HAART from the beginning of the study if HAART had been available. In compliance with the WHO guidelines and recommendations (WHO 2006), in the Mitra plus study, women with a CD4+ cell counts <200 cells/µL were started on HAART as soon as they were found to be eligible for treatment and HAART was to be continued for the rest of the women's lives. In the follow up of this sub-group of Mitra Plus women, we have observed much lower mortality compared to that in the un-treated women in the Petra cohort in Dar es Salaam (unpublished data).

6 CONCLUSIONS

Abundant knowledge on prevention of MTCT of HIV infection has been accumulated in the last decade. This has almost eliminated pediatric HIV infection in infants and young children in resource-rich countries, where correct and effective strategies including routine VCT, use of prophylactic HAART, delivery by caesarean section and avoidance of breast-feeding have been applied in a public health programming approach. Because of poverty, stigma and slow behaviour change, measures similar to those applied in the developed world have not yet been applied widely in developing countries. However, studies of short-course ARV regimens in breast-feeding populations in developing countries have demonstrated important reductions in early MTCT of HIV. The Petra trial (Paper I) reduced the HIV transmission rate at 6 weeks after birth by 63% when ZDV and 3TC were given for 2 weeks antepartum, intrapartum and for 7 days postpartum to mother and infant (Petra trial arm A). However, after 18 months of follow up of the breast-fed children most of the effectiveness demonstrated at 6 weeks had disappeared. This opened up another phase of PMTCT research to reduce breast milk transmission of HIV by exclusive breast-feeding and by ART of the infant or the mother during breast-feeding. The Mitra and Mitra Plus studies have contributed to new scientific knowledge in this field. These studies showed that infant and maternal ARV prophylaxis for 6 months during breast-feeding resulted in similar low transmission rates at 6 months and also very low and similar cumulative risks of acquisition of HIV infection between 6 weeks and 6 months. The Mitra Plus study also showed an increase in child HIV-free survival at 18 months of follow up. The dilemma now is to determine which prophylaxis to recommend for prevention of breast milk transmission if the mother does not need HAART for her own health. The infant ARV prophylaxis may be preferable to maternal prophylaxis during breast-feeding for those women who do not require HAART for their own health. However, more scientific data is required to enable policy makers to formulate policies and recommendations which are evidence based.

We observed increased acceptance of HIV testing and receipt of results in the Mitra and Mitra Plus studies compared to the Petra trial most likely due to the use of rapid HIV testing with same day results in the Mitra and the Mitra Plus studies.

During the Petra trial and the Mitra study access to ARV drugs had not yet been introduced in Tanzania. The proportion of women who would have benefited from ART for their own health

was 15% in the Petra trial in Dar es Salaam as well as in the Mitra study. The mortality 24 months after delivery among the HIV-1-infected women with CD4+ cell counts <200/µL at enrolment in the Petra trial in Dar es Salaam was high (30%). In the Mitra Plus study HIV-infected women with CD4+ cell counts <200/µL at enrolment were given continuous HAART. Preliminary results show a significantly lower mortality 24 months after delivery in these women compared to that in the un-treated women in the Petra cohort in Dar es Salaam.

A challenge is to translate the findings in these studies into clinical practice particularly where they are needed most.