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Prevention of Mother-To-Child Transmission of HIV-1 by Antiretroviral Treatment and the Impact on Maternal Health in Dar es Salaam, Tanzania

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ABSTRACT

This thesis describes the results of the Mitra Plus study, including the outcome of antiretroviral therapy (ART) for preventing mother-to-child transmission (PMTCT) of HIV-1 in breastfeeding women and improving HIV- free survival (Paper I), treatment outcome of women initiated on ART for life (Paper II) and reasons for poor drug adherence (Paper III) in Dar es Salaam, Tanzania. Another study (qualitative in nature) explored women's preferred treatment option for the prevention of breast milk transmission of HIV (Paper IV).

In the Mitra Plus study, 501 HIV-1 infected pregnant women were treated with Zidovudine + Lamivudine + Nevirapine /Nelfinavir from 34 weeks of gestation. Treatment of mothers was stopped at six months post-delivery except for those who needed ART for their own health (CD4 cell count $\leq$ 200/μL or WHO stage III or IV). Mothers were advised to exclusively breastfeed and to wean abruptly when the infant was between five and six months. The cumulative HIV-1 rates determined by Kaplan Meier survival analysis of transmission of 441 infants were 4.1% (95% confidence intervals (CI) 2.2-6.0%) at six weeks, 5.0% (95% CI 2.9-7.1%) at six months and 6.0% (95% CI 3.7-8.3%) at 18 months post-delivery. The cumulative risk of HIV transmission between six weeks and six months was 1.0% and between six months and 18 months was 1.1%. The cumulative HIV infection or death rate was 13.6% (95% CI 10.3-16.9%) at 18 months after delivery. Thus extended maternal prophylaxis with ART resulted in low HIV-1 transmission during breastfeeding and a high HIV-free child survival at 18 months.

Follow-up of the Mitra Plus women on ART for life revealed that, following an initial treatment success at three and six months, virologic and immunologic failure were common at 12 and 24 months postpartum. A high proportion of viremic mothers also had drug resistance mutations. The mortality rate was fairly low, 5.9% (95% CI 2.5-13.7%). The probability of virologic and immunologic failure was associated with reported non-perfect adherence to ART at month 24 postpartum. In-depth interviews revealed that women's main motivation for ART adherence was to protect the infant from HIV infection. HIV-related stigma, poverty and overwhelming daily demands were other important barriers to ART adherence.

Among the currently recommended WHO Options for preventing breast milk transmission (A- infant prophylaxis, B- maternal prophylaxis and B+- maternal treatment for life) women preferred Option B as they thought it was better than Option A because of less risk for HIV-associated stigma, less drug side effects for the child and better logistics for postnatal adherence. Women were not in favour of Option B+ as they anticipated loss of motivation after protecting the child, fearing drug side effects and many did not feel ready to embark on lifelong medication when asymptomatic regardless of CD4 count. In conclusion, women should be counselled about the possibility to "opt-out" of ART after cessation of breastfeeding. Drug adherence counselling, drug safety and benefits, economic concerns and available resources for laboratory monitoring and evaluation should be addressed during B+ implementation to enhance long-term feasibility and effectiveness.

LIST OF PUBLICATIONS

I. Charles Kilewo, Katarina Karlsson, **Matilda Ngarina**, Augustine Massawe, Eligius Lyamuya, Andrew Swai, Rosina Lipyoga, Fred Mhalu, Gunnel Biberfeld.
Prevention of Mother to Child Transmission of HIV-1 through Breastfeeding by Treating Mothers with triple Antiretroviral Therapy in Dar es Salaam, Tanzania: The Mitra Plus study.
Journal of Acquired Immune Deficiency Syndromes. 2009, 53: 406-41

II. **Matilda Ngarina**, Charles Kilewo, Katarina Karlsson, Said Aboud, Annika Karlsson, Gaetano Marrone, Anna Mia Ekström, Gunnel Bibberfeld.
Virologic and immunologic responses to antiretroviral therapy, drug resistance and mortality during the first 24 months postpartum in a cohort of HIV-1 infected mothers in Dar es Salaam, Tanzania.
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III. **Matilda Ngarina**, Rebecca Popenoe, Charles Kilewo, Gunnel Biberfeld, Anna Mia Ekström.
Reasons for poor adherence to antiretroviral therapy postnatally in HIV-1 infected women treated for their own health: experiences from the Mitra Plus study in Tanzania.
BMC Public Health. 2013, 13:450

IV. **Matilda Ngarina**, Edith A.M Tarimo, Helga Naburi, Charles Kilewo, Mary Mwanyika-Sando, Guerino Chalamilla, Gunnel Biberfeld, Anna Mia Ekström.
Women's preferences between infant or maternal antiretroviral prophylaxis for prevention of mother-to-child transmission of HIV during breastfeeding and their views on Option B+ in Dar es Salaam, Tanzania.
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CONTENTS

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ANC	Antenatal clinic
ART	Antiretroviral therapy
ARV	Antiretroviral
CD	Cluster of differentiation
CD4 T cell	Helper T Lymphocyte
CD8 T cell	Cytotoxic T Lymphocyte
DNA	Deoxyribonucleic acid
EFV	Efavirenz
ELISA	Enzyme linked immunosorbent assay
FGDs	Focus Group Discussions
FTC	Emtricitabin
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
IDIs	In-depth interviews
KI	Karolinska Institutet
MDH	Management and Development for Health
MNH	Muhimbili National Hospital
MTCT	Mother-to-child transmission
MUHAS	Muhimbili University of Health and Allied Sciences
NNRTI	Non-nucleotide reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PCR	Polymerase chain reaction
PMTCT	Prevention of mother-to-child transmission
RCH	Reproductive and child health
RNA	Ribonucleic acid
SSA	Sub-Saharan Africa
TDF	Tenofovir
THMIS	Tanzania HIV/AIDS and Malaria Indicator Survey
3TC	Lamivudine
UN	United Nations
UNAIDS	Joint United Nations programme on HIV/AIDS
UNICEF	United Nations Children's Fund
VCT	Voluntary counseling and testing
WHO	World Health Organization
ZDV	Zidovudine

1 GENERAL BACKGROUND

1.1 Introduction

The pandemic caused by the human immunodeficiency virus (HIV), which leads to acquired immunodeficiency syndrome (AIDS), is one of the most serious health challenges the world has ever faced. Cases of AIDS characterised by low levels of CD4 T-cells and by opportunistic infections and/or certain malignancies such as Kaposi's sarcoma were first described in 1981 (CDC 1981, Gottlieb et al. 1981). The two retroviruses causing AIDS, HIV type 1 (HIV-1) and type 2 (HIV-2) were identified a few years later (Barre-Sinoussi et al. 1983, Gallo et al. 1984, Clavel et al. 1986, Albert et al. 1987).

By the end of 2012, 75 million people had been infected with HIV and 36 million had died from AIDS. Despite substantial progress since AIDS was first reported, the HIV epidemic remains an extraordinary human catastrophe inflicting enormous suffering on countries, communities and families throughout the world, but in particular in sub-Saharan Africa (SSA) where two-thirds of all HIV-infected people live (UNAIDS global epidemic report 2013).

The global community has engaged in multi-targeted approaches to prevent and treat HIV, which has had a notable impact particularly on countries in southern and eastern Africa over the past decade. Among the measures to combat HIV infection is the Global plan of eliminating new HIV infections among children by 2015 and keeping their mothers alive (UNAIDS 2011 Count down to Zero). More than 90% of children who acquired HIV in 2012 live in SSA. Implementation of the Global plan is based on a four-pronged strategy including: i) prevention of HIV among women of reproductive age; ii) preventing unplanned pregnancies among women living with HIV; iii) prevention of mother-to-child transmission (PMTCT) of HIV by use of antiretroviral therapy (ART) during pregnancy, delivery and breastfeeding and iv) HIV care, treatment and support for women and children living with HIV and their families. Attainment of the Millennium Development Goals (MDGs) 4, 5, 6 (UN, 2008) i.e. reducing child mortality, improving maternal health, combating HIV/AIDS, malaria and other diseases respectively in SSA countries, is doubtful unless there is a significant increase in the effective coverage of PMTCT and paediatric HIV treatment programmes.

1.2 The epidemiology of HIV infection

1.2.1 Global situation

The 2013 global AIDS epidemic report by UNAIDS showed that 35.3 million (32.2-38.8 million) people were living with HIV at the end of 2012. It was also estimated that 3.3 million (3.0-3.7 million) children under 15 years of age were living with HIV in the same year (UNAIDS global epidemic report 2013). Worldwide, the number of people newly infected by HIV by the end of 2012 was 2.3 million (1.9-2.7 million), which was 33% lower than in 2001 when incidence rates peaked. Among them were 260,000 (230,000-320,000) children below the age of 15 years, which represents a 52% drop since the peak in 2001. It is also estimated that about 6,300 new HIV infections occurred every day in 2012, of which 95% occurred in low-and middle-income countries. Of these new infections, 700 were children below 15 years of age, 5,500 were adults of whom 47% were women and 39% were young people (15-24 years). In the same year, there were 1.6 million (1.4-1.9 million) deaths due to AIDS, a fall of 30% since the peak in 2005. There were approximately 210,000 (190,000-250,000) deaths among children below 15 years old. ART has averted around 6.6 million AIDS-related deaths worldwide between 1996 and 2012, including 5.5 million deaths in low-and middle-income countries (UNAIDS global epidemic report 2013).

1.2.2 HIV infection in sub-Saharan Africa

The number of adults and children living with HIV in the SSA region at the end of 2012 was 25 million (23.5-26.6 million), of whom 1.6 million (1.4-1.8 million) were newly infected. Around 230,000 (200,000-280,000) children below 15 years old were newly infected by HIV in this region, which is home to 92% of all pregnant women living with HIV worldwide. The number of deaths caused by HIV/AIDS-related illnesses was 1.2 million (1.1-1.3 million), which declined by 32% from 2005-2012. This is a result of several interventions implemented over a number of years. For instance, the coverage of PMTCT services in SSA countries reached 65% (57%-70%) in 2012 (UNAIDS global epidemic report 2013).

1.2.1 HIV and AIDS in Tanzania

According to a census done in 2012, the United Republic of Tanzania had a population of 44.9 million people. It is a relatively young population with the majority (45%) being under the age of 15. Tanzania has one of the world's poorest economies in terms of income per capita (ranked at 52) with 36% of its population living below the poverty line of less than US$ 1.25 a day. The gross domestic product (GDP) per capita was US$ 1,600 in 2012. Despite this, Tanzania has achieved high overall economic growth rates, mainly attributable to gold production and tourism. In 2011, the government spent 7.3% of GDP on health (The World Fact book 2013).

HIV prevalence among adults aged 15-49 years was estimated at 5.1% (4.6-5.7%) of which women accounted for 6.2% and men 3.8% at the end of 2012 (Tanzania 2011-12). Around 1.5 million (1.3-1.6 million) people are currently living with HIV in Tanzania (UNAIDS global epidemic report 2013). This includes 730,000 (660,000-810,000) women above and 230,000 (200,000-270,000) children under the age of 15 years (UNAIDS global epidemic report 2013, WHO 2013 Data on the size). It was estimated that 83,000 (69,000-100,000) Tanzanians, of whom 14,000 (8,600-21,000) are children, were newly infected with HIV by the end of 2012, which is over 200 new infections a day. Around 80,000 (69,000-94,000) Tanzanians died of AIDS in the same year (UNAIDS global epidemic report 2013). There is a substantial difference in HIV prevalence from region to region in the country. In some regions, it is as high as 14.8% (Njombe) while in other regions it is as low as 1.5% (Manyara) (TACAIDS 2013, NMSF 2013).

The government has taken several success measures to confront the epidemic but the main drawback is that the Tanzanian HIV and AIDS response is heavily reliant on foreign funding (almost 95%), of which more than two thirds is from the Global Fund and PEPFAR (TACAIDS 2008). The current levels of funding from donors may not be sustainable. Another drawback to ensuring sustained reduction of HIV transmission is human resource shortages and the stigma of HIV-positive people.

1.3 HIV virology

1.3.1 HIV structure and replication

The HIV particle is spherical with a diameter of about 100-120 nm. It has three main parts: 1) a lipid bilayer envelope; 2) HIV matrix proteins; and 3) The viral core (Figure 1). The lipid bilayer envelope (of host cell origin) surrounds a nucleocapsid (core) containing genomic RNA and enzymes. Envelope proteins form spikes consisting of glycoprotein (gp)120 and transmembrane gp41. The gp120 is responsible for viral attachment to host cells and gp41 is important for the cell fusion process. The HIV matrix lies between the envelope and the core. It consists of the p17 proteins. The viral core contains the viral capsule p24 which surrounds two single strands of HIV RNA and the enzymes needed for HIV replication, such as reverse transcriptase, protease, ribonuclease, and integrase (Figure 1). Three out of the nine virus genes, namely *gag*, *pol* and *env*, contain the information needed to make structural proteins for new virus particles (Rubbert et al. 2007).

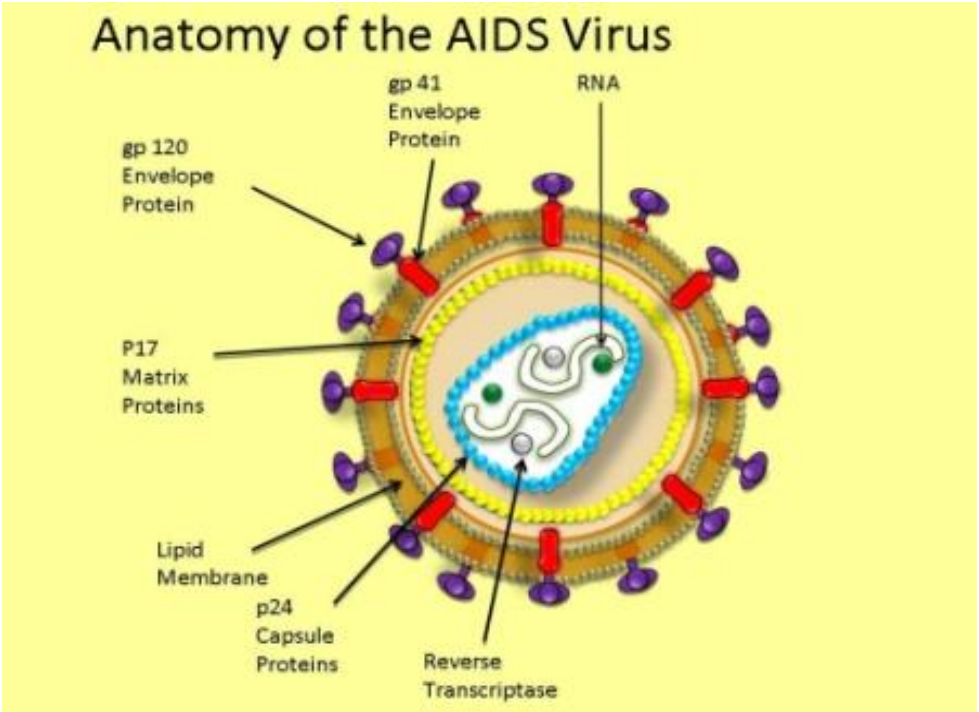

Figure 1: Human Immunodeficiency Virus structure. Accessed and adapted from the following website

http://www.itg.be/internet/e-learning/written_lecture_eng/1_hiv_structure.html

HIV targets the CD4 molecule expressed at the surface of T-helper cells, monocytes, macrophages and dendritic cells. The virus's gp120 molecules bind tightly to CD4 molecule(s) on the cell's surface resulting in a conformational change in the gp120 molecule which then allows gp120 to bind to one of the main co-receptors, CCR5 or CXCR4. Following binding of the virus to the host cell, fusion takes place under the influence of the viral gp41 molecule which results in the release of the viral core into the cytoplasm. The enzyme reverse transcriptase is responsible for the transcription of the viral RNA to double-stranded DNA. The viral DNA is transported to the cell nucleus and integrated into the host chromosomal DNA. The integrated provirus serves as a template for viral transcription which is then translated into viral proteins which are cleaved by the HIV protease enzyme. The virion assembles and then buds through the cell membrane to form a mature infectious virus (Rubbert et al. 2007).

1.3.2 HIV genetic diversity and HIV subtypes

HIV is characterised by a large genomic diversity within and between infected individuals. The genetic variation is due to the high replication and mutation rates of HIV. The reverse transcriptase enzyme is responsible for generating mutations during the reverse transcription of RNA to DNA. The high variability of HIV has implications for disease progression and drug resistance and is one major reason for the difficulty in developing an effective vaccine against HIV (Hemelaar 2013).

HIV-1 and HIV-2 are lentiviruses and belong to the family of retroviruses. There is evidence that HIV-1 and HIV-2 originated from transmission of simian immunodeficiency viruses (SIV) from non-human primates to humans. HIV-1 is related to SIV $_{cpz}$ in Chimpanzees and HIV-2 to SIV $_{sm}$ from sooty mangabey monkeys (Hahn et al. 2000, Hemelaar 2012). Most HIV infections are due to HIV-1 which occurs worldwide, whereas HIV-2 infections are mainly prevalent in West Africa. HIV-1 is divided into three groups: major (M), outlier (O), and non M/non O (N). Group M is further divided into nine subtypes: A, B, C, D, F, G, H, J and K and many circulating recombinant forms (CRFs) which have different geographical distribution. Subtype C is common in southern and eastern Africa, India and Nepal, while in Europe and North America subtype B is the most frequent (Hemelaar 2012). In Tanzania, the prevalent subtypes are A, C, D and CRFs (Lyamuya et al. 2000, Arroyo et al. 2004, Nyombi et al. 2008, Mosha et al 2011, Kiwelu et al. 2012).

1.3.3 Natural history of HIV-1 infection

The typical clinical course of HIV infection can be divided in three phases; the primary infection, chronic asymptomatic phase, and symptomatic (AIDS) phase (Pantaleo et al 1993).

Primary acute infection may last from one to three months. This is the period from initial infection to when the immune response to HIV gains some control over viral replication. During the acute infection, the viral RNA level in blood is very high and there is a rapid decrease of CD4 T-cells. This is a very infectious stage and the risk of HIV transmission is high. After the initial peak, the viral RNA level decreases and reaches a set point after two to six months (Vergis and Mellors 2000, Altfeld and Walker 2007). The initial decline of viral load coincides in time with the appearance of HIV-specific CD8 T-cells whereas HIV-specific-neutralising antibodies appear later (Koup et al. 1994). Clinically, the primary infection phase is characterised by fever, myalgia, maculopapular non-pruritic rash, malaise, lymphadenopathy and oral ulcers in 40-90% of the individuals (Vergis and Mellors 2000, Altfeld and Walker 2007).

