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

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Objective: The main aim of this study was to reduce breast-milk transmission of HIV-1 by treating HIV-1–infected women with highly active antiretroviral therapy (HAART) during breastfeeding.

Methods: Mitra Plus was an open-label, nonrandomized, prospective cohort study. HIV-1–infected pregnant women in Dar es Salaam were treated with zidovudine (ZDV) + lamivudine (3TC) + nevirapine (NVP). NVP was later replaced by nelfinavir for mothers with CD4 cell counts >200 cells per microliter or with adverse reaction to NVP. HAART was initiated at 34 weeks of gestation. For women with symptomatic HIV infection or CD4 cell counts below 200 cells per microliter, HAART was started earlier if possible. Treatment of the mothers was stopped at 6 months except for those mothers who needed HAART for their own health. The infants received ZDV + 3TC for 1 week after birth. Mothers were advised to exclusively breastfeed and to wean abruptly between 5 and 6 months. Transmission of HIV-1 was analyzed using the Kaplan–Meier survival technique. Cox regression was used for comparison with the breastfeeding population of the Petra trial arm A.

Results: There were 441 infants included in the analysis of HIV-1 transmission. The cumulative transmission of HIV-1 was 4.1 % [95% confidence interval (CI): 2.2 to 6.0] at 6 weeks, 5.0% (95% CI: 2.9 to 7.1) at 6 months, and 6.0% (95% CI: 3.7 to 8.3) at 18 months after delivery. The cumulative risk of HIV transmission between 6 weeks and 6 months was 1.0% and between 6 months and 18 months 1.1%. The cumulative HIV infection or death rate was 8.6% (95% CI: 6.0 to 11.2) at 6 months and 13.6% (95% CI: 10.3 to 16.9) at 18 months after delivery. Viral load at enrollment and duration of HAART before delivery were significantly associated with transmission but CD4 cell count at enrollment was not. The median time of breastfeeding was 24 weeks. The transmission in the Mitra Plus study was about half of the transmission in the breastfeeding population in the Petra trial arm A at 6 months after delivery (adjusted relative hazard = 0.49, $P < 0.001$). The combined outcome HIV infection or death was significantly lower in the Mitra Plus study than in the breastfeeding population in the Petra trial arm A at 18 months (adjusted relative hazard = 0.61, $P = 0.007$). NVP-related mucocutaneous rash was demonstrated in 6.5% of 429 NVP-exposed women. The incidence of NVP-related grade 3 or 4 hepatotoxicity was low (0.5%).

Conclusions: HAART given to HIV-infected mothers in late pregnancy and during breastfeeding resulted in a low postnatal HIV transmission similar to that previously demonstrated in the Mitra study in Dar es Salaam using infant prophylaxis with 3TC during breastfeeding. The extended maternal prophylaxis with HAART for prevention of mother-to-child transmission of HIV-1 for breastfeeding mothers who do not need HAART for their own health should be further evaluated and compared with the use of infant postnatal antiretroviral prophylaxis regarding safety and cost-effectiveness.

Key Words: Africa, antiretroviral treatment, breastfeeding, HIV, mother-to-child transmission, prevention

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The members of the Mitra Plus Study Team are listed in Appendix 1.

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INTRODUCTION

Mother-to-child transmission (MTCT) of HIV-1 infection is the major source of HIV infection in infants and young children younger than 5 years. In the absence of HIV prevention measures, the rates of MTCT of HIV-1 have been estimated to range from 25% to 48% in breastfeeding populations in resource-poor settings.[1] Breastfeeding accounts

for about 40% of MTCT of HIV-1 in developing countries.[2–4] In resource-rich countries, the use of highly active antiretroviral treatment (HAART) together with elective cesarean section and avoidance of breastfeeding has reduced the rate of MTCT of HIV to below 2%.[5,6] Several clinical trials in resource-poor countries have shown that short-course perinatal antiretroviral (ARV) treatment regimens can reduce the risk of early MTCT of HIV by 35%–60 %,[7–13] but additional interventions are needed to prevent postnatal HIV-1 transmission through breastfeeding. Exclusive replacement infant feeding would be appropriate for averting breast-milk transmission of HIV-1, but this option is not safe, affordable, and sustainable in many low resource-limited settings. The majority of mothers in developing countries will choose to breastfeed their infants even when they know that they are themselves HIV infected and that there is a risk of transmitting the infection to the infant. Morbidity and mortality in infants due to infectious diseases, fear of stigmatization as a result of not breastfeeding, and lack of economic resources are the main obstacles against raising children without breastfeeding in resource-poor settings.