The chronic asymptomatic phase may last for eight to ten years, without ART, in industrialised countries (Vergis and Mellors 2000). After the acute infection phase, the CD4 cell count in the peripheral blood increases again, although not to the same level as before infection. Individuals are asymptomatic despite chronic immune activation, persistent viral replication and continued infection of CD4 T-cells (Vergis and Mellors 2000).

Symptomatic disease and the AIDS phase may last two to three years before death without ART. It is characterised by a rapid increase in HIV RNA copies and a decline in CD4 cell counts in peripheral blood (Levy 1993). Decline of CD4 cells causes severe immunosuppression, predisposing the individual to opportunistic infections like cryptococcal infections, varicella-zoster, tuberculosis and to malignancies, especially Kaposi's sarcoma and lymphomas (Onen 2002, Biberfeld et al 2008).

WHO has clinically classified HIV progression into four main stages (WHO 2005).

1.3.4 Diagnosis of HIV infection

Counselling and testing for HIV is the entry point to HIV interventions. The most common method of HIV diagnosis is based on the demonstration of HIV antibodies by enzyme-linked immunosorbent assays (ELISAs) and by different types of rapid simple assays (Read 2007). However, antibodies usually do not appear until three to four weeks after initial HIV infection (the window period). The newer generation ELISAs can detect both HIV p24 antigen and antibodies, which allow early detection of acute HIV infection by reducing the window period (Burst et al. 2000). Screening for HIV antibodies is done by ELISA and reactive samples confirmed by Western blot assay or other types of immunoblot assays in resource-rich countries. The Western blot assay is very expensive, hence rarely used in resource-limited countries, where a combination of two or three rapid simple antibody assays or ELISAs is used. The rapid simple tests offer results within 30 minutes from sample collection. They have sensitivity and specificity that is similar to ELISA-based assays (CDC, WHO/AFRO, APHL. 2002). Infants younger than 18 months carry maternal antibodies, hence HIV antibody assays are not suitable. HIV diagnosis in infants is usually done by a qualitative HIV-1 DNA polymerase chain reaction (PCR) assay using peripheral blood mononuclear cells or by HIV-1 RNA PCR assays which detect plasma viral RNA or by detection of p24 antigen (Reed 2007). The use of filter paper to transfer specimens (dried blood spots) from one remote area to a more advanced lab where diagnosis can be done is a major step forward in early diagnosis of HIV infection in children.

1.3.5 Prevention of HIV infection

Although the incidence of HIV has declined as compared to the peak of the epidemic, 2.3 (1.9-2.7) million new HIV infections occurred worldwide in 2012 (UNAIDS global epidemic report 2013). While waiting for a potential vaccine the spread of HIV infection can be prevented by using biomedical and behavioural interventions. These include: giving health education on modes of transmission and how to prevent transmission, counselling and testing, PMTCT, condom use, pre- and post-exposure ART prophylaxis, male circumcision and HIV screening of blood and blood products. Early detection and treatment of infected individuals are very important in preventing the spread of HIV (Cohen et al. 2011). Behavioural change includes delayed sexual debut, partner fidelity, exclusive breastfeeding and couple HIV testing (Vermund et al. 2013). The male condom may reduce HIV transmission by 80% when used properly (Weller et al. 2002). Male circumcision has also shown to reduce HIV transmission by 50-60% in trials in

Kenya and Uganda (Bailey et al. 2007, Gray et al. 2007). Behavioural change and a safe, effective and affordable vaccine would be the most effective way to prevent the spread of HIV.

1.4 Mother-to-child transmission of HIV infection

1.4.1 MTCT of HIV

Mother-to-child transmission (MTCT) of HIV is one mode of transmission, others being sexual intercourse (oral, vaginal or anal), exposure to infected blood and blood products and use of HIV-contaminated medical devices including non-sterile injection equipment. MTCT accounts for 90% of childhood HIV infections, hence prevention in this context has a huge impact on the spread of the virus among children (WHO 2010-PMTCT strategic vision). Transmission occurs either in utero, intrapartum or postpartum via breastfeeding (Charurat et al. 2009, Cavarelli and Scarlatti. 2011). Numerous socio-economical, clinical, viral and host (maternal) factors increase the risk of transmission to the baby. Some of these factors include non-disclosure of HIV status, mixed feeding, prolonged duration of breastfeeding, prolonged rupture of membranes, vaginal delivery vs Caesarean section, high maternal viral load, low maternal CD4 count and maternal co-infections like tuberculosis, malaria and syphilis (Mmiro et al. 2009, Bucagu et al. 2013, Selvaraj et al 2013). Without any intervention, the risk of vertical transmission ranges from 25-48% in breastfeeding women in resource-limited settings and from 14-32% in non-breastfeeding populations (De Cock et al. 2000). Maternal and infant HIV-immune suppression as well as innate immune factors have been shown to be associated with reduced infant infection rates (Lehman and Farquhar 2007, Tiemessen et al. 2009, Lohman-Payne et al. 2012, Mabuka et al. 2013).

1.4.2 Breastfeeding and MTCT

Breast milk provides all of the nutrients, agents and antibodies needed during the first few months of life, but unfortunately breastfeeding transmits HIV from the mother to the child. The additional risk caused by breastfeeding is 5%-20% with an attributable risk of 40% (De Cock et al. 2000). Breast milk can transmit HIV any time during lactation, hence the rate of HIV infection in breastfed infants is cumulative and increases with the duration of breastfeeding. National health agencies and the WHO 2013 guidelines recommend that HIV-positive mothers in high-income countries should avoid

breastfeeding, give replacement feeds and ARV drugs to the infant for 4-6 weeks while women in low-income countries should provide infants with NVP for 4-6 weeks, practise exclusive breastfeeding for 6 months, introduce complementary food and stop breastfeeding at 12 months (WHO 2013, consolidated ART guidelines).

The pathogenesis of breast milk transmission is yet to be well understood as not all breastfed children get infected. This suggests that HIV-1 transmission through breast-milk is relatively inefficient and supports the protective role of breast milk in preventing viral and other infections (Aldrovandi and Kuhn 2010). Breast milk has both cell-free virus and HIV-infected cells (Rousseau et al. 2003, 2004).

Several studies have been done to investigate the role of breast milk in HIV-1 infectivity. Maternal factors shown to increase the risk of HIV transmission through breast milk include: high plasma viral load; low CD4 count; breast pathology (including abscesses and mastitis); mode of infant feeding; and prolonged duration of breastfeeding - more than six months. The infant factors include damage to mucous membranes (e.g. by oral thrush), damage to the intestinal mucosa by cow's milk or allergic reactions to complementary foods and impaired intestinal permeability by mixed feeding (Kourtis et al. 2007, WHO 2007a). Studies done in South Africa, Malawi and West Africa have shown that women with high viral loads in plasma and breast milk were more likely to transmit HIV compared to those with undetectable virus (Semba et al. 1999, Leroy et al. 2003, Shapiro et al. 2010). The use of ART in HIV-infected women during pregnancy and breastfeeding has been shown to be associated with a rapid decrease in the levels of cell-free HIV RNA in breast milk (Shapiro et al. 2005, Mabuka et al. 2013).

Both cellular and humoral HIV-specific immune responses in breast milk may play a role in reducing the rate of breast milk transmission of HIV. A study among breastfeeding women in Kenya showed that breast milk HIV-gag-specific interferon-γ cellular immune response was associated with an approximately 70% reduction in infant HIV infection (Lohman-Payne et al. 2012). Another study in Kenya showed that breast milk HIV-specific antibodies with antibody-dependent cellular cytotoxicity activity were associated with a reduced risk of MTCT whereas the presence of neutralising antibodies in breast milk was not associated with a reduced risk of infant infection (Mabuka et al. 2013).

1.4.3 Prevention of MTCT of HIV

The first trial of ART prophylaxis for PMTCT performed in the US and France (ACTG 076) used short-course ZDV during late pregnancy, delivery and for six weeks to the infant after birth, which resulted in a significant reduction of MTCT from 25% to 8% (Connor et al. 1994). Current, highly successful PMTCT interventions in resource-rich countries, including the use of ART, elective Caesarean delivery and avoidance of breast-milk, have been proven to reduce MTCT to less than two percent (The European Mode of Delivery Collaboration. 1999, Cooper et al. 2002, Townsend et al. 2008, Mepham et al. 2010). In many low-income countries in SSA including Tanzania, most women cannot implement or sustain replacement feeding on a large scale for several reasons, including costs, access to replacement feeding options, stigma associated with not breastfeeding, and lack of safe water sources. Breastfeeding is very important in these settings, as it reduces the risk of life-threatening infections and malnutrition (WHO 2002, Becquet et al. 2006, Kuhn et al. 2008, 2012, Kagaayi et al 2008, Mepham et al. 2010).

Following the ACTG 076 trial, several PMTCT studies using short-course perinatal ARV prophylaxis in low-income countries showed a reduction of MTCT 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). In the Petra trial conducted in South-Africa, Tanzania and Uganda, 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 six weeks after delivery (The Petra Study Team 2002). However after 18 months of follow-up, little prevention effect remained because of transmission during breastfeeding.

Subsequent PMTCT studies in SSA, in which prophylactic ART of mother or infant was used during the latter stages of pregnancy and during breastfeeding have shown substantial reduction of MTCT rates, down to five percent or less at six months after delivery (Thior et al. 2006, Palombi et al. 2007, Kilewo et al. 2008, 2009, Study team SWEN 2009, Shapiro et al. 2010, Chasela et al. 2010,Thomas et al. 2011, The Kesho Bora 2011, Taha et al. 2011, Jameison et al. 2012, Coovadia et al 2012) and also a low transmission rate 12 to 24 months after delivery (Kilewo et al. 2009, Thomas et al. 2011).

The PMTCT guidelines have been revised several times since 2000 in response to rapidly changing evidence and programme experience. The most current guidelines on PMTCT aim to eliminate new HIV infections among children by 2015 and to keep their mothers alive (UNAIDS 2011 Countdown to Zero). Since most of MTCT occurs in resource-limited regions, WHO issued PMTCT guidelines in 2010 recommending prophylactic ART either for the infants (Option A) or mothers (Option B) during breastfeeding for pregnant women with a CD4 count of >350 cell/μL in low-income countries (WHO 2010a). Furthermore, in 2012, WHO proposed that all HIV-infected pregnant women receive triple ART for life (B+) irrespective of CD4 count. This is a recommendation in the 2013 PMTCT guidelines (WHO 2013, consolidated ART guidelines). The current Global plan can only be achieved by having a positive national trend towards the efforts to accelerate HIV prevention and treatment programmes. Between 2005 and the end of 2012, expansion of PMTCT programmes and the use of more efficacious ART regimen have helped to prevent 800,000 children globally from becoming newly HIV infected (WHO 2013).

1.4.4 PMTCT in Tanzania

HIV prevalence among women of reproductive age in Tanzania (15-49 years) is 6.2% (Tanzania 2011-2012) and around 6.9% among pregnant women (Tanzania eMTCT 2012). It is estimated that there are around 1.7 million annual births in Tanzania, of which 119,000 are from HIV-positive women (Tanzania eMTCT 2012). There are around 14,000 new paediatric infections each year due to a very high MTCT transmission rate of 15% (UNAIDS global epidemic report 2013).

Tanzania started PMTCT activities in 1996 by participating in a multicentre PMTCT trial (Petra study team, 2000) and since 2000, Tanzania has made considerable progress in the scale-up and implementation of PMTCT services (Tanzania eMTCT 2012, NMSF 2013/14). By the end of 2011, 96% of all reproductive and child health (RCH) facilities were capable of providing PMTCT services, reaching about 64% of pregnant women and 56% of their babies with ARV prophylaxis, and 19% of those with advanced HIV infection were started on lifelong ART (Tanzania eMTCT 2012). Guidelines for early infant diagnosis and early initiation of ART in children have been developed. As a result, four referral hospitals (MNH, KCMC, Bugando and Mbeya) can now perform early infant diagnosis using DNA-PCR testing. In 2011, Tanzania adopted Option A

from the WHO 2010 PMTCT recommendations, but has now changed to Option B+ (lifelong treatment for HIV-positive women diagnosed during pregnancy).

The Tanzanian Government together with other stakeholders in the country (MOHSW, CDC, USAID, UNAIDS, UNICEF, WHO, FHI, m2m, CHAI, JHPIEGO, MDH, TACAIDS, AMREF, PSI, AIDS Relief, and NACOPHA) have appointed a taskforce to plan how best to eliminate MTCT in Tanzania (NMSF 2013/14). This includes implementation of Option B+. The government (in cooperation with the public, NGO and private sectors alike) is striving to integrate PMTCT services into routine RCH services so that clients can receive counselling and testing, ART (antenatally, intrapartum and postpartum), modified obstetric care and counselling for safer infant feeding options, paediatric care for exposed children and monitoring, evaluation and linkage of HIV-positive mothers and their families to HIV care and treatment clinics for continuum of care under one roof.

1.5 Antiretroviral therapy and PMTCT

1.5.1 Treatment options

ART results in profound suppression of HIV replication, improved immune function and reduced HIV-associated morbidity and mortality (Pallela et al. 1998, ART CC AC. 2008, Volberding and Deeks. 2010). ART is a successful preventive strategy for PMTCT (Kilewo et al 2008, 2009, Shapiro et al. 2010, Chasela et al. 2010, Thomas et al. 2011, Taha et al. 2011, Jameison et al. 2012, Coovadia et al. 2012) and can also be used as pre-exposure and post-exposure prophylaxis to prevent HIV transmission (Williams et al. 2011, Cohen et al. 2012, Kiselinova et al. 2014).

As of today, there are six major types of ARV drugs (NIH 2013):

- Entry Inhibitors (e.g. maraviroc)
- Fusion Inhibitors (e.g. enfuvirtide T-20)
- Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (e.g. ZDV, 3TC and emtricitabine)
- Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) like NVP and EFV)
- HIV Integrase Strand Transfer Inhibitors (e.g. raltegravir and dolutegravir)

- Protease Inhibitors (e.g. lopinavir, saquinqvir and ritonavir) (FDA 2013).

Each of these groups of drugs interferes with the HIV replication cycle at different stages (Figure 2).

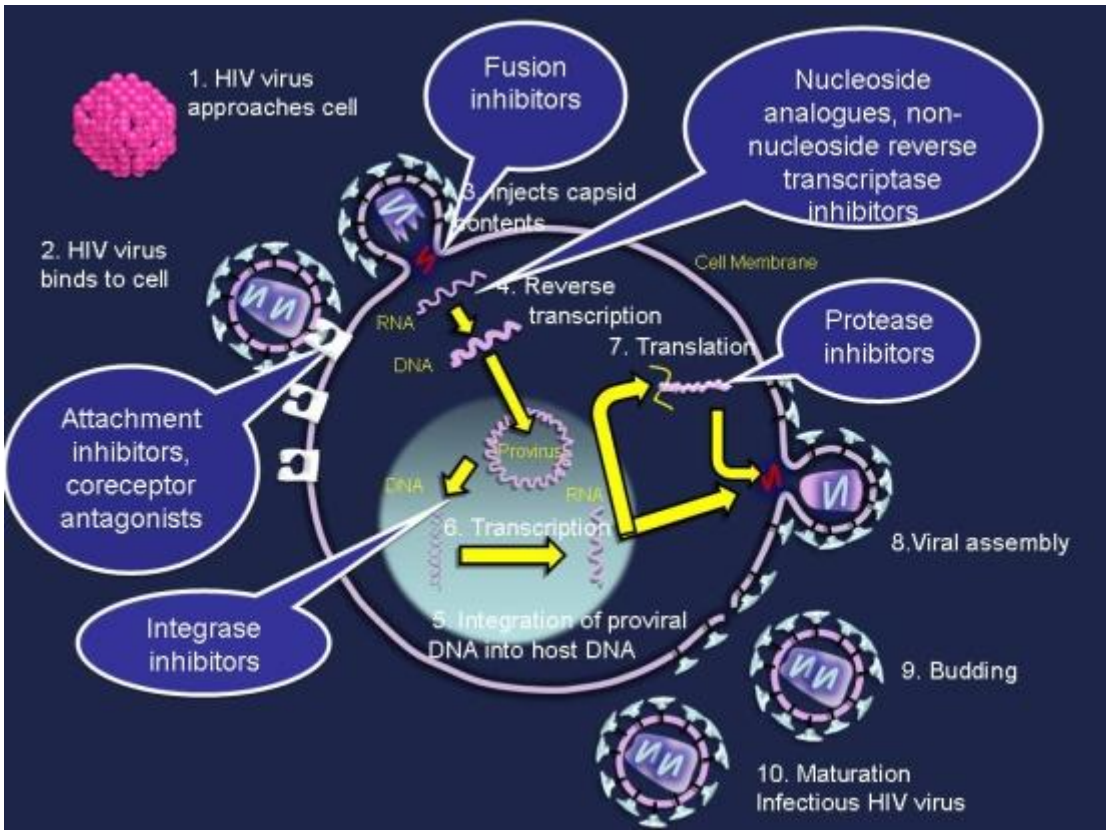

Figure 2: HIV replication cycle. Accessed and adapted from this website http://www.itg.be/internet/elearning/written_lecture_eng/3_virus_life_cycle__where_drugs_interact.html

The treatment options for prevention of MTCT of HIV differ from one resource setting to another. WHO recommends a combination of at least three different ARV drugs (2 NRTIs +NNRTI) for PMTCT in resource-limited settings as follows:

- TDF+3TC (or FTC)+EFV
- TDF+3TC (or FTC)+NVP
- ZDV+3TC+EFV
- ZDV+3TC+NVP (WHO 2013, consolidated ART guidelines)

These combinations are also used by pregnant women on ART for their own health (Option B+). Women on Option A use AZT alone during pregnancy, while those on Option B use the above combination during pregnancy, delivery and breastfeeding (WHO 2012. ART Programme update).

1.5.2 Scale up of access to and patient retention in ART programmes

Access to ART has increased rapidly over the last few years. By the end of 2012, the global target of reaching 15 million people receiving ART by 2015 was achieved to 65% (UNAIDS 2013, Global update on HIV treatment).

Studies in SSA show that almost half of people who test HIV-positive in the general population are lost before being assigned for treatment eligibility and 32% are lost before initiating treatment (UNAIDS 2013, Global update on HIV treatment). Retention in ART programmes is a big challenge in the fight against HIV/AIDS (UNAIDS 2013, Global update on HIV treatment). A systematic review of studies done in SSA on patient retention in ART programmes between 2007-2009 showed that the retention rate declined from 86% at six months to 80% at 12 months and 77% at 24 months (Fox and Rosen. 2010).

1.5.3 Retention in PMTCT programmes

1.5.3.1 The PMTCT cascade

The PMTCT cascade (flow of interventions important in achieving perfect PMTCT) has the following components: all pregnant women attend ANC; all pregnant women are offered and accept HIV testing; all HIV-infected women receive CD4 testing and clinical staging; and all HIV-infected women enrol on a PMTCT/HIV treatment programme, adhere to ART, give birth with a skilled attendant, follow safe infant feeding practices, bring infants for HIV testing and return for results, adhere to option A, B or B+ and use postpartum family planning methods (Stringer et al. 2008).

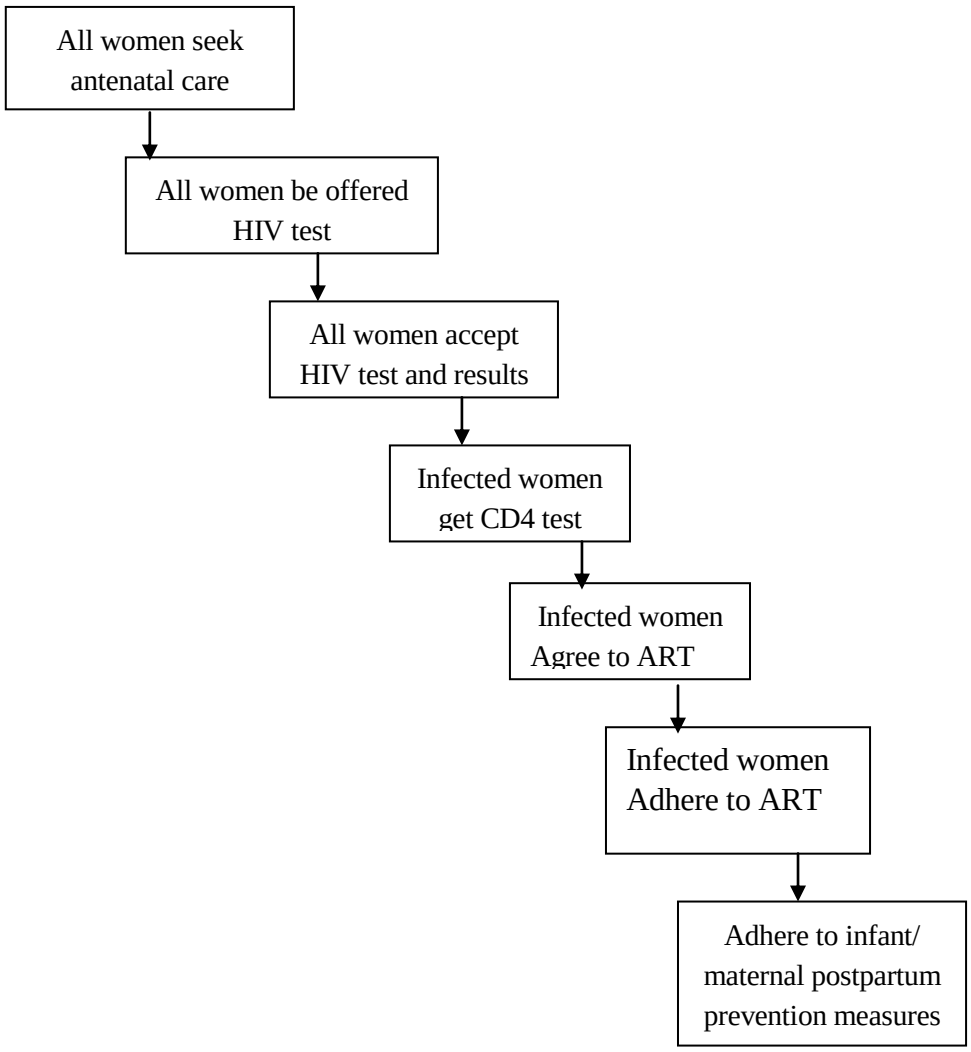

Figure 3: The PMTCT cascade

1.5.3.2 Deviation from the PMTCT cascade

Despite increasing access to PMTCT services, there are still many ways of dropping out of any step of the PMTCT cascade. A review of the impact of stigma on PMTCT programmes in low-income countries revealed that 54-58% of HIV-infected pregnant women receive CD4 testing and clinical staging, 93-94% enrol on a PMTCT/HIV programme, 66-80% adhere to ART during pregnancy, 34-41% give birth with a skilled attendant and 31-37% bring infants for HIV testing and return for results (Turan et al. 2013).

On average, 65% [55%-71%] of pregnant women in the 21 African countries in the global plan received ART for PMTCT in 2012 compared with 59% in 2011 and 49% in

2009. In Tanzania, 77% of pregnant women living with HIV received ART for PMTCT by the end of 2012. (UNAIDS 2013 Global update HIV treatment).

By the end of 2012, only 35% of infants born to mothers living with HIV received an HIV test within the first two months of life indicating that more effort is needed to identify and treat infected children (UNAIDS 2013 Global update HIV treatment). A review of 44 studies of retention of mother-child pairs in SSA PMTCT programmes showed that the loss to follow-up ranged from 19% to 89% (Kalembo et al. 2012).

Barriers to access to, uptake of, and retention in ART and PMTCT programmes include structural, operational, logistical and social obstacles (like stigma and discrimination) and disciplinary laws and policies (Mills et al. 2006, Kalembo et al. 2012, Nachega et al. 2012, UNAIDS 2013 Global update HIV treatment). Some of these barriers have been overcome by increasing the number of ART sites and decentralising ART sites (UNAIDS 2013 Global update HIV treatment).

1.5.4 Treatment failure

Treatment failure is a term used to describe the failure of ARV drugs to control/contain the infection in terms of viral suppression, which in turn could lead to disease progression and eventual death. There are three types of treatment failure: virologic failure; immunologic failure; and clinical progression.

Virologic failure is defined by the failure of ARVs to reduce the amount of virus in the blood (viral load) to undetectable levels depending on the cut-off points agreed in different settings (< 50 or 400 or 1000 copies/mL) after three months on therapy or rebound of detectable viral load after a previous decrease (Hull et al. 2009).

Immunologic failure happens when the immune system does not respond to ART, that is when CD4 counts do not rise significantly or keep decreasing. In 2006, WHO defined immunologic failure as: either a fall of CD4 cell count to pre-therapy baseline or below or a 50% fall of the CD4 cell count from the on-treatment peak value, or persistent CD4 levels below 100 cells/µL (WHO 2006).

Clinical progression refers to a situation where a person has HIV symptoms despite ART for 6 months and/or develops a new or recurrent stage IV condition (WHO 2006). Virologic failure happens earlier followed by immunologic failure and then clinical progression. They may happen months to years apart. Unfortunately, viral load determination, which is the quickest and most reliable indicator of treatment failure, is expensive and usually not available in low-income countries, except in research settings. The WHO 2013 ARV guidelines recommend each country to phase in viral load testing for monitoring treatment response (WHO 2013 consolidated ARV guidelines). Treatment failure can occur as a consequence of poor drug adherence, pre-existing resistant virus, acquired drug resistance, drug interactions, altered drug metabolism, decreased drug absorption and advanced disease stage with very high viral load and/or very low CD4 count (Fletcher et al. 2000, Rotger et al. 2006, Nachega et al. 2007, Paredes et al. 2010, Kwobah et al. 2012.)

1.5.5 Side effects

Despite the significant benefits of ARV drugs in delaying the disease progression and preventing MTCT, like most other drugs, ARV drugs have multiple side effects among the general population as well as among both mothers and children when used during pregnancy. Side effects include nausea, diarrhoea, body malaise, headache, dizziness, skin rash, strange dreams, difficulty in sleeping and elevated liver enzymes. Less common though life-threatening side effects (especially in relation to long-term use) include allergic shock, hepatitis, pancreatitis, bone marrow suppression, peripheral neuropathy, diabetes mellitus, dyslipidermia, renal insufficiency, Steven-Johnson syndrome and toxic epidermal necrolysis (McNicholl 2012). For pregnant women, some of the serious maternal complications include hepatotoxicity caused by NVP (Bera and Mia. 2012), anaemia and neutropenia caused by ZDV (Connor 1994) and renal dysfunction caused by prolonged use of Tenofovir (Striuk et al. 2011). Premature delivery, stillbirth and small-for-gestation-age babies have been observed among pregnant mothers who have been on a combination ART for a long time (Rudin et al. 2011, Chen et al. 2012, Lopez et al. 2012).

Management of side effects differs individually. It is important to give proper counselling on side effects to patients who are about to start treatment as well as to those already on treatment, as most of these symptoms subside with time or may evolve slowly. Patients need proper instructions of what to do when they experience adverse

reactions if we seriously want to contain the infection. Adverse effects can impair drug adherence and this leads to emergent of drug-resistant strains (O'Brien et al. 2003). However, research on ART adverse side effects on pregnancy outcome and infants is needed as the number of pregnant women on ART and HIV exposed uninfected infants is increasing (Newell and Bunders. 2013).

1.5.6 Drug resistance

HIV drug resistance is the ability of the virus to survive and continue to replicate despite the use of ARV drugs (Clavel and Hance 2004). Drug resistance could either be primary (transmitted), i.e. found in treatment-naïve patients infected by a viral strain with pre-existing or acquired resistance to ARVs, i.e. resistant mutations emerge within the same individual during ART or ARV prophylaxis. Development of drug resistance could be secondary to suboptimal adherence to treatment regimens, drug stock-outs, inadequate patient monitoring mechanisms or insufficient knowledge among patients and health workers (Clavel and Hance 2004, Hamers et al. 2012, Nachega et al. 2011, Fokam et al. 2013). These factors lead to treatment failure and eventually drug resistance. Although WHO surveys in Japan, Europe and United States showed a higher rate of 10-17%, it was between 5.1-8.3% in 40 low and middle-income countries by the end of 2011 (WHO 2012a). A multicentre study done in SSA revealed that transmitted resistance doubled the risk of virologic failure and further acquisition of drug-resistant mutations in patients on first-line drugs (Hamers et al. 2012). In Tanzania for instance, the transmitted drug resistance was found to be 11.9% in treatment-naïve HIV-1 infected pregnant women (Vairo et al. 2013) and nine percent among treatment-naïve youths (Mosha at al. 2011). Since the access to ART for HIV has dramatically increased in low- and middle-income countries over the past decade, the emergence of more drug-resistant mutations is foreseeable followed by a need of a second-line regimen, which is more expensive (Gupta et al. 2012).

ARV drug resistance is dynamic and resistance testing is of importance to define the type and level of drug resistance. There are high-level, intermediate-level and low-level reduced susceptibility viral responses. There are two types of ARV resistance testing; genotypic and phenotypic. Genotypic assays are done to detect mutations in the key viral genes, whereby the genetic code of a patient virus is compared to a wild-type (non-mutated). Phenotypic testing, on the other hand, is done to access the susceptibility of

the virus to different drugs in tissue-culture systems and compares it to the wild-type. Phenotypic testing is a very expensive and time-consuming test (Clavel and Hance 2004, Panel of ARV guidelines 2013). Genotypic testing is the recommended test to guide therapy in all pregnant women before initiation of ART, patients with suboptimal virologic responses or virologic failure and ART-naïve patients. Phenotypic testing is done on top of genotypic testing on persons (including pregnant women) with suspected or known to have complex drug-resistance mutation patterns particularly to protease inhibitors (Panel of ARV guidelines 2013). In pregnant women, where the aim of ART is to achieve maximum viral suppression in order to prevent MTCT of HIV, resistance testing helps the clinician to select the optimal regimen for the patient.

Drug resistance is of a particular interest in PMTCT. It has been observed that NVP-drug-resistant mutations are frequently detected after a single dose NVP (sdNVP), arise early and decay slowly and are detectable as major or low-frequency variants for up to three years after sdNVP in both mother and child (Flys et al. 2007, 2008, Persaud et al. 2011). HIV-drug-resistance mutations in HIV-infected infants have been shown to occur between two weeks and six months postpartum, most likely due to exposure to maternal ARV drugs through breast milk. The common drug-resistant virus mutations identified were M184V/I (3TC) and K103N (NVP) (Zeh et al. 2011). Studies have also shown that multiclass resistance mutations to both NNRTI and NRTI are detected in breastfeeding infants of women on ART within 14 weeks of delivery (Fogel et al. 2011). The current WHO proposal of treating pregnant HIV-positive women for life may be a cost-effective option to prevent both HIV transmission and resistance but only if optimal adherence, assured constant drug supply and proper human and logistic issues management in healthcare systems are guaranteed (Parades et al. 2013).

1.5.7 Adherence to ART

WHO defines drug adherence as “the extent to which a person’s behaviour - taking medication, following a diet, and/or executing lifestyle changes corresponds with agreed recommendations from a health-care provider” (WHO 2003). Approximately 50% of patients with chronic illness do not take medication as prescribed (Sabate 2003). As former surgeon General C. Everett Koop reminded us: “Drugs don’t work in patients who don’t take them”, thus physicians must know that increasing adherence may have a greater effect on improving the treatment outcome than modifications to specific medical treatment (Sabate 2003, Osterberg and Blaschkle 2005). Adherence to ART is

also a major challenge as HIV is one of the chronic illnesses. The reasons for poor adherence can be grouped into four categories: characteristics of the patient (stigma, fear of disclosure, age, psychosocial issues, level of education, cultural/traditions practices and beliefs, substance abuse, forgetfulness, work and poor quality of life); drug regimen (high pill burden, frequent dosing, side effects, food requirement); clinical setting (distance, availability of drugs, cost of treatment, privacy); and provider/patients relationship (trustworthiness, work load, provider technical knowhow) (Mills et al. 2006, Unge et al. 2010, Gourlay et al. 2013). A systematic review and meta-analysis of 51 studies of ART adherence during and after pregnancy showed that the adherence level was lower postpartum (53%) than antepartum (76%) (Nachega et al. 2012).

There is still no gold standard for the assessment of adherence, but there are many validated ways and strategies that one can choose from (Chesney 2006). These can be divided into direct and indirect methods. The direct methods include: direct observed therapy; measurement of levels of metabolites or medicine in blood; and measurement of biological makers in blood. Indirect methods include: patients self-report; pill-count; rate of prescription refills; assessment of patient's clinical response; electronic medication monitor and patient diaries. The direct methods are the best but they are expensive and burdensome to healthcare workers. Self-reports, pharmacy refills and patient diaries are commonly used to assess adherence as they are cheap and relatively simple but they may be misrepresented by the patient and result in overestimation of adherence by the healthcare provider (Osterberg and Blaschkle 2005). Patient's self-reports have commonly been used to assess adherence by several researchers and have shown to be significantly associated with viral load, CD4 counts and weight gain (Nieuwkerk and Oort 2005, Ross-Degnan et al 2010). Although self-reports have been found to over-estimate adherence by 20% (Arnsten et al 2001), they are still associated with viral load responses and thus when a patient reports sub-optimal adherence, it is a strong indicator of poor adherence and should be taken seriously (Simoni et al. 2006).

There is a strong correlation between drug adherence, HIV viral suppression, reduced rates of drug resistance, increased survival and improved quality of life (Nachega et al. 2011). Treatment success depends significantly on good adherence otherwise the virus may quickly develop therapy-limiting drug resistance (Nachega et al. 2011). Earlier studies suggested that virologic failure was much less likely to occur in patients who adhered to at least 95% of the prescribed dose (Paterson et al. 2000) but more recent

studies with ART combinations containing boosted protease inhibitors and EFV which have longer half-lives have shown that viral suppression can be achieved at 70%-80% adherence to the prescribed dose (Nachega et al. 2007, Martin et al. 2008, Kobin and Sheth 2011). Despite this knowledge, physicians should encourage their clients to adhere to the prescribed drug regimen as much as possible.

2 RATIONALE OF THE STUDY

As one of the resource-limited countries in SSA, Tanzania has suffered the consequences of the HIV/AIDS pandemic. MTCT, which accounts for 90% of HIV infections in infants and children below 15 years of age, has resulted in 230,000 (200,000-270,000) children living with HIV and 1.2 (1-1.3) million children orphaned by AIDS in Tanzania at the end of 2012 (UNAIDS global epidemic report 2013). Most of the people cannot afford replacement feeding for their infants, hence breastfeeding remains the most safe, acceptable and feasible mode of infant feeding. Tanzania is struggling hard to implement both the PMTCT guidelines and the global plan of elimination of MTCT by 2015. In order to achieve this, it is very important to understand how feasible and acceptable the current PMTCT programmes in Tanzanian settings are.

This thesis studied the prevention of MTCT through the use of ART during late pregnancy, labour and breastfeeding, treatment response in women who needed ART for their own health and reasons for treatment failure among women in this cohort in Dar es Salaam Tanzania. The preferences between infant and maternal ART prophylaxis among breastfeeding women (WHO 2010 guidelines) and their views on the proposed recommendation for lifelong treatment for pregnant women (WHO 2013 guidelines) have also been included in this thesis.

3 OBJECTIVES

3.1 Broad objective

To reduce MTCT of HIV-1 by prophylactic treatment of mothers-child pairs using ARV drugs during pregnancy, labour and breast-feeding and to determine ARV treatment outcomes and preferred options for PMTCT by women in Dar-es-Salaam, Tanzania.