It has been shown that the risk of postnatal HIV transmission is lower with exclusive breastfeeding than with mixed feeding,[14–17] and the World Health Organization (WHO) has issued guidelines recommending exclusive breastfeeding for 6 months for HIV-infected women when other infant feeding options are not affordable, feasible, acceptable, sustainable, and safe.[18] Further research on additional interventions to make breastfeeding safer is ongoing. Recent studies, including the Mitra study in Dar es Salaam, have shown that infant ARV prophylaxis with lamivudine (3TC), nevirapine (NVP), or zidovudine (ZDV) or NVP plus ZDV can reduce postnatal HIV-1 infection in breastfed infants.[19,20–23] Another approach to prevent postnatal HIV transmission is to use maternal prophylaxis with HAART during breastfeeding.

The present study was an open-label, nonrandomised, prospective cohort study conducted in Dar es Salaam, Tanzania, with the aim to investigate the possibility to reduce breast-milk HIV-1 transmission by treating HIV-infected women with HAART in late pregnancy and for 6 months during breastfeeding. This approach also aimed to treat mothers eligible for HAART to improve their own health.

METHODS

Study Design

Mitra Plus was an open-label, nonrandomised, prospective cohort study. The study was conducted at the Dar es Salaam site used for the Petra trial[11] and the Mitra study.[19] Enrollment into the Mitra Plus study started although the follow-up of Mitra mothers and children was still going on in the same study clinic.

Study Population and Setting

The study population consisted of HIV-infected pregnant women enrolled at the study clinic in Dar es Salaam, Tanzania. Women were recruited from 3 primary health care clinics providing antenatal care services and from the antenatal clinic at the Muhimbili National Hospital in Dar es Salaam. Counseling and voluntary testing for HIV as part of the National prevention of mother-to-child transmission (PMTCT) program was being offered routinely to all pregnant women attending antenatal services in these clinics. All HIV-seropositive women were then introduced to the Mitra Plus study and invited to participate if they wished and met the eligibility criteria.

The eligibility criteria included HIV seropositivity determined by testing of 2 blood samples, intention to breastfeed, hemoglobin (Hb) level not less than 7 g/dL, being 18 years or older, 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. The women had to give written informed consent to participate in the study and were free to withdraw from the study at any stage if they wished to do so. HIV-seropositive women who were not eligible or did not want to participate in the Mitra Plus study continued to be managed within the National PMTCT program at the antenatal clinics where mothers and children received a single-dose NVP during labour/birth.

Enrollment into the Mitra Plus study including starting HAART was at 34 weeks of gestation. For women with symptomatic HIV infection (WHO clinical stage 3 or 4) or with CD4 T-cell counts <200 cells per microliter, HAART started earlier than 34 weeks of gestation if possible. Enrolled women received normal antenatal, labour, and delivery care. In addition, they received ARV treatment according to the study protocol: a combination of ZDV twice daily + 3TC 150 mg twice daily + NVP 200 mg lead dose for 14 days and then escalated to 400 mg per day administered in 2 doses during the rest of the treatment period. The same regimen was continued intrapartum and postnatally for 6 months and was then stopped (treatment with ZDV + 3TC was continued for 1 week after stopping NVP). In women who were eligible for ARV treatment for their own health, HAART was continued within the Mitra Plus study for 3 years. The women were then 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 (NFV). When NFV was not available, NVP was suspended and the mother continued with ZDV + 3TC (n = 26). Toward the end of enrollment (from October 1, 2005), women with CD4 cell counts >200 cells per microliter received a regimen containing NFV 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 cells per microliter.[24–27]

Infants were treated with ZDV (4 mg/kg twice daily) + 3TC (2 mg/kg twice daily) from birth to 1 week of age (as in the Petra trial arm A). All mothers were advised to deliver at Muhimbili National Hospital. Mothers and infants received free medical care within the study. A special postnatal clinic was used for 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. At each visit, clinical examinations of the mothers and children were done, adverse events since the last visit were recorded, and detailed information on feeding practices and changes since the last visit was collected.

The protocol included monitoring of both clinical and laboratory levels of toxicity in mothers and infants.