3.2 Specific objectives

1. To reduce MTCT of HIV-1 through breast milk by maternal prophylaxis/treatment with triple ARV drugs during late pregnancy and breastfeeding in Dar-es-Salaam, Tanzania – **Paper I**

2. To determine the virologic and immunologic responses to antiretroviral therapy, drug resistance and mortality during the first 24 months postpartum in a cohort of HIV-1 infected mothers in Dar-es Salaam, Tanzania – **Paper II**

3. To identify the reasons for poor adherence to antiretroviral therapy postnatally in HIV-1 infected women treated for their own health in Dar-es-Salaam, Tanzania – **Paper III**

4. To determine the most preferred PMTCT option (infant-Option A vs maternal-Option B ART prophylaxis) during breastfeeding among HIV-1 infected women and their views on Option B+ (treatment for life) in Dar-es-Salaam, Tanzania – **Paper IV**

4 METHODS

Table 1: Summary of methods for the four papers

	Study design	**Data collection**	**Main outcome indicator(s)**	**Main analysis**
Paper I	Open-label, non-randomised, prospective cohort of 501 HIV-1 positive pregnant women and their infants. (Mitra Plus study)	Interviews, follow-up and collection of infant's blood samples at 6 weeks, 6, 12, 18 months postnatally.	Breastfed infants, HIV-1 negative and alive at 6 weeks, 6, 12 and 18 months postnatally.	Kaplan-Meier survival technique and Cox regression analysis.
Paper II	Open-label, non-randomised, prospective cohort of 84 HIV-1 positive pregnant women within the Mitra Plus study put ART for life.	Interviews, follow-up and collection of blood samples for women put on ART for life at recruitment, 3, 6, 12, 24 months postnatally	Virologic and immunologic suppression at month 3, 6, 12, 24 postnatally, mortality and development of drug resistance.	Descriptive analysis and multivariate analysis by Generalised Estimated Equations (Repeated measures)
Paper III	Qualitative interviews among women with virologic failure in a cohort of women in the Mitra Plus study put on ART for life.	In-depth interviews.	Reasons for poor drug adherence.	Content analysis (Manifest)
Paper IV	Qualitative interviews among pregnant and post-delivery women attending RCH clinics.	Focus group discussions (pregnant women with un-known HIV status). In-depth interviews (HIV positive pregnant and post-delivery women in either Option A or B)	Preferred option for PMTCT in breastfeeding populations and reasons for their choices.	Content analysis (both manifest and latent)

4.1 Study design, population and setting (Paper I, II, III)

The Mitra Plus study was an observational prospective cohort study conducted from 2004-2009 in the former Petra Study site located within the Muhimbili National Hospital (MNH) compound, in Dar es Salaam, Tanzania. Tanzania is divided into 26 administrative regions. Dar es Salaam is one of the regions on the east coast of Tanzania and has a population of 4.4 million (Census 2012). This region is administratively divided into three municipalities: Ilala, Temeke and Kinondoni (Figure 4). There is a mixture of around 120 ethnic groups, 70% of the population are Muslims and Kiswahili is the major language in this region. The majority of the population works for small businesses or do manual labour; very few have office jobs (DSS report. 2007). The Mitra Plus study which constitutes Papers I, II, III and the study that makes up Paper IV were all carried out in Dar es Salaam.

Figure 4: Location of Dar es Salaam, adapted from the DSS site map, Tanzania. Source http://www.idrc.ca/ev_en.php?ID=43009_201&ID2=DO_TOPIC

The Mitra Plus study was a collaborative project between the Muhimbili University of Health and Allied Sciences (MUHAS), MNH in Dar es Salaam, Tanzania and the Swedish Institute for Communicable Disease Control (SMI) and Karolinska Institutet (KI) in Stockholm, Sweden. The study aimed at evaluating the efficacy of ART to prevent breast milk HIV-1 transmission by treating mothers with ART in late pregnancy, intrapartum and postnatally during the first six months of breastfeeding for women who were not eligible for treatment and to continue with ART for eligible mothers (Paper I). We also aimed at assessing the impact of ART on maternal health (Paper II, III).

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

The Mitra Plus study enrolled 501 HIV-1 infected pregnant mothers recruited from four antenatal clinics providing antenatal care services in Dar es Salaam, i.e. one clinic from each of the three municipals and from the antenatal clinic at the MNH in Dar es Salaam. Routine counselling and testing for HIV-1 was offered to all pregnant women reporting for antenatal care by the National PMTCT program. Initial screening was done by nurse/midwife counsellors or by health laboratory technicians using the Capillus rapid simple assay (Trinity Biotech, Ireland) followed by Determine rapid simple assay (Abbott Laboratories) which was done on reactive samples. HIV sero-positive women were invited to join the Mitra Plus study and for those who agreed to participate, a second sample was collected for confirmation of reactivity at the research laboratory in the Department of Microbiology/Immunology at MUHAS by two consecutive anti-HIV enzyme-linked immunosorbent assays (ELISAs), Enzygnost anti-HIV 1+2 Plus ELISA (Behring, Marburg, Germany) and Vironostika HIV uniform II plus ELISA (Biomerieux, the Netherland). 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.

Women with the following characteristics were eligible for enrolment: HIV sero-positivity confirmed by testing of two blood samples as evidence of HIV-1 infection; intention to breastfeed; haemoglobin level not less than 7g/dl; being 18 years or older; absence of life-threatening disease; absence of severe foetal anomalies; willingness to take drugs as prescribed; willingness to deliver at the study site; availability for 18 months of follow-up; and being an accessible resident of Dar es Salaam. Eligible women had to give written informed consent to participate in the study and were free to withdraw at any stage if they wished to do so. Women with a CD4 cell count >200 /μL were enrolled at 34 weeks gestation while those with a CD4 count ≤200 /μL or WHO stage 3 or 4 were enrolled earlier (as soon as they were diagnosed and consented).

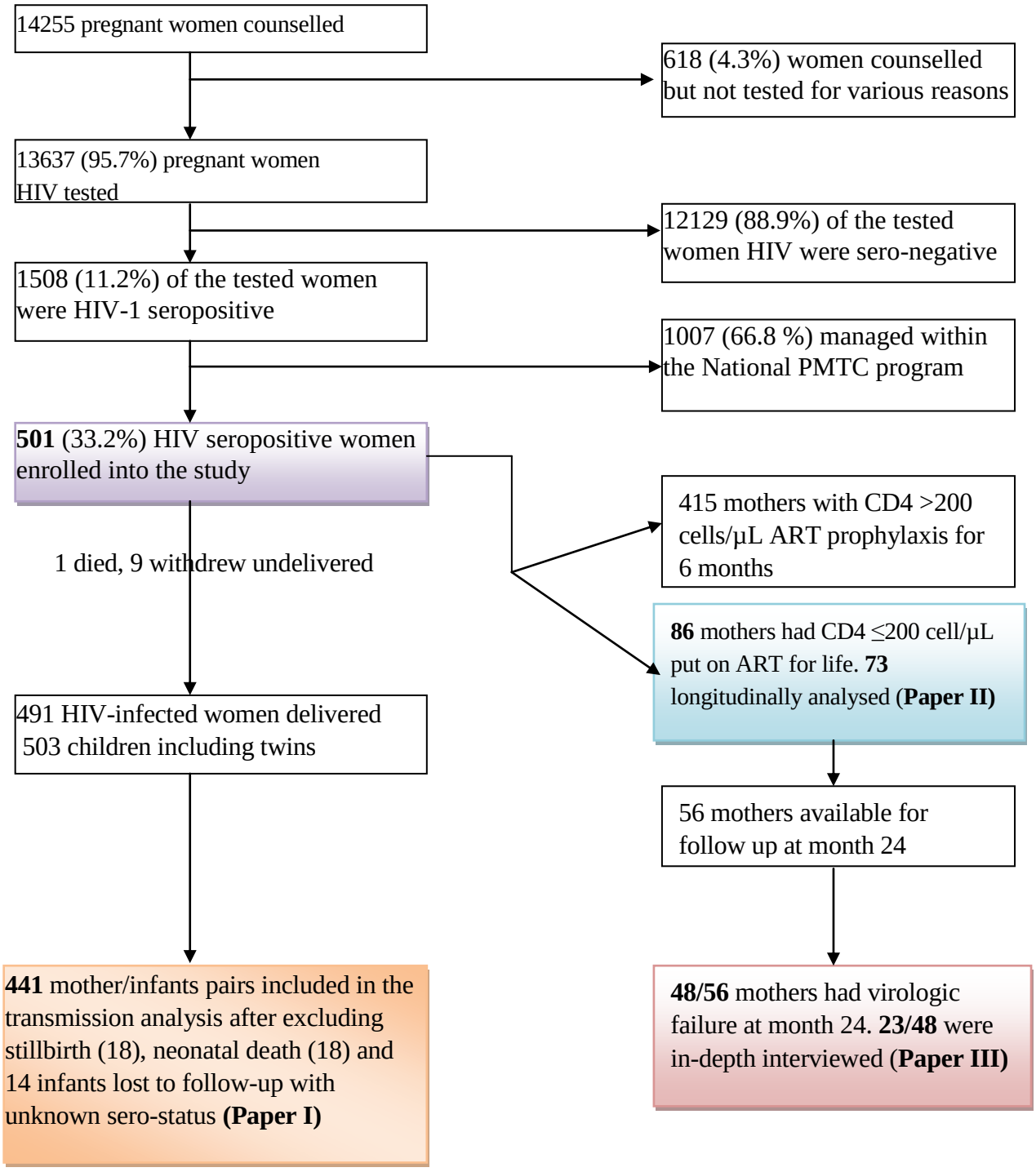

Figure 5: Enrolment of mothers in the Mitra Plus to show the study population of each sub-study.

The study population for Paper II consisted of 24 months postnatal follow-up of treatment response (virologic and immunologic suppression, mortality and drug resistance) of 86 (17%) of the 501 women enrolled in the Mitra Plus study with a CD4 cell count of ≤200 cells /µL and put on treatment for life. Among these 86 women, one

died and one withdrew from the study before delivery, eight were excluded from the longitudinal analysis as they were lost to follow-up very early before they attended the visit at three months. Three women who died early before they could give a second blood sample post-delivery were also excluded from the treatment response analysis. Hence 84 women were included in the mortality analysis (excluding the two women lost before delivery) and 73 women were included in the longitudinal analysis (Figure 5).

For Paper III, out of the 86 women enrolled with CD4 cell counts ≤200/μL, 56 women were available for follow-up at 24 months post-delivery visit. Forty-eight of them (85.7%) had detectable viral load at the end of 24 months. Home visits for women who were lost to follow-up revealed that most of them had moved out of town and none was reported to be dead. We wanted to know the reasons for the virologic failure and we employed qualitative research design (in-depth interviews), which allows close interactions enabling the interviewer to explore the interviewee's perception of the research question. All 48 were traced and invited for interviews. Only 23 viremic women agreed to be interviewed and tape-recorded after signing an informed consent form (Figure 5).

4.3 Study procedures (Paper I, II, III)

At enrolment, the socio-demographic data, medical and pregnancy history were recorded in each mother's case file. Enrolled women received normal antenatal care at the Mitra Plus clinic located in the compound of MNH. They were encouraged to deliver at the labour ward of MNH, where a study nurse could ensure that they got the required care during labour and delivery. They also received ARV treatment according to the study protocol: a combination of Zidovudine (ZDV) 300mg twice daily, + Lamivudine (3TC) 150mg twice daily, + Nevirapine (NVP) 200mg lead dose for 14 days and then escalated to 400mg per day administered in two doses during the rest of the treatment period. The same regimen was continued intrapartum and postnatally for six months and was then stopped (treatment with ZDV + 3TC was continued for one week after stopping NVP). In women who were eligible for ARV treatment for their own health (CD4 cell count ≤ 200/μL), ART was continued within the Mitra Plus study for three years. Thereafter, women were managed at the Care and Treatment Clinics in Dar es Salaam. For women who showed adverse reaction to NVP, this drug was replaced by Nelfinavir (NLF). Towards the end of enrolment, women with CD4 cell

counts > 200/μL received a regimen containing NLF instead of NVP from the beginning of therapy because of new information regarding NVP-related side effects in women with a CD4 cell count >250/μL (Hitti et al. 2004, Dao et al. 2007).

Infants were treated with ZDV (4mg/kg bid) + 3TC (2mg/kg bid) from birth to one week of age. Mothers and infants received free medical care within the study. Postnatal care was given at the Mitra Plus clinic where the follow-up appointments of mother-child pairs given at weeks 1, 3 and 6 and at months 3, 4, 5, 6, 9, 12, 15, 18, 21 and 24 after delivery. Clinical examinations of the mothers and children were done at each visit. Adverse events since the last visit were registered and detailed information on feeding practices and changes since the last visit were recorded in the files. Haemoglobin, leucocytes, lymphocyte and thrombocyte counts, hepatic transaminases (ALT and AST) and serum creatinine were determined in the pregnant women before administration of ART and repeated in the follow-up two weeks after enrolment, at delivery, week 6, month 3 and then after every three months until the mothers were discharged from the study.

4.4 HIV laboratory diagnosis for infants (Paper I)

Infants born from Mitra plus mothers were tested for HIV-1 infection at week 6, month 3, 6 and 9 using the Amplicor HIV-1 DNA v 1.5 qualitative PCR assay (Roche Diagnostics, Rand burg, South Africa) and at months 12, 15 and 18 using Enzygnost anti-HIV 1+2 plus ELISA done at MUHAS in Tanzania and/or at SMI in Sweden. Samples reactive by this ELISA were tested by Vironostika HIV uniform II antigen/antibody ELISA (Biomerieux, the Netherland). Children with a positive HIV test by PCR or ELISA were retested at the next scheduled visit. Children with two positive HIV tests were diagnosed as being HIV infected. Children with a positive HIV test at six weeks and from whom a later sample was not available had their birth or week 1 sample tested using the Amplicor HIV-1 RNA Monitor v1.5 assay (Roche Diagnostics, Randburg, South Africa). A viral load of ≥1000 copies/mL was considered positive when a RNA PCR test was done at birth or one week postnatally. Elisa antibody tests were done at month 12, 15 and 18. HIV-1 RNA PCR assay was done at month 12 and 15 and the diagnostic threshold for the test was 10,000 copies/mL (Read 2007).

4.5 Laboratory assessment of viral load, CD4 cell count and resistance (paper II)

For Paper II, the virologic and immunologic responses to ART and drug resistance among women enrolled with a CD4 cell count < 200/μL (put on treatment for life) were monitored by laboratory testing of plasma viral load, CD4 cell count and resistance testing. Blood samples for viral load testing were collected at enrolment, months 3, 6, 12 and 24 postnatally and were analysed when all women had completed 24 months of follow-up. Plasma viral load was quantified by the Amplicor HIV-1 Monitor assay version 1.5 (Roche Diagnostics, Randburg, South Africa.) The detection limits of the assay using standard protocol testing were 400-750,000 copies/mL.

Determination of T-lymphocyte subsets was done at enrolment and every three months postnatally until the women were discharged from the clinic. The SimulSET flow cytometry method (Becton Dickinson, San Jose, CA) was used (Urassa et al. 2003).

Drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) was tested at 12 months postnatally using the Agence Nationale de Researches Sur le SIDA (ANRS) algorithm (July 2009, version 18), an in-house method (Lindstrom and Albert 2003, Murillo et al. 2010).

4.6 Study population, settings and recruitment procedures (Paper IV)

The aim of the fourth sub-study was to explore women's preferences for Option A and B respectively and their views on Option B+ for PMTCT of HIV-1 during breastfeeding as recommended by the WHO guidelines. It was a qualitative study conducted at the RCH clinics in Kinondoni and Ilala districts between July 2012 and June 2013 in Dar es Salaam, Tanzania. At the time of the study, Tanzania was implementing Option A but has recently adopted Option B+. However, some RCH sites implemented Option B for research purposes conducted by the Ministry of Health.

Interviews were conducted at six purposefully selected clinics (three in each district), i.e. clinics that had enough clients (at least a minimum of ten new cases per day),

confidential rooms or places with privacy and located close to public transport to facilitate the homeward journey of the key informant afterwards.

During recruitment of participants for focus group discussions (FGDs), women followed the normal RCH routine care where nurses give all women health education on pregnancy-related issues including PMTCT. Just after the health education session and HIV screening for the first time attendees, we approached women eligible for FGDs. They were informed about the research before being asked if they would like to participate. Consenting women were interviewed and tape-recorded. Four FGDs (two among primigravida and two among multigravida with unknown HIV status each group having 4-8 participants) were conducted as it was more practical to find women in these sub-groups (primigravida and multigravida) than other groups like age, education and occupation in a day.

In-depth interviews (IDIs) were also conducted among consenting HIV-positive pregnant women who had given birth and who had been enrolled in either Option A or Option B postnatally. Participants were purposefully selected so that the sample included women with different characteristics (pregnant, primiparous, multiparous, post-delivery but before HIV-screening of the infant and post-delivery after testing the infant for HIV). Thirty-one IDIs were conducted; ten with women using Option A, one of whom was also asked to give her views on Option B+ and 21 with women using Option B, 17 of who were asked to give their views on Option B+. With these interviews, we reached a saturation point where we felt that adding more interviews would not bring forth any new information.

4.7 Statistical analysis (Paper I, II)

For Paper I, the calculation of sample size for Mitra Plus was based on the assumption that triple ARV treatment of mothers during breastfeeding would decrease the HIV-1 transmission rate at six months from 14% (Turnbull estimate for the breastfeeding population of the arm A in the Petra trial) to 7% in the Mitra Plus study. A significance level of 5% and a power of 80% were used. In order to compare the 222 children remaining in follow-up at six months in the breastfeeding population in arm A of the Petra trial (where HIV-infected pregnant women were initiated on ZDV and 3TC from 36 weeks gestation to one week postpartum), we would then need 324 children in the

Mitra Plus study at six months. To allow for deaths and loss to follow-up, we planned to enrol at least 450 mothers in the Mitra Plus study.

Data analysis for Paper I was done using the SPSS software system 15 (Statistical Package for Social Sciences, SPSS Inc., Chicago Illinois, USA). The firstborn baby in case of twins was included in the analyses. HIV-1 transmission, mortality, the combined outcome ´HIV infection or death´, and breastfeeding were analysed using the Kaplan-Meier survival technique. Time for HIV-1 infection was estimated as 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. Differences in distributions were tested with the chi-square test. Differences between means were tested with Student's t-test and differences between medians with the Mann-Whitney test.

For Paper II, data analysis was performed using the STATA software 11(Stata Corp. College Station, Texas, USA). Mean, median, interquartile range and standard deviation were used for descriptive analysis of numerical variables. Frequencies and percentages were used for categorical variables. Generalised estimating equations were used to determine the association between virologic and immunologic failure with the baseline (age, marital status, education, and disclosure) and clinical (haemoglobin, gravidity, partner's HIV status) characteristics at 3, 6, 12, and 24. The command 'xtgee' in STATA was used to run the regression models. Selection of the best model and variance-covariance structure was done based on quasi likelihood under the independence model criterion (QIC). Multivariate analysis for drug resistance and mortality were not performed due to small numbers of mothers with this endpoint. For the virologic and immunologic failure models, p-values < 0.05 were considered significant.

4.8 Qualitative – content analysis (Paper III, IV)

Papers III and IV employed qualitative research methods. The data was analysed using manifest (Paper III) and both manifest and latent content analysis (Paper IV). Manifest analysis involves analysis of visible and obvious component of the text while latent analysis involves interpretation of the underlying meaning of a text (Graneheim and Lundman 2004). Data analysis in qualitative studies begins and evolves during data

collection and continues throughout the analysis process as guided by Kvale (Kvale 1996). The analysis was inductive, as in both papers we started with a research question and we chose content analysis because we wanted to understand the reasons, meaning, reasoning and variation of views our informants had regarding poor ART adherence and the preferred PMTCT options.

I (MN), a clinician (gynaecologist) and two other public health specialists (AB and EAM), all female, familiar with qualitative research methods and fluent in both Swahili and English, conducted the interviews in both papers. We (MN and AB) interviewed the women at the Mitra Plus clinic for Paper III. For Paper IV we (MN and EAM) interviewed the women at their respective RCH clinics. Interviews were conducted in Kiswahili (national language) using an interview guide.

According to Graneheim and Lundman 2004, content analysis focuses on selecting the unit of analysis, meaning units, codes, categories and themes. A meaning unit is a group of words or sentences or paragraphs containing related aspects through their content and context. A code is a word/group of words that can be assigned to a phenomenon and should be understood in relation to the context. A category is a group of codes that have something in common regarding the context in question. Categories express the manifest content of the text. Themes emerge as a thread of underlying meaning throughout the whole analysis process at an interpretive level and this is now latent analysis. Tape-recorded interviews were transcribed verbatim and translated into English. The transcripts (unit of analysis) were read through several times and meaning units were identified. The meaning units were then condensed and codes were developed and from them categories were made manually (Figure 6). For Paper IV, the categories were compared and then merged into themes. Quotes were selected to illustrate the informants' views.

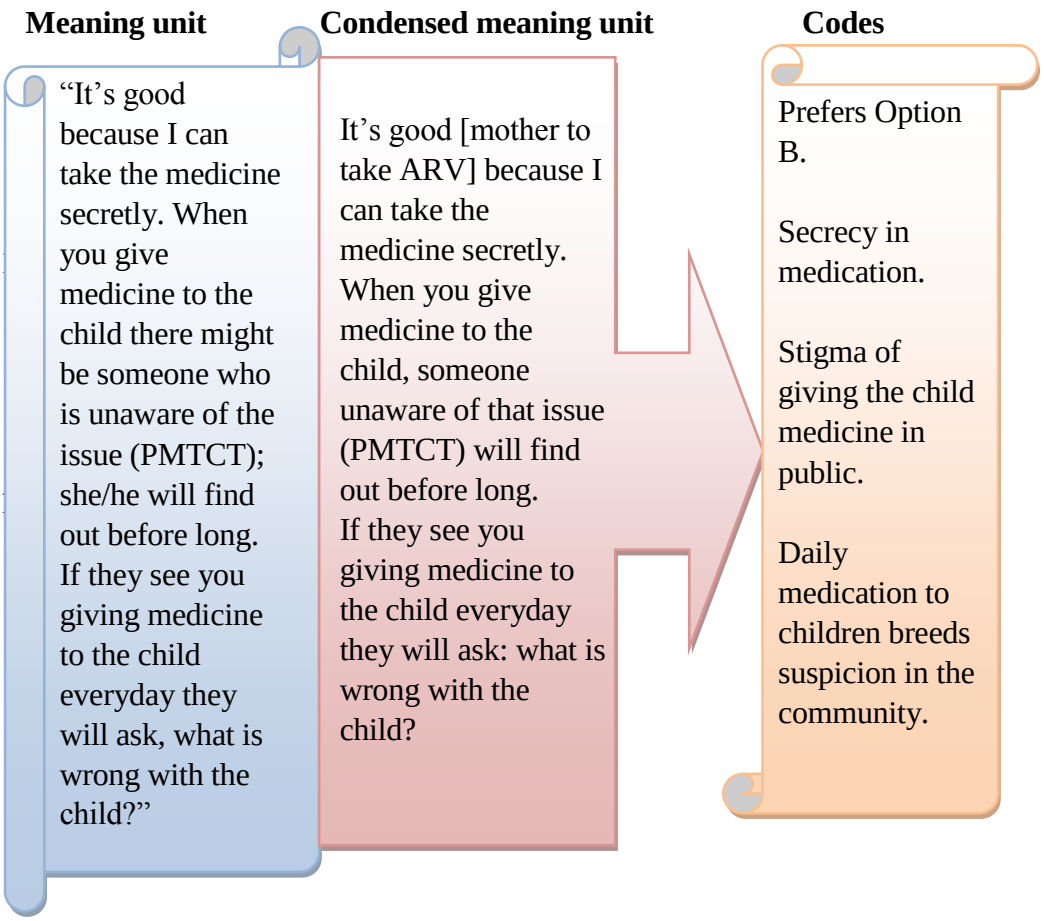

Figure 6: Example of the analysis process; identification of meaning units, condensed meaning units and codes.

4.9 Ethical consideration

The ethical permit for the Mitra Plus study was approved by the Institutional Review Boards (IRBs) of Tanzania National Institute for Medical Research, MUHAS, and Karolinska Institutet, Stockholm, Sweden.

The ethical permit for Paper IV was also obtained from the Institutional Review Board (IRB) of the Muhimbili University of Health and Allied Sciences (MUHAS).

5 RESULTS AND DISCUSSION

My PhD work is based on the Mitra Plus study (Paper I) which was a clinical intervention study that included two sub-studies (Papers II and III) and another qualitative sub-study (Paper IV) using the set-up of an ongoing cluster-based intervention study. The overall aim of this work was to determine how well ART can prevent breast milk transmission of HIV in a resource-poor urban setting such as Dar es Salaam, the treatment outcome for those initiated on ART for life, the impact it has on women and children and the acceptability of PMTCT programmes in this part of the world. Cross-cutting results from the four papers that make up this PhD thesis will be presented and discussed under the same sub-headings.

5.1 Prevention of postnatal MTCT of HIV-1 through breastfeeding and improving HIV-free survival in children by perinatal ARV prophylactic treatment (Paper I)

Breastfeeding is the most effective way of saving the lives of young children especially infants of less than six months in resource-limited countries (WHO 2002, Becquet et al. 2006, Kuhn et al. 2007, 2012, Kagaayi et al 2008). Most of the women in these settings have no alternative to breast milk despite the fact that it transmits HIV from mother-to-child. Mixed feeding (combining breastfeeding with other feeds) which is a common practice in most low-income countries was found to have a higher risk of HIV transmission than exclusive breastfeeding (Coutsoudis et al. 2001, WHO 2007, Kuhn et al. 2007). This challenge has necessitated the need of further research and recommendations to improve infant-feeding practices so as to prevent breast milk transmission of HIV.

The Mitra Plus study explored the likelihood of preventing breast milk transmission of HIV-1 by prophylactic treatment of HIV-1 positive women with triple ART irrespective of their WHO clinical stage from 34 weeks of gestation (or earlier) to six months of exclusive breastfeeding. We enrolled 501 women and 441 mother/infant pairs were suitable for inclusion in the transmission analysis (Figure 4). The median age of women enrolled in the study was 26 years, with a median haemoglobin level of 10.0g/dl. The majority (82%) had a CD4 cell count >200cells/µL and 94% were in WHO Stage I.

Women enrolled with a low CD4 counts (≤200 cells/µL) were started on treatment for life. The median duration of breastfeeding was 24 weeks.

Kaplan-Meier survival analysis was used to determine the transmission rates and the HIV-free survival at different time points (Table 2). The transmission rates were lower compared to the short perinatal intervention of the Petra trial arm A – [a PMTCT study also done at site but not part of this thesis] (The Petra Study Team 2002); 4.1% (95%CI: 2.2-6.0) vs 5.4% (95% CI: 2.7-8.1) at six weeks; 5.0% (95% CI: 2.9-7.1) vs 11.9% (95% CI: 7.9-15.8) at six months and 6.0% (95% CI: 3.7-8.3) vs 17.7% (95% CI: 12.8-22.6) at 18 months. The cumulative risk of HIV transmission between six weeks and six months was 1.0% and 1.1% between six and 18 months.

The combined outcome HIV infection or death at 18 months after delivery was also lower in the Mitra Plus study, 13.6% (95% CI 10.3-16.9) than in the breastfeeding population in the Petra trial arm A, 21.9% (95% CI 16.7-27.1). Apart from the neonatal deaths, 32 children died between day 29 and month 18 in the Mitra Plus study and all except six were HIV-negative at their last HIV test before death. They died of pneumonia, malaria, diarrhoeal diseases and other febrile illnesses.

Table 2: Kaplan-Meier estimated transmission of HIV-1 and HIV-free survival in the Mitra Plus study.

	HIV-1 Infection			HIV-1 Infection or Death		
Age	**No. at Risk**	**Cumulative No. Infected**	**Cumulative Infection. Rate% (95% CI)**	**No. at Risk**	**Cumulative No. Infected or Dead**	**Cumulative HIV Infection or Death Rate% (95% CI)**
6 wks	423	18	4.1 (2.2-6.0)	425	27	5.9 (3.7-8.1)
3 mo	418	19	4.3 (2.4-6.2)	419	30	6.6 (4.3-8.9)
6 mo	397	22	5.0 (2.9-7.1)	400	39	8.6 (6.0-11.2)
9 mo	387	23	5.3 (3.2-7.4)	388	50	11.2 (8.2-14.2)
12 mo	368	25	5.8 (3.6-8.0)	369	57	12.8 (9.6-16.0)
18 mo	333	26	6.0 (3.7-8.3)	334	60	13.6 (10.3-16.9)

This study, together with several other studies done in low-income countries, has demonstrated low postnatal transmission rates when ART was given to women during pregnancy and the breastfeeding period (Palombi L et al. 2007, Tonwe-Gold et al 2007, Shapiro RL et al. 2010, Kesho Bora study. 2011, Thomas KT et al 2011). In a PMTCT

study done at Kisumu Kenya, where triple ART was initiated in HIV-infected women from 34 weeks to six months postpartum, the transmission rates were similar to our Mitra Plus study findings i.e. 4.2% at six weeks, 5.0% at six months, 5.7% at 12 months and 7.0% at 24 months (Thomas KT et al. 2011).

Lower transmission rates have been observed when treatment is started earlier in pregnancy (Palombi et al. 2007, Shapiro et al. 2010). In a study on ARV regimens in pregnancy and breastfeeding in Botswana, where three different types of ART regimen were assigned to HIV-infected women from 26 weeks gestation until breastfeeding was stopped, MTCT of HIV was found to be 1.1% at six months (Shapiro et al 2010). In a multi-centre randomised controlled trial done in five countries in Africa where two different regimens of ART (triple and dual therapy) were given to HIV-infected women from 28 weeks gestation, the HIV transmission rate in women who declared they intended to breastfeed was 5.6% in the triple therapy group vs 10.7% in the dual therapy group at 12 months (The Kesho Bora study. 2011).

Low MTCT rates have also been demonstrated in studies in which postnatal ARV prophylaxis was given to the infants (Thior et al. 2006, Kilewo et al. 2008, Kumwenda et al. 2008, Study team SWEN. 2008, Chasela et al. 2010, Taha et al. 2011, Jameison et al. 2012, Coovadia et al. 2012). In the Mitra study performed at the same site in Dar es Salaam as the Mitra Plus study, HIV-1 infected women were given short course ART with ZDV and 3TC from 36 weeks gestation to one week postpartum and infants were given ZDV and 3TC for one week and then 3TC alone during breastfeeding for six months. The cumulative infection rate was 4.9% at six months and the postnatal transmission rate between six weeks and six months was 1.2%, which is very similar to the corresponding transmission rates in the Mitra Plus study (Kilewo et al. 2008). In a study done in Malawi, mother-child pairs were randomised into maternal, infant or no prophylaxis arms. At 28 weeks postnatally, the HIV transmission rates were 1.7% in the infant prophylaxis group, 2.9% in the maternal prophylaxis group and 5.0% in the arm without extended postnatal ART (Chasela et al. 2010). In a randomised, double-blinded placebo-controlled trial (HPTN 046) done in four African countries, breastfeeding infants without HIV infection were randomised into extended NVP prophylaxis or placebo. The HIV transmission rates from six weeks to six months were 1.1% in the extended NVP group compared to 2.4% in the placebo group, equating to a 54% reduction in transmission ($p=0.049$) (Coovadia et al. 2012).

5.2 Treatment outcomes, drug adherence, motivation and barriers to adherence among women initiated on ART for life during pregnancy (Paper II, III)

In the Mitra Plus study, women enrolled with a CD4 cell count of >200/μL were given ART during pregnancy, delivery and the breastfeeding period, and after weaning, the treatment was stopped. These women and their children were followed for 24 months post-delivery and thereafter discharged to care and treatment centres. Women who were diagnosed with HIV during pregnancy with lower CD4 counts (≤ 200/μ/L) were, according to the prevailing guidelines, initiated on ART for life and followed up together with their infants for 36 months before discharge to care and treatment centres.

5.2.1 Treatment failure, drug adherence and loss to follow-up

Retrospective viral load testing of blood samples collected at enrolment and at months 3, 6, 12 and 24 postpartum from the women initiated on ART for life showed that many of these women had developed virologic failure (viral load ≥ 400 RNA copies/μL) at 12 and 24 months postpartum (Figure 7). The proportion of women with immunologic failure also increased significantly at 12 and 24 months (p=0.032 and 0.035 respectively) (Figure 7).

Treatment failure is often a result of poor drug adherence (Coffie et al. 2008, Datay et al. 2010, El-Khatib et al. 2011). In our study, self-reports also revealed declining drug adherence over time (from 95% at three months to 65% at 24 months). We found that the probability of having virologic failure was ten times higher among women who reported non-perfect adherence (AOR=10.00; 95% CI: 2.29-43.66), adjusting for age, education, marital status, haemoglobin at enrolment, disclosure, gravidity, and partner's HIV status. The increased relative risk of immunologic failure was also much higher compared to women who reported perfect adherence, but with a very wide confidence interval. In many resource-poor settings, patients' self-reports (being the cheapest method to assess drug adherence) have been used to show an association between adherence and treatment outcomes (Ross-Degnan et al 2010).

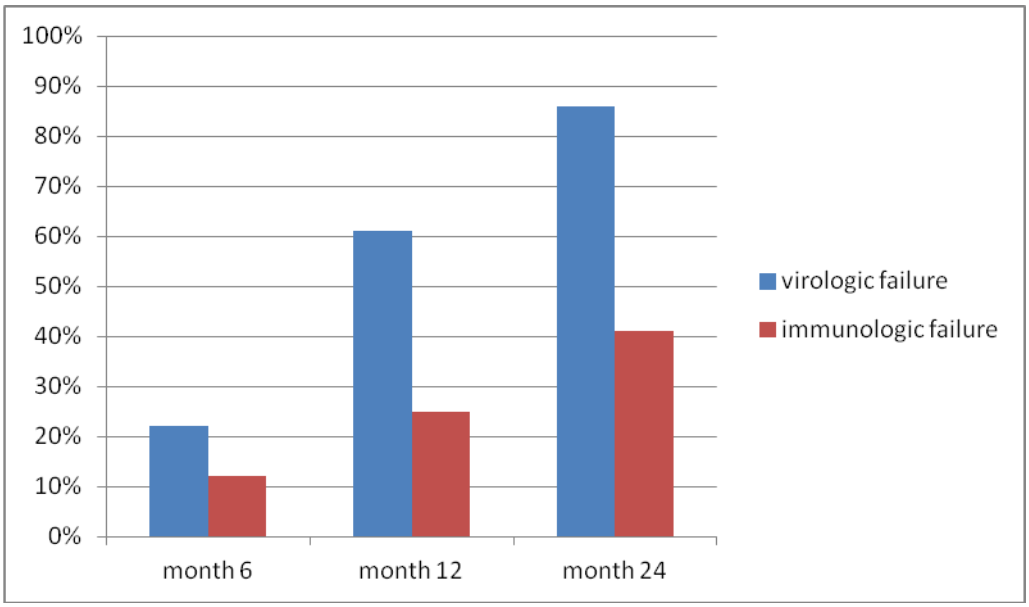

Figure 7: Proportions of women with treatment failure on ART at 6, 12 and 24 months post-delivery.

Repeated CD4 count assessment every three months proved not to be a good predictor of viral treatment failure given the delay compared to virologic failure (Moore et al. 2008, Badri et al. 2008, Reynolds et al. 2009, Rawizza et al. 2011). The 24-month mortality rate in this Tanzanian cohort of women with CD4 counts below 200 was as low as 5.9% (95% CI 2.5-13.7%). All women who died had HIV-related complications. The fact that most of the surviving women were doing very well at clinical assessment made it difficult to anticipate that they were failing virologically.

Drug resistance testing was also performed retrospectively on blood samples collected at 12 months post-delivery. It revealed that 34% of the women available for follow-up had resistance mutations, a number equivalent to 56% of the women with detectable viral load at this time point. As much as 60% of the women with detectable viral load (>400 copies) had dual resistance against the two most frequently used first-line drugs (3TC and NVP). We did not perform baseline resistance testing but studies done in Tanzania among ART-naïve clients have shown a prevalence of primary ART resistance mutation of 9% among youth (Mosha et al. 2011) and of 12% among pregnant women (Vairo et al. 2013). Assuming similar proportions at baseline among the treatment-naïve enrolled in our Mitra Plus study, it is most likely that the high proportion of drug resistance detectable at 12 months was secondary to suboptimal drug adherence, as previously shown by other studies (Nachega et al. 2011, Hamers et al. 2012, Fokam et al. 2013).

Despite close monitoring and follow-up as part of a research study including home visits to all clients who had missed more than two consecutive appointments, loss to follow-up was high in this population. Out of 73 women eligible for follow-up after delivery, only 56 returned for their 24 month visit (77%). Previous studies in low-income countries have also shown that postnatal drop-out from the PMTCT cascade is common (Nachega et al. 2012, Kalembo et al. 2012). Loss to follow-up has been a major drawback to the efforts of combating MTCT of HIV in resource-limited regions. This makes it pertinent to discuss how feasible and realistic it is to implement the 2013 WHO guidelines to initiate all HIV-positive pregnant women on ART for life. A study done in Malawi, which was the first country to implement the Option B+ recommendations, has shown that 17% of women were already lost to follow-up within six months post-delivery (Tenthani et al. 2014).

5.2.2 Motivation of adhering to ART

We employed qualitative methods (Paper III) to explore why women stopped taking ART despite the fact that they knew it was for their own health and that it was important for them to survive for the sake of their young children. Qualitative in-depth methods are the best when one needs to gain a deeper insight into sensitive issues such as this and we wanted to understand women's own reasons for poor drug adherence leading to poor treatment outcomes.

We found that despite the high quality of care given under the Mitra Plus research set-up and the confidentiality of the services offered to all participants, the women faced many other important barriers to optimal drug adherence. It was clear from the interviews that the main and almost exclusive motivation for them to take ART was **the need to protect their child from HIV infection**;

> *"It was the same when I was breastfeeding. But I was afraid to stop taking them* [the ARVs]*, as I didn't want to transmit the virus to my baby. So when I stopped breastfeeding, I was now sure that I cannot infect my child and I could now have drug holidays. I could go for 3-4 days without taking the drugs." (34 years, cohabiting, primary education)*

All of these women were diagnosed as HIV-infected during the antenatal screening. Most of them were asymptomatic despite CD4 counts of ≤200, and most of those who did experience some symptoms became asymptomatic after taking ART during

pregnancy and the six months of breastfeeding. Being asymptomatic, their own motivation to adhere to ART decreased after they knew they had protected the baby from HIV infection. We are not aware of other studies that agree or disagree with this finding.

5.2.3 Barriers to ART adherence

We also found that loss of motivation to adhere to ART after protecting the child was compounded with other strong barriers to ART adherence that women had little power to influence or change. These were categorised into **stigma**, **poverty** and **the constant demands of daily life.**

5.2.3.1 Stigma

Thirty years into the HIV epidemic and ten years after the scale-up of life-saving combination ART began in SSA, the stigma attached to HIV infection in Tanzania and many other low-income countries is still extremely strong. The great majority of people living with HIV hide their sero-status and treatment even from their partners and family members. HIV infection is sometimes considered to be a punishment for promiscuity and people living with HIV are sometimes thought of as dangerous or "almost dead" individuals in the society (Rankin et al. 2005, Simbayi et al. 2007, Amuli et al. 2011, Agnarson et al. 2013). The impact of stigma has been one of the major obstacles to drug adherence and effective implementation of PMTCT guidelines (Turan et al. 2013).

> "*This is a secret disease. You should not go around spreading news that you are infected because once people get to know that you are infected they discriminate you. People try to avoid you and would prefer to stay far from you." (33 years, married, no formal education)*.

5.2.3.2 Poverty

Most of the women in this study (like most women in Tanzania) were poor. They had a low level of education with no or very little income-generating activities (Rwebangira 1996, Feinstein et al. 2010). They depended on their partners for survival, making disclosure of their HIV status even more difficult and risky. Many were not sure of their next meal and had to run around to earn money for food.

"*They tell us that we should eat fruits and vegetables but here in Dar es Salaam one has to buy all these things. They should maybe give us some assistance on this. At the moment, a whole day may pass without a meal and one is not even able to afford an orange." (37 years, widow, primary education).*

5.2.3.3 Constant demands of daily life

Women also told us how complicated it was for them to keep their jobs, which was most important for their daily survival, and at the same time keep their appointments for clinics and drug refills in such an HIV-stigmatised society. In Tanzania, it is common to have regular hospital visits for vaccination until the child is nine months old but after that it may be very complicated to get regular permission from work for clinical check-ups and drug refills unless the woman disclosed her HIV status, which is risky since she may lose her job.

> *"I did not have time to go and get the drugs when I was employed. I used to hide and go to get the drugs but when I got here they had either closed or the queue was too long and I could not wait because I had the keys for my employers' house. Sometimes I would ask the nurse to serve me first but the other clients would complain." (28 years, cohabiting, primary education)*

The influence on ART adherence of stigma, poverty, lack of disclosure and a busy daily schedule has been well described by other researchers in this field (Murray et al. 2009, Duff et al. 2010, Do et al 2010, Awiti et al 2011, Mepham et al. 2011.) Lifelong ART adherence will likely continue to be a challenge in Tanzania and most other low- and middle-income countries unless efforts to combat HIV-related stigma are addressed. Poverty and women's empowerment is another complex area that should be dealt with in order for ART and PMTCT programmes to reach their full potential.

5.3 Women's views and preferences towards the current recommended WHO options for prevention of breast milk HIV transmission (Paper IV)

The WHO guidelines from 2010 indicate two treatment options for preventing HIV transmission in breastfeeding women (Options A and B) and the guidelines from 2013 provide a third option (B+). In all three options, the women start ART from 14 weeks or as soon as they register for antenatal care, but the regimens are different during the breastfeeding period. For Option A, the infant is given daily NVP syrup until breastfeeding stops (infant prophylaxis), whereas for Option B, the mother takes ART throughout the breastfeeding period (maternal prophylaxis) and for Option B+, the mother continues with ART for life regardless of the WHO stage or the CD4 count at initiation of treatment.

The WHO has advised each country to make some initial assessments to understand women's attitudes, perception and preference regarding the three treatment options before implementation (UNICEF 2013). At the time of this study, Tanzania was practicing Option A, although there were a few reproductive and child health clinics that were practicing Option B. The qualitative study reported in Paper IV, which included IDIs and FGDs, was done to explore women's own insight regarding the preferred treatment option for PMTCT of HIV and to get their views on Option B+ (treatment for life), which was going to be adopted in Tanzania. Implementation of Option B+ started in October 2013 after the end of this study.

5.3.1 Embracing Option B to minimise stigma and enhance ART adherence during breastfeeding

Analysis of the interviews in sub-study IV revealed that most of the women (both those who were HIV-positive and those who did not yet know their HIV status) preferred Option B since most women thought it would be easier to cope with the three main challenges they considered to be barriers to ART adherence in society. These three barriers were categorised into: 1) HIV-associated stigma; 2) fear of drug side-effects in the infant; and 3) challenging logistics of postnatal drug adherence for the infant. These three categories formed the theme above, "Embracing Option B to minimise stigma and enhance ART adherence during breastfeeding."

5.3.1.1 HIV-associated stigma

It became clear from the interviews that giving medicine every day to a child who is not sick is considered very stigmatising in the community and this can easily make someone refrain from doing it. Women thought it was easier for them to keep their HIV status a secret if they were to take medicine themselves, rather than if they had to give medicine to their infants.

> *"……so when they see the child taking medicine every day, they will point fingers at the child when it is very young and may discriminate against him when he goes from house to house…….. This will make the child very uncomfortable and he won't be free to play with other children."(34 years, married, completed primary education, housewife)*

Breastfeeding can also be stigmatising especially for women who do not breastfeed at all or who practise exclusive breastfeeding, as in these settings mixed feeding (especially water) is introduced very early in life.

The findings of our study indicate that HIV-associated stigma and discrimination remain a major challenge in Tanzania. This is similar to other studies done in Tanzania, where it was found that people believed HIV/AIDS was a punishment for sinning and stigma was significantly associated with poverty, a lack of education and living in rural areas (Amuli et al. 2011). A population-based study done in Tanzania also showed that more than half of the participants thought that people living with HIV were a threat to the society, dangerous and should be isolated (Agnarson et al. 2013). A study in Cape Town found that 40% of people living with HIV/AIDS had experienced discrimination related to their HIV infection and identified an urgent need for social reforms to reduce HIV-related stigma and help people to adjust and adapt to social conditions of HIV/AIDS in South Africa (Simbayi et al. 2007). In sub-study IV, we found the same attitudes as previously presented in Paper III (section 5.2.3), but the magnitude and impact of stigma and discrimination was perhaps even more profound than expected. Stigma (self and community) is one of the major obstacles to effective implementation of PMTCT and sustained ART. A review of HIV-related stigma as a barrier to PMTCT in low-income countries found that it negatively impacts service uptake and adherence at each stage of the PMTCT cascade. There is hence a need to integrate stigma-reduction component into PMTCT, maternal, neonatal and child health services (Turan et al. 2013).

5.3.1.2 Drug side-effects to children

We also learned that women were very worried that their children would suffer from side effects of the ARVs.

> *"….Just because I feel it is not good to give medicine to a small child; it may affect him in the future. Because his body system is not matured…so I think it is better for the mother to take medicine instead of giving it to the child…." (27 years, married, college, agriculturalist)*

Many argued that young children cannot complain when they are not feeling well and some (especially those who had some drug-related complications) were sure that the child will also feel what they felt when they started taking drugs. They argued that it may be difficult for the mother to differentiate between drug side effects and other illnesses when the baby cries. There were no serious side effects reported in the Mitra study (performed at our site) where children were given 3TC for six months of breastfeeding (Kilewo et al. 2008).

5.3.1.3 Challenging logistics for postnatal drug adherence

Another major challenge that came up under this theme was how to combine one's routine activities with the logistics of postnatal drug adherence. Women explained how inconvenient it was for them to resume their daily routines after delivery if they also had to adhere to issues like exclusive breastfeeding and timely medication of the infant. It is even more complicated when there is no disclosure to anyone (which is usually the case) in a stigmatising neighbourhood.

> *"I can't stay with the baby every day. Sometimes emergencies happen and may need to be away for long periods and it may be time to take the drugs and you are not there. So I think it's much better if I take the drugs myself because I will know when and how to take them."(34 years, married, completed primary education, housewife)*

Women were also concerned about what to do if the child vomited up the drug.

We conclude that women preferred maternal prophylaxis since they considered adherence to infant prophylaxis to be more problematic. To our knowledge, there are no

other reported studies that have compared women's attitudes to Options A, B and B+ for PMTCT.

5.3.2 Feared obstacles to Option B+ adherence

Eighteen women were asked about their opinion on Option B+, i.e. starting ART for life during pregnancy regardless of CD4 count, whether they needed it yet or not. We grouped their answers into three main categories: 1) loss of motivation after protecting the child from HIV infection, 2) fear of drug side effects, and 3) the challenges of lifelong medication. The three categories formed the theme above "feared obstacles to option B+ adherence".

It became clear from the interviews that women were abiding by the medication and breastfeeding regulations with one main focus in sight: that of **saving their children from HIV infection**. This finding supports the results described in Paper III (Section 5.2.2), and could be one of the main reasons for loss to follow-up after the cessation of breastfeeding at six months postpartum. A meta-analysis of studies on retention in PMTCT programmes in SSA revealed a loss to follow-up of mother-child pairs ranging from 19-89% (Kalembo et al. 2012). In this meta-analysis, stigma and discrimination, home deliveries, socioeconomic factors and fear of HIV-1 test were some of the reasons for loss to follow-up (Kalembo et al. 2012).

Another meta-analysis showed that ART adherence among pregnant women and post-delivery mothers was higher during the antenatal period (74%) compared to the postnatal period (53%). However adherence to ART was significantly higher in low-and middle-income countries (76%) as compared to high-income countries (62%) (Nachega et al. 2012). Nevertheless, the ART adherence observed in this meta-analysis was significantly lower than that recommended for adequate virologic suppression. Of specific interest is the 17% loss to follow-up after six months post-delivery in a cohort of women enrolled on Option B+ in Malawi and the finding that women who were started on ART during pregnancy were five times more likely never to return after their initial visit, compared to women who were enrolled on ART due to low CD4 count or with advanced disease (Tenthani et al. 2014).

ART-related side effects were another serious concern that affected adherence to ART negatively. Women explained how hard it was sometimes to cope with the side effects of treatment at the same time as they had to care for their young infants:

> *"I've tried to take and get used to the night dose but duh….I'm not good at all after the night dose but I tried to take them but I can't manage…. It's disturbing a lot!!! Sometimes you become weak, you have problems sleeping and in the head it feels as if there is fire… (28 years, single, completed primary education, small-scale businesswoman)*

We observed very low levels of NVP related drug toxicity in the Mitra Plus study (Paper I). Only 6.5% of 429 NVP exposed women had adverse skin reactions and 1.6% had grade 3 and 4 mucocutaneous rash. Grade 3 and 4 hepatotoxicity was seen in only 0.5% of the exposed women. Women and the population at large need to be educated about and counselled on drug safety if programmes to combat MTCT are to be successful.
Women were also concerned about the **realities and challenges of lifelong daily medication.**

> ….."*The challenge is not only taking the same medicine but also taking it every day... At least it should be once per month or week….. But you have to take it every day! It's very tiring…Sometimes I forget to take them at the right time and other medicines. I have tried to take them but I can't. (41 years, married, completed primary education, small-scale businesswoman)*

> …. *"if you tell that person to take medicine every day… they will not use them, instead they throw them away under the bed. There are so many people doing that. Taking medicine every day is confusing people. Whenever they come to the clinic, they will make sure they take medicine and the next visit you do the same but I know when you give us them to take home, others do not take them. (33 years, married, completed primary education, small-scale businesswoman)*

Our findings are supported by previous reviews (Kalembo et al. 2012, Nachega et al. 2012, Tenthani et al 2014.) This raises concern about the feasibility and expected

success of Option B+ which is now being implemented in Tanzania and several other countries in SSA despite lack of evidence of real-life effectiveness and women's preferences.

Similar concerns have been raised by other parties questioning the sustainability of Option B+ in low- and middle-income countries, for example Coutsoudis et al, recommending that a number of ethical, medical safety, programme feasibility and economical concerns should be considered before countries decide to adopt Option B+ (Coutsoudis et al. 2013). Some of these include why only pregnant women with a high CD4 count should be treated for life, drug adherence and development of resistance, sustainability of the programme in economically constrained countries and ART-related adverse treatment outcomes. The same issues (ART adherence, treatment monitoring and funding) have been brought up by other reviewers (e.g. Van de Perre et al. 2013). In light of our own as well as other ART adherence studies in Tanzania, we think long-term adherence may be challenged and is hard to achieve due to barriers that cannot be solved rapidly. Future financing to sustain life-long treatment in low-income countries with high prevalence of HIV is another major challenge as a large proportion of ART and PMTCT programmes are donor-funded (UNAIDS global epidemic report, 2013).

We realised that, even though women favoured Option B, the majority were not in favour of treatment for life (Option B+) for the reasons discussed above. It is well-known that up to 50% of patients with chronic illnesses do not take their medication as prescribed (Sabate 2003). Since HIV is a chronic illness that in addition is highly stigmatised, it is not surprising that patients show treatment fatigue especially when being asymptomatic. However, there is potential to identify, educate and motivate those women who are eager to prolong life through implementation of Option B+, and they exist so that they can help other women living with HIV to adhere to ART also after the cessation of breastfeeding.

In order to prevent treatment failure caused by low motivation or other social and health systems-related barriers to ART adherence, our findings in Papers II, III, IV also demonstrate the need for better surveillance of treatment failure (such as more affordable monitoring of viral loads) as well as better support to clients on ART in these settings. Repeated side-effect and drug adherence counselling, case follow-up of lost clients and allowing freedom of choice for women to make their own decisions of what

PMTCT option they prefer depending on their circumstances are important for the future sustainability and success of these life-saving programmes.

5.4 Methodological reflections (Paper III and IV)

5.4.1 Generalisability

Generalisability refers to the extent to which research findings apply to a wider population and/or to a different context. In qualitative research, findings are considered in light of the participants' context since the sampling procedure is not random. The participants of both Papers III and IV came from the same study area (urban) and the majority were of the same socio-economic status (small scale businesswomen or unemployed housewives) with primary education (seven years schooling). Such features are common for many women in low-income countries. The findings provide insight of what is expected of women of such background. Some of the findings on reasons for poor ART adherence in our two studies (stigma, poverty, fear of side effects, busy daily schedule) were similar to quantitative studies cited in Section 5.2 above. The advantage of qualitative methods is that they give a "thick and deep" understanding of why people think and behave the way they do.

5.4.2 Reflexivity

The researcher is a tool through which the meaning of data is interpreted. Reflecting on what the researcher contributes, on his or her qualifications and experience is hence critical in qualitative studies (Graneheim et al. 2004). I (a middle aged, female gynaecologist and obstetrician who also worked as a clinician and a researcher in the Mitra Plus study) conducted the majority of the interviews (70%) in both Paper II and Paper III. I tried as much as I could to put my professionalism and experience aside so that I could understand the actual meaning of the information given by participants. On the other hand, women knowing who I was could have influenced the quality of the information that they gave (social desirability bias), perhaps giving a more positive modification of reality.

5.4.3 Trustworthiness

The trustworthiness of qualitative research is measured by three things; **credibility** (truth value) which refers to having an adequate engagement in the research setting thus recurrent patterns in data can be properly identified and verified, **transferability** which

refers to allowing readers to be able to apply the findings of the study to their own situations and **dependability** which means being able to demonstrate how research findings were reached. One way of assessing credibility is to seek for agreement among co-researchers, experts and participants (Graneheim et al. 2004). In both sub-studies III and IV, adjusted pilot-tested interview guides were used and interviews were tape-recorded and transcribed verbatim. Peer-debriefing sessions by interviewers and other co-authors were done at the end of the day which shaped the subsequent interviews. Codes were discussed among co-authors (who had multiple professions) to reach a consensus of the main results. We feel that the results of this study can well be applied in similar settings.

6 CONCLUSIONS

- ART of HIV-infected women during pregnancy, labour, delivery and breastfeeding significantly reduced breast milk transmission of HIV from mother to child and increased the HIV-free survival rate in infants in the Mitra Plus PMTCT study in Dar-es-Salaam.
- Virologic failure and drug resistance mutations were already common one year post-delivery among women with sub-optimal adherence to ART. However, the 24-month mortality rate was still fairly low.
- Immunologic failure was not a good predictor of virologic failure among women put on ART for life.
- The main motivation to adhere to ART among asymptomatic HIV-infected women diagnosed during pregnancy was to protect their children from acquiring HIV infection during pregnancy and breastfeeding. This may explain why many women had showed sub-optimal ART adherence after the breastfeeding period.
- Stigma, poverty, busy daily life and fear of drug side effects were found to be significant and hard to tackle barriers of lifelong ART adherence.
- The majority of interviewed Tanzanian women preferred maternal ARV prophylaxis that ended after the breastfeeding period was over (Option B) for PMTCT.
- Long-term adherence to ART among asymptomatic women diagnosed with HIV during pregnancy is still a challenge in low-income settings such as Tanzania.

7 RECOMMENDATIONS

- Viral load and drug resistance testing is required for monitoring treatment response among HIV-infected individuals put on ART for life.
- There is a need for repeated ART adherence counselling, health education on ART and related side effects, and more community and male involvement in all ART and PMTCT activities.
- Programmes to alleviate poverty, empower women and reduce HIV-related stigma should go hand in hand with ART and PMTCT programmes.
- Women should be given the chance to choose the PMTCT Option they think is most feasible and sustainable according to their own circumstances.
- More studies are needed to understand the acceptability, feasibility, sustainability and long-term outcomes of each treatment option for PMTCT (drug side effects, treatment outcomes, MTCT and HIV-free survival rates) before countries decide which one to recommend and implement.

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9 REFERENCES

Aldrovandi, G.M., Kuhn L. (2010). "What infants and breasts can teach us about natural protection on HIV infection." J Infect Dis **1;202**(3): S366-70.

Agnarson, A.M., F. Levira, et al. (2013). "Antiretroviral treatment knowledge and stigma-implications for programs and HIV treatment interventions in rural Tanzanian population." PLoS ONE **8**(1): e53993. Doi:101371/journal.pone.0053993

Albert, J., U. Bredberg, et al. (1987). "A new human retrovirus isolation of West African origin (SBL-6669) and its relationship to HTLV-IV, LAV- II, and HTLV-IIIB." AIDS Res Hum Retroviruses Spring, **3**(1): 3-10.

Altfeld, M. and B.D. Walker (2007). Acute HIV-1 infection. HIV Medicine 2007 **15th edition**: 33-39 access. www.HIVmedicine.com

Amuli, M., S. Mitchell, et al. (2011). "Socio-economic status and HIV/AIDS stigma in Tanzania." AIDS Care **23**(3): 378-382.

Arnsten, J.H., P.A. Demas, et al. (2001). "Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. Clin Infect Dis **33**(8):1417-1423.

Arroyo, M.A., M. Hoelscher, et al. (2004). "HIV type 1 subtypes among blood donors in the Mbeya region of Southwest Tanzania." AIDS research hum retroviruses **8**: 895-901

ART CC AC. (2008). "Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies." Lancet **372**(9635):293-299.

Awiti, U.O., A. M. Ekström, et al. (2011). "Reasoning and deciding PMTCT-adherence during pregnancy among women living with HIV in Kenya." Cult Health Sex **13**(7):829-40.

Badri, M., S.D. Lawn, et al. (2008). "Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting." BMC Infect Dis **8**:89.

Bailey, R.C., S. Moses, et al. (2007). "Male circumcision for HIV prevention in young men in Kisumu Kenya: a randomised controlled trial." Lancet **369**(9562): p.643-56.

Barre-Sinoussi, F., J.C. Chermann, et al. (1983). "Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS)." Science **220**(4599): 868-71.

Becquet, R., V. Leroy, et al. (2006). "Complementary feeding adequacy in relation to nutritional status among early weaned breastfed children who are born to HIV-infected mothers: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. Pediatrics **117**: e701-10.

Bera, E., R. Mia. (2012). "Safety of nevirapine in HIV-infected pregnant women initiating antiretroviral therapy at higher CD4 counts: a systematic review and meta-analysis." South Afr Med J **102**: 855-859.

Biberfeld, P., P. Pyakurel, et al. (2008). "Human herpes virus 8.In. Viral Coinfection in HIV. G. Moyle, J, and Lalezari J. P. Remedica. **Second edition:** pg 137-167

Brust, S., H. Duttmann, et al. (2000). "Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV-1/2/0 screening test." J Virol Methods **90**(2): 153-65.

Bucagu, M., J. D. Bizimana, et al. (2013). "Socio-economic, clinical and biological risk factors for mother - to - child transmission of HIV-1 in

Muhima health centre (Rwanda): a prospective cohort study." Arch Public Health **71**(1):4. doi: 10.1186/0778-7367-71-4.

Cavarelli, M. and G. Scarlatti. (2011). "Human immunodeficiency virus type 1 mother-to-child transmission and prevention: successes and controversies." J Intern Med **270**: 561-579.

CDC (1981). Center for Disease Control and Prevention. "Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men - New York city and California." MMWR **30**: 305-308

CDC, WHO/AFRO, APHL. (2002). "Guidelines for appropriate evaluation of HIV testing technologies in Africa." Accessed at http://www.who.int/hiv/pub/vct/testing_africa/en/

Census (2012). Population distribution by age and sex. National Bureau of statistics, United Republic of Tanzania. Assessed at http://ihi.eprints.org/2169/1/Age_Sex_Distribution.pdf

Charurat, M., P. Datong, et al. (2009). "Timing and determinants of mother-to-child transmission of HIV in Nigeria." Int. J. Gynecol. Obstet **106**(1): 8-13.

Chasela, C.S., M.D. Hudgens, et al. (2010). "Maternal or infant antiretroviral drugs to reduce HIV-1 transmission." N Engl J Med **362**:2271-81.

Chen, J.Y., H.J. Ribaudo, et al. (2012). "Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana." J Infect Dis **206**:1695-1705.

Chesney, M.A. (2006) "The elusive gold standard. Future perspectives for HIV adherence assessment and intervention." J Acquir Immune Defic Syndr **43**(1):S149-155.

Clavel, F., D. Guetard,et al. (1986). "Isolation of new human retroviruses from West African patients with AIDS." Science **233**: 343-6.

Clavel, F., A.J. Hance. (2004). "HIV drug resistance." N Engl J Med **350**: 1023-1035.

Coffie, P.A., D.K. Ekouevi, et al. (2008). "Maternal 12-month response to antiretroviral therapy following prevention of mother-to-child transmission of HIV type 1, Ivory Coast. 2003-2006." Clin. Infect Dis **46**(4):611-21.

Cohen, M.S., Y.Q. Chen YQ, et al. (2011). "Prevention of HIV-1 infection with early antiretroviral therapy." N Engl J Med **365**(6):493-505.

Cohen, M.S., K.E. Muessig, et al. (2012). Antiviral agents and HIV prevention: controversies, conflicts, and consensus." AIDS **26**(13): 1585-1589.

Cooper, E.R., M. Charurat, et al. (2002). "Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission." J Acquir ImmuneDefic Syndr **29**(5):484-94.

Connor, E.M., R.S. Sperling, et al. (1994). "Pediartric AIDS Clinical Trials Group Protocol 076 Study Group. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment." N Eng J Med **331**: 1173-1180.

Coovadia, H.M., E.R. Brown, et al. (2012). Efficacy and safety of an extended Nevirapine regimen in infant children of breast-feeding mothers with HIV-1 infection for prevention of postnatal HIV-1 transmission (HPTN 046): a randomized, double blind, placebo-controlled trial. Lancent **379**: 221-28.

Coutsoudis, A., K. Pillay, et al. (2001). "Method of feeding and transmission of HIV-1 from mother-to-child by 15 months of age: prospective cohort study from Durban, South Africa." AIDS **15**(3): 379-87.

Coutsoudis, A., A. Goga, et al. (2013). "Is Option B+ the best choice?" Lancet **381**: 267-271.

Dabis, F., P. Msellati, et al. (1999). "6-month efficacy, tolerance, and acceptability of short regimen of oral Zidovudine to reduce vertical transmission of HIV in breastfed children in Cote d'Ivoire and Burkina Faso: a double-blind placebo-controlled multicentre trial. DITRAME Study Group. Diminution de la transmission mere-enfant." Lancet **353**(9155): 786-92.

Dao, H., L.M., Mofenson, et al. (2007). "International recommendations on antiretroviral drugs for treatment of HIV-1 infected women and prevention of mother-to-child HIV transmission in resource-limited settings: 2006 update." Am J Obstet Gynecol **197**(3 Suppl): S42-55.

Datay, M.I., A. Boulle, et al. (2010). "Associations with virologic treatment failure in adults on antiretroviral therapy in South Africa." J Acquir Immune Defic Syndr **54**(5):489-495.

De Cock, K.M., M.G. Fowler, et al. (2000). "Prevention of mother-to-child HIV transmission in resource poor countries: translating research into policy and practice." JAMA **283**(9): 1175-82.

Do, N.T., K. Phiri, et al. (2010). "Psychosocial factors affecting medication adherence among HIV-1 infected adults receiving combination antiretroviral therapy (cART) in Botswana." AIDS Res Hum Retrovir **26**(6):685-91.

DSS (2007). Assessment of the country health information system in Tanzania. Assessed at http://www.who.int/healthmetrics/library/countries/HMN_TZA_Assess_Draft_2007_05_en.pdf

Duff, P., W. Kipp, et al. (2010). "Barriers to accessing highly active antiretroviral therapy by HIV- positive women attending an antenatal clinic in a regional hospital in western Uganda. J Int AIDS Soc **23**:13:37

El-Khatib, Z., D. Katzenstein , et al. (2011). "Adherence to drug re-fill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa." PLoS ONE **6**(3): e17518.

European Mode of Delivery Collaboration. (1999). "Elective caesarean-section versus vaginal delivery in prevention of vertical HIV-1 transmission: a randomised clinical trial." Lancet **353**(9158):1035-9.

Feinstein S, Feinstein R, Sabrow S. (2010) "Gender inequality in the division of household labour in Tanzania**.** African sociological review **14**:98-109.

Fletcher, C.V., E.P. Acosta, et al. (2000). "Competing drug-drug interaction among multidrug antiretroviral regimen used in the treatment of HIV-infected subjects: ACTG 884. AIDS **14**:2495-2501.

Flys, T.S., D. Donnell, et al. (2007). "Persistence of K103N-containing HIV-1 variants after single-dose Nevirapine for mother-to-child transmission." J Infect Dis **195**: 711-5.

Flys, T.S., M.S. McConnell, et al. (2008). "Nevirapine resistance in women and infants after first versus repeated use of single-dose Nevirapine for prevention of HIV-1 vertical transmission." J Infect Dis **198**:465-9.

Fogel, J., Q. Li., et al. (2011). "Initiation of antiretroviral treatment in women after delivery can induce multiclass drug resistance in breastfeeding HIV-infected infants." Clin Infect Dis **52**: 1069-76.

Fokam, J., S. C. Billong, et al. (2013). "Declining trends in early warning indicators for HIV drug resistance in Cameroon from 2008-2010: lessons and challenges for low-resource settings." BMC Public Health **13**: 308.

Fowler, M.G., M.A. Lampe, et al. (2007). "Reducing the risk of mother-to child human immunodeficiency virus transmission: past success, current progress and challenges, and future directions." Am J Obstet Gynecol **197**(3 supply): S3-9.

Fox, M.P and S. Rosen. (2010). "Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review." Trop Med Int Health **15**(1):1-15.

Gallo, R.C., S.Z. Salahuddin, et al. (1984). "Frequent detection and isolation fo cytopathic retroviruses (HTLV-III) from patients with AIDS and a risk of AIDS." Science **224**(4648):500-3.

Gottlieb M., R. Schroff, et al. (1981). "Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency." N Eng J Med **305**:1425-1431.

Gourlay, A., I. Birdthistle, et al, (2013). "Barriers and facilitating factors to the uptake of antiretroviral drugs for prevention of mother-to-child transmission of HIV in sub-Saharan Africa: a systemic review." J Int AIDS Soc **16**: 18588.

Graneheim, U.H. and B. Lundman. (2004). "Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness." Nurse Educ Today **24**(2): 105-112.

Gray, R.H., G. Kigozi et al. (2007). "Male circumcision for HIV prevention in men in Rakai, Uganda: a randomized trial." Lancet **369**(9562): p. 657-66.

Guay, L.A., P. Musoke, et al. (1991). "Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial." Lancet **354**: 795-802

Gupta, R.K., M.R. Jordan, et al. (2012). "Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis." Lancet **380**: 1250-58.

Hahn, B.H., G.M. Shaw, et al. (2000). "AIDS as a Zoonosis: Scientific and Public Health Implications." Science **287**:607-614.

Hamers, R.L., K.C.E Sigaloff, et al. (2012) "Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies." Clin. Infec. Dis **54**(11):1660-9.

Hemelaar, J. (2012). "The origin and diversity of the HIV-1 pandemic." Trends in Molecular Med **18**:182-192.

Hemelaar, J. (2013). "Implications of HIV diversity for the HIV-1 pandemic." J of Infect **66**: 391-400.

Hitti, J., L.M. Frenkel, et al. (2004). "Maternal toxicity with continuous nevirapine in pregnancy: results from PACTG 1022." J Acquir Immune Defic Syndr **36**(3): 772-6. .

Hull, M.N., V.D. Lima, et al. (2009). "Epidermiology of treatment failure: a focus in recent trends. Curr Opin HIV AIDS **4**: 467-473.

Jameison, D.J., C.S. Chasela, et al. (2012) "Maternal and infant antiretroviral regimens to prevent postnatal HIV-1 transmission: 48-week follow-up of the BAN randomized controlled trial. Lancet **379**: 2449-2458.

Kagaayi, J., R.H. Gray, et al. (2008). "Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda." PLoS ONE **3**(12): e3877. doi:10.1371/journal.pone.0003877.

Kalembo, W.F., M. Zgambo. (2012). "Loss to Follow-up: A major challenge to successful implementation of prevention of mother-to-child transmission of HIV-1 programs in sub-Saharan Africa." ISRN AIDS **2012**: 589817 doi:10.5402/2012/589817.

Kilewo, C., K. Karlsson, et al. (2008). "Prevention of mother-to-child transmission of HIV-1 through breast-feeding by treating infants prophylactically with lamivudine in Dar es Salaam, Tanzania: the Mitra Study." J Acquir Immune Defic Syndr **48**(3): 315-323.

Kilewo, C., K. Karlsson, et al. (2009). "Prevention of mother-to-child transmission of HIV-1 through breast-feeding by treating mothers with triple antiretroviral therapy in Dar es Salaam, Tanzania: The Mitra Plus study." J Acquir Immune Defic Syndr **52**: 406-416.

Kiselinova, M., W. De Spiegelaere, et al. (2014). "Antiretrovirals for HIV prevention: when should they be recommended?" Expert Rev. Anti Infect. Ther. **12**(4): 431-445.

Kiwelu , I.E., V. Novitsky, et al. (2012). "HIV-1 Subtypes and Recombinants in Northern Tanzania: Distribution of Viral Quasispecies." PLoS ONE **7**(10): e47605. doi:10.1371/journal.pone.0047605.

Kobin, A.B., N.U. Sheth. (2011). "Levels of adherence required for virologic suppression among newer antiretroviral medications." Ann Pharmacother **45**: 372-379.

Koup, R.A., J.T. Safrit, et al. (1994). "Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome." J Virol **68**(7): 4650-5.

Kourtis, A.P., D.J. Jamieson, et al. (2007). "Prevention of human immunodeficiency virus-1 transmission to the infant through breastfeeding: new developments." Am J Obstet Gynecol **197**: S113-122.

Kuhn, L., M. Sinkala, et al. (2007). "High uptake of exclusive breast-feeding and reduced early post-natal HIV transmission." PLoS One **2**(12): e1363.

Kuhn, L., M.G. Aldrovandi, et al. (2008). "Effects of early abrupt weaning on HIV-free survival of children in Zambia." N Eng J Med **359**: 130-41.

Kuhn, L., M.G. Aldrovandi. (2012). "Pendulum swings in HIV-1 and infant feeding policies: now halfway back." Adv Exp Med Biol **743**:273-287.

Kumwenda, N. I., D.R. Hoover, et al. (2008). "Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission." N Eng J Med **359**:119-129.

Kvåle, S. (1996). "Interviews: An introduction to qualitative research interviewing." Thousand oaks, California: Sage.

Kwobah, C.M., A.W. Mwangi, et al. (2012). "Factors associated with first-line antiretroviral therapy failure against HIV-infected African patients: A case control study." World Journal of AIDS **2**(4): 271-278.

Lehman, D.A. and C. Farquhar. (2007). "Biological mechanisms of vertical human immunodeficiency virus HIV-1 transmission." Rev Med Virol. **17**(6):381-403.

Leroy, V., J.M. Karon, et al. (2003). "Postnatal transmission of HIV-1 after a maternal short-course zidovudine peripartum regimen in West Africa." AIDS **17**(10): 1493-1501.

Leroy, V., C. Sakarovitch, et al. (2005). "Is there a difference in the efficacy of peripartum antiretroviral regimen in reducing mother-to-child transmission of HIV in Africa?" AIDS **19**(16): 1865-75.

Levy, J.A. (1993). "Pathogenesis of human immunodeficiency virus infection." Microbiol Rev **57**(1):183-289.

Lindstrom. A and J. Albert. (2003). "A simple and sensitive 'in-house' method for determining genotypic drug resistance in HIV-1." J Virol Methods **107**:45-51.

Lohman-Payne, B., J.A. Slyker, et al. (2012). "Breast milk cellular HIV-specific interferon γ responses are associated with protection from peripartum HIV transmission." AIDS **26**:2007-2016.

Lopez, M., F. Figueras, et al. (2012). "Association of HIV infection with spontaneous and iatrogenic preterm delivery: effect of HAART. AIDS **26**: 37-43.

Lyamuya, E., E. Olausson-Hansson, et al. (2000). "Evaluation of a prototype Ampicor PCR assay for determination of human immunodeficiency virus type 1 DNA in blood samples from Tanzanian infected with HIV-1 subtypes A, C and D." J Clin Virol **17**(1): 57-63.

Mabuka, J., R. Nduati, et al. (2012). "HIV-specific antibodies capable of ADCC are common in breastmilk and are associated with reduced risk of transmission in women with high viral loads." PLoS ONE **8**(6): e1002739.

Martin, M., E. Dei Cacho, et al. (2008). "Relationship between adherence level, type of the antiretroviral regimen, and plasma HIV type 1 RNA viral load: a prospective cohort study." AIDS Res HumRetroviruses **24**: 1263-1268.

McNicholl, I. (2012). "Comprehensive, up-to-date information on HIV/AIDS treatment, prevention and policy from the University of California San Francicso: Adverse effects of Antiretroviral drugs." Accessed at http://hivinsite.ucsf.edu/InSite?page=ar-05-01

Mepham, S.O., R.M. Bland, et al. (2010). "Prevention of mother-to-child transmission of HIV in resource-rich and –poor settings." BJOG **118**: 202-218.

Mepham, S., Z. Zondi, et al. (2011). "Challenges in PMTCT antiretroviral adherence in northern KwaZulu-Natal, South Africa." AIDS Care **23**(6):741-7.

Mills, E.J., J.B. Nachega, et al. (2006). "Adherence to HAART: A systematic review of developed and developing nation patient-reported barriers and facilitators." PLoS Med **3**(11): e438. doi:10.1371/journal.pmed.0030438

Mmiro, A.F., J. Azire, et al. (2009). "Predictors of early and late mother-to-child transmission of HIV in a breastfeeding population: HIV Network for Prevention Trials 012 experience, Kampala, Uganda." J AcquirImmune Defic Syndr **52**: 32-9.

Mock, P.A., N. Shaffer, et al. (1999). "Maternal viral load and timing of mother-to-child HIV transmission, Bangkok, Thailand." AIDS **13**: 407-414.

Moore, D.M., A. Awor, R. Downing , et al. (2008). "$CD4^+$ T-cell count monitoring does not accurately identify HIV-infected adults with

virologic failure receiving antiretroviral therapy." J Acquir Immune Defic Syndr **49**(5):477-484.

Mosha, F., W. Urassa, et al. (2011). "Prevalence of genotypic resistance to antiretroviral drugs in treatment-naive youths infected with diverse HIV type 1 subtypes and recombinant forms in Dar es Salaam, Tanzania. AIDS Res Hum Retroviruses **27**(4):377-382.

Murillo, W., I.L. de Rivera, et al. (2010). Prevalence of drug resistance and importance of viral load measurement in Honduran HIV-infected patients failing antiretroviral treatment. HIVMed **11**(2):95-103.

Murray, L.K., K. Semrau, et al. (2009). "Barriers to acceptance and adherence of antiretroviral therapy in urban Zambian women: a qualitative study." AIDS Care **21**(1): 78-86.

Nachega, J.B., M. Hislop, et al. (2007). "Adherence to non-nucleoside reverse transcriptase inhibitors-based HIV therapy and virological outcomes." Ann Intern Med 146(8): 564-73.

Nachega, J.B., V.C. Marconi, et al. (2011). "HIV treatment adherence, drug resistance, virologic failure: evolving concept." Infect Disord Drug Tergets **11**: 167-174.

Nachega, J.B., O.A. Uthman, et al. (2012). "Adherence to antiretroviral therapy during and after pregnancy in low-income, middle-income, and high-income countries: a systemic review and meta-analysis. AIDS **26**(16):2039-52.

Newell, M.L. and M.J. Bunders. (2013). "Safety of antiretroviral drugs in pregnancy and breastfeeding for mother and child." Curr Opin HIV AIDS **8**:504-510.

Nieuwkerk, P.T., F.J. Oort. (2005). "Self-reported adherence to antiretroviral therapy for HIV-1 infection and virologic treatment response - A meta-analysis." J Acquir Immune Defic Syndr **38**(4):445-448

NIH (2013). Types of HIV/AIDS antiretroviral drugs. National Institute of allergy and infectious diseases. Assessed at http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Treatment/Pages/arvDrugClasses.aspx

(NMSF 2013/14). Tanzania third national multi-sectral strategic framework for HIV and AIDS 2013/14 – 2017/18. Accessed at http://tacaids.go.tz/index.php?option=com_docman&task=doc

Nyombi, B.M., K.I. Kristiansen, et al. (2008). "Diversity of human immunodeficiency virus type 1 subtypes in Kagera and Kilimanjaro regions, Tanzania. AIDS Res Hum Retroviruses **24**(6): 761-9. doi: 10.1089/aid.2007.0311.

O'Brien, M.E., R.A Clark, et al. (2003). "Patterns and correlates of discontinuation of the initial HAART regimen in an urban outpatient cohort." J Acquir Immune Defic Syndr **34**(4):407-414.

Onen, C.L. (2002). "Clinical diagnosis of AIDS and HIV-related diseases." AIDS in Africa. Kluwer Academic Plenum/Publisher. **Second edition:** 297-321.

Osterberg, L., T. Blaschkle. (2005) "Adherence to medication." N Engl J Med **353**: 487-497.

Palella, F.J., K.M. Delaney, et al. (1998). "Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators." N Engl J Med **338**(13):853-860

Palombi, L., M.C. Marrazi, et al. (2007). "Treatment acceleration program and the experience of the DREAM program in prevention of mother-to-child transmission of HIV. AIDS **21** (4): S65-71.

Panel on antiretroviral guidelines for adults and adolescents. (2013). "Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents." Accessed at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf

Pantaleo, G., C. Graziosi, et al. (1993). New concepts in the immunopathogenesis of human immunodeficiency virus infection. N Engl J Med **328**(5): 327-35.

Paredes, R., C.M. Lalama, et al. (2010). "Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure." J Infect Dis **201**(5):662-671.

Paredes, R., V.C. Marconi, et al. (2013) "Impact of antiretroviral drugs in pregnant women in Africa: HIV resistance and treatment outcomes." J Infect Dis **207**(S2): S93-100.

Paterson, D.L., S. Swindells, et al. (2000). "Adherence to protease inhibitor therapy and outcomes in patients with HIV infection." Ann Intern Med **133**(1):21-30.

Persaund, D., A. Bedri, et al. (2011). "Slower clearance of Nevirapine resistant virus in infants failing extended Nevirapine prophylaxis for prevention of mother-to-child HIV transmission." AIDS Res Hum Retroviruses **27**: 823-827.

Rankin, W., S. Brennan, et al. (2005). "The stigma of being HIV-positive in Africa." PloS Med **2**(8): 702-704.

Rawizza, H.E., B. Chaplin, et al. (2011). "Immunologic criteria are poor predictors of virologic outcome: Implications of HIV treatment monitoring in resource-limited settings. Clin. Infect. Dis **53**(12):1283-90.

Read, J.S. (2007). "Diagnosis of HIV-1 infection in children younger than 18 months in the United States." Paediatrics **120**(6): e1547-62.

Reynolds, S.J., G. Nakigoza, et al. (2009). "Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda." AIDS **23**(6):697-700.

Rotger, M., C. Csajka, et al. (2006). "Genetic, ethnic and gender differences in the pharmacokinetics of antiretroviral agents." Curr HIV/AIDS Res **3**:118-125.

Ross-Degnan, D., D.M. Pierre-Jacques, et al. (2010). "Measuring adherence to antiretroviral treatment in resources-poor settings: The clinical validity of key indicators." BMC Health Service Research **10**: 42.

Rousseau, C.M., R.W. Nduati, et al. (2003). "Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease." J Infect Dis **1;187**(5): 741-7.

Rousseau, C.M., R.W. Nduati, et al. (2004). "Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission." J Infect Dis **190**(10): 1880-8.

Rubbert, A., G. Behrens, et al. (2007). "Pathogenesis of HIV-1 infection." **15th Edition**: 33-39 access www.HIV medicine.com.

Rudin, C., A. Spaenhauer, et al. (2011). "Antiretroviral therapy during pregnancy and premature birth: analysis of a Swiss data." HIV medicine **12**: 228-235.

Rwebangira MK. (1996). "The legal status of women and poverty in Tanzania". Nordiska Afrika institutet: The Scandinavian Institute of African Studies; *research report number* 100, page 7-13, 44-56. http://urn.kb.se/resolve?urn=urn:nbn:se:nai:diva-604

Sabate, E., ed. (2003). Adherence to long term therapies: Evidence for action. Geneva, Switzerland; WHO.

Selvaraj, S., E. Paintsil. (2013). "Virologic and host risk factors for mother-to-child transmission of HIV." Curr HIV Res **11**(2): 93-101.

Semba, R.D., N. Kumwenda, et al. (1999). "Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1." J Infect Dis **180**(1): 93-98.

Shapiro, R.L., M.D. Hughes, et al. (2010). "Antiretroviral regimens in pregnancy and breast-feeding in Botswana." N Engl J Med 17; **362**(24): 2282-94.

Shapiro, R.L., T. Ndung'u, et al. (2005). "Highly active antiretroviral therapy started during pregnancy or postpartum suppresses HIV-1RNA, but not DNA, in breast milk." J Infect Dis **192**(5): 713-9.

Simbayi, L., S. Kalichman et al. (2007). "Internalised stigma, discrimination and depression among men and women living with HIV/AIDS in Cape Town, South Africa." Soc Science & Med **64**: 1823-1831

Simoni, J.M., A.E. Kurth, et al. (2006). "Self-report measures of antiretroviral therapy adherence: A review with recommendations for HIV research and clinical management." AIDS Behav **10**(3):227-245.

Stringer, E.M., B.H. Chi, et al. (2008). "Monitoring effectiveness of programmes to prevent mother-to-child HIV transmission in lower-income countries." Bulletin of the WHO **1**:1-80.

Struik, G.M., R.A. den Exter, et al. (2011). "The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi." Int JSTD AIDS **22**(8): 457-462. doi: 10.1258/ijsa.2011.010521.

Study Team SWEN, A. Bedri, et al. (2008). "Extended-dose nevirapine to 6 weeks of age of infant to prevent HIV transmission via breastfeeding in Ethiopia, India and Uganda: an analysis of the three randomised controlled trials." Lancet **372**: 300-313.

TACAIDS (2008). Tanzania Public expenditure Review Multi-Sectoral review: HIV-AIDS December 2007. Accessed at http://www.tacaids.go.tz/home/13-example-category/177-tz-public-expenditure-multisectorial-hiv-aids-2007.html

TACAIDS (2013). Tanzania: HIV infection rates seen dropping. Accessed at http://allafrica.com/stories/201303280069.html

Taha, T.E., Q. Li, et al. (2011). "Postexposure prophylaxis of breastfeeding HIV-exposed infants with antiretroviral drugs to age 14 weeks:updated efficacy results of the PEPI-Malawi trial." J Acquir Immune Defic Syndr **57**(4):319-25.

Tanzania (2011-12). HIV/AIDS and malaria indicator survey. Accessed at http://www.measuredhs.com/pubs/pdf/SR196/SR196.pdf

Tanzania eMTCT (2012). United republic of Tanzania. Ministry of health and social welfare. Tanzania elimination of MTCT of HIV plan 2012-2015. Accessed at http://www.emtct-iatt.org/wp-content/uploads/2012/11/Costed-eMTCT-Plan-Final-Nov-20121.pdf

Tenthani, L., A.D. Haas, et al. (2014) "Retention in care under universal antiretroviral therapy for HIV-infected pregnant and breastfeeding women ('Option B+') in Malawi." AIDS **28**:589-598.

The Kesho Bora Study Group: (2011). "Triple antiretroviral compared with single-dose Nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child-transmission of HIV-1 (Kesho Bora Study): a randomized controlled trial." LancetInfect Dis **11**: 171-80.

The Petra study team. (2002). "Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda [Petra study]: a randomized, double-blind, placebo-controlled trial. Lancet **359**:1178-1186.

The World Fact Book: Tanzania 2013. Accessed at http://www.cia.gov/library/publications/the-worldfactbook/geos/tz.html

Thior, I., S. Lockman, et al. (2006). "Breast-feeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: A randomized trial: The Mashi Study." JAMA **296**:794-805.

Thomas, K.T., R. Masaba, et al. (2011). "Triple antiretroviral prophylaxis to prevent mother to child transmission through breastfeeding-Kisumu breastfeeding study, Kenya: a clinical trial." PLoSMed **8**(3):e1001015.

Tiemessen, C.T., S. Shalekoff, et al. (2009). "Unusual NK cell responses to HIV-1peptides are associated with protection against maternal-infant transmission of HIV-1." The Journ of Immunol **182**: 5914-5918.

Tonwe-Gold, B., D.K. Ekouevi, et al. (2007). "Antiretroviraltreatment and prevention of peripartum and postnalal HIV transmission in West Africa: evaluation of a two-thread approach." PLoS Med **4**(8): e257.

Townsend, C.L., M. Cortina-Borja, et al. (2008). "Trends in management and outcome of pregnancies in HIV-infected women in the UK and Ireland, 1990-2006." BJOG **115**(9):1078-86. doi: 10.1111/j.1471-0528.2008.01706.x.

Turan, J.M., J. Nyblade. (2013). "HIV-related stigma as a barrier to achievement of global PMTCT and maternal health: a review of the evidence." AIDS Behav **17**: 2528-2539.

UN (2008). The millenium development goals. Accessed at http://unstats.un.org/unsd/mdg/Host.aspx?Content=Indicators/OfficialList.htm

UNAIDS (2011). Count down to zero. "Global plan towards the elimination of new HIV infections among children by 2025 and keeping their mothers alive." Accessed at http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20110609_J

UNAIDS (2013). Global Report. UNAIDS report on the global AIDS epidemic. Accessed at http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

UNAIDS (2013). Global update on HIV treatment 2013: Results, Impact and Opportunities. Accessed at http://www.who.int/hiv/pub/progressreports/update2013/en/

Unge, C., B. Sodergard, et al. (2010). "Long-term adherence to antiretroviral treatment and program drop-out in a high risk urban setting in sub-Saharan Africa: A prospective cohort study." PLoS ONE **5**(10): e13613.

UNICEF/UNFPA/WHO (2007). "HIV transmission through breastfeeding: A review of available evidence. Accessed at http://www.who.int/maternal_child_adolescent/documents/9789241596596/en/index.html

UNICEF (2013). Options B and B+: Key considerations for countries to implement an equity-focused approach. Eliminating new HIV infections among children and keeping mothers living with HIV alive and well. Accessed at http://www.unicef.org/aids/files/hiv_Key_considerations_options_ B.pdf

Urassa, W.K., E.M. Mbena, et al. (2003). "Lymphocyte subsets enumeration in HIV sero-negative and HIV-1 sero-positive adults in Dar es Salaam Tanzania: determination of reference values in males and females and comparison of two flow cytometric methods." J Immunol Methods **277**:65 – 74.

U.S. Food and drug Administration (2013). "Antiretroviral drugs used in the treatment of HIV infection." Accessed at http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm

Vairo, F., E. Nicastri, et al. (2013). "HIV-1 drug resistance in recently HIV-infected pregnant mother's naïve to antiretroviral therapy in Dodoma urban, Tanzania." BMC Infect Dis **13**:439.

Van de Perre, P., T. Tylleskar, et al. (2013). "How evidence based are public health policies for prevention of mother to child transmission of HIV?" BMJ **346**:f3763

Vergis, E.N. and J.W. Mellors (2000). "Natural history of HIV infection." Infect Dis Clin North Am **14**(4): 809-25, v-vi.

Vermund, S.H., J.A. Tique, et al. (2013). "Translation of biomedical prevention strategies for HIV: prospects and pitfalls." J Acquir Immune Defic Syndr **63**:(1) s12-25.

Volberding. P.A. and S.G. Deeks. (2010). "Antiretroviral therapy and management of HIV infection." Lancet **376**: 49-62.

Weller, S. and K. Davis. (2002). "Condom effectiveness in reducing heterosexual HIV transmission." Cochrane Database Sys Rev **1**:CD003255

WHO (2002). Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. "Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis." Lancet **355**: 451-5.

WHO (2003). Adherence to long term therapies. Evidence for action. Assessed at http://apps.who.int/medicinedocs/pdf/s4883e/s4883e.pdf

WHO (2005). Interim WHO staging of HIV and AIDS and HIV/AIDS case definition for surveillance. Accessed at http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf

WHO (2006). Antiretroviral therapy for HIV infection in adult and adolescents in resource-limited settings: towards universal access. Recommendations for a public health approach. Accessed at: http://www.who.int/hiv/pub/guidelines/adult/en/index.html

WHO (2007a). HIV transmission through breastfeeding. A review of available evidence. Accessed at http://whqlibdoc.who.int/publications/2008/9789241596596_eng.p df

WHO (2007). HIV and infant feeding: new evidence and programmatic experience report of a technical consultation held on behalf of the interagency task team on prevention of HIV infections in pregnant

women, mothers and their infants, Geneva, Switzerland, 25-27 October 2006. Accessed at http://whqlibdoc.who.int/publications/2007/9789241595964_eng.pdf?ua=1

WHO (2010a). HIV/AIDS Programme. "Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants; Recommendations for a public health approach." Accessed at www.whqlibdoc.who.int/publications/2010/9789241599818_eng. pdf

WHO (2010). PMTCT Strategic Vision 2010-2015: Preventing mother-to-child transmission of HIV to reach the UNGAASS and Millennium Development Goals. Moving towards the elimination of pediatric HIV. Accessed at http://www.who.int/hiv/pub/mtct/strategic_vision.pdf

WHO (2012a). WHO drug resistance report. Assessed at http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf

WHO (2012). HIV/AIDS Programme. "Programmatic update; Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants; Executive summary, April 2012." Accessed at www.who.int/hiv/PMTCT_update.pdf

WHO (2013). Consolidated ARV guidelines. Accessed at http://www.who.int/hiv/pub/guidelines/arv2013/intro/rag/en/index4.html

WHO (2013). Data on the size of the HIV/AIDS epidemic: number of adults, women and children living with HIV by country. Accessed at http://apps.who.int/gho/data/node.main.621

Wiktor, S. Z., E. Ekpini, et al. (1999). "Short-course oral zidovudine for prevention of mother-to-child transmission of HIV-1 in Abidjan, Cote d'Ivore: a randomised trial." Lancet **353**(9155):781-5.

Williams, B., R. Wood, et al. (2011). "Treatment as prevention: preparing the way." J Int AIDS Soc **14**(1): S6

Zeh, C., P.J. Weidle, et al. (2011). "HIV-1 drug resistance emergence among breastfeeding infants born to HIV-infected mothers during a single-arm trial of triple-antiretroviral prophylaxis for prevention of mother-to-child transmission: a secondary analysis." PLoS Med **8**(3): e1000430. Doi:10.1371/journal.pmed.1000430.