Hemoglobin, leucocytes, lymphocyte, and thrombocyte counts, hepatic transaminases (alanine aminotransferase and aspartate aminotransferase), and serum creatinine were determined in the pregnant women before administration of HAART and repeated in the follow-up 2 weeks after enrollment, at delivery, at week 6, month 3, and then after every 3 months until the mothers were discharged from the study. Adverse reactions were defined according to the AIDS Clinical Trial Group adult and pediatric adverse experiences grading scale.[28] Indication to suspend NVP was grade 3 or 4 hepatotoxicity (any of the transaminase measurements greater than 5 times the upper normal limit or life threatening condition) which had not resolved at the next visit or if clinical symptoms suggested liver toxicity or skin reactions at any level that made the mother uncomfortable to continue the treatment. The clinic physicians evaluated women complaining of skin adverse events, particularly itching and rash, to judge whether they were associated with NVP treatment or not.

Blood samples were drawn from the infants at each planned visit except the visit at week 3 and months 4 and 5. Determination of Hb level, leucocytes, lymphocyte and thrombocyte counts, serum creatinine, and liver enzymes was done at birth, week 1, months 3 and 6. Counseling on infant feeding was done at every visit to the clinic. Home tracing of study participants was conducted if 2 consecutive appointments were missed. Children whose mothers died were brought to the clinic by relatives. Mothers whose children died continued to come to the clinic for follow-up, and for those who moved up country, information was obtained from relatives and friends.

The study did not provide replacement feeding for infants except in case of failure to thrive after cessation of breastfeeding. The mothers were advised to exclusively breastfeed and to wean abruptly between 5 and 6 months. All infants were given prophylactic treatment with cotrimoxazole for prevention of opportunistic infections from 6 weeks of age to the time when they were shown to be HIV negative after having stopped breastfeeding. Children diagnosed as HIV infected continued with cotrimoxazole prophylaxis after cessation of breastfeeding and were put on treatment according to the national guidelines for initiating ARV treatment in HIV-infected children.

The main study protocol was approved by the institutional review boards of the Tanzania National Institute for Medical Research, Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania, and Karolinska Institutet, Stockholm, Sweden.

Laboratory Methods

Screening for HIV antibodies in the pregnant women was done at the recruitment site by nurse/midwife counselors or by health laboratory technicians using the Capillus rapid simple assay (Trinity Biotech, Bray Ireland) for initial testing followed by testing of reactive samples on the Determine rapid simple assay (Abbott Laboratories, Tokyo, Japan). A second sample was collected for confirmation of reactivity at the research laboratory in the microbiology/immunology Department, Muhimbili University of Health and Allied Sciences (MUHAS), before recruitment into the study. The second sample was tested for HIV antibodies by 2 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, Boxtel, 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.

Children were tested for HIV-1 infection at week 6 and months 3, 6, and 9 by the Amplicor HIV-1 DNA v 1.5 qualitative polymerase chain reaction (PCR) assay (Roche Diagnostics, Rand burg, South Africa) and at months 12, 15, and 18 by Enzygnost anti-HIV 1+2 plus ELISA. Samples reactive by this ELISA were tested by Vironostika HIV uniform II antigen/antibody ELISA (Biomerieux, The Netherland). Samples positive by antibody ELISA at 12 and/or 15 months were also tested by the Amplicor HIV-1 RNA Monitor v 1.5 assay (Roche Diagnostics). The diagnostic threshold of the RNA PCR at 12 or 15 months was 10,000 copies per milliliter.[29] Children with a positive HIV test by PCR and/or ELISA were retested at the next scheduled visit. Children with 2 consecutive positive HIV tests were diagnosed as being HIV infected. Children with a positive HIV test at 6 weeks were tested at 1 week after birth by the Amplicor HIV-1 RNA Monitor v 1.5 assay. The RNA PCR test at 1 week was considered positive if the viral load was more than 1000 copies per milliliter because the RNA levels have been shown to be lower at birth than at 2 months or later.[30] Samples for RNA PCR were available at 1 week after birth from all of 18 infants who were HIV DNA PCR positive at 6 weeks, but only 12 of them had a sample at birth. Plasma HIV-1 RNA was quantified by the Amplicor HIV-1 RNA Monitor v 1.5 assay.

Determination of T-lymphocyte subsets was done using the SimulSET flow cytometry method (Immunocytometry System; Becton Dickinson, San Jose, CA) as described previously.[31]

The MUHAS laboratory participated in quality assurance programs for HIV-1 DNA and RNA PCR assays (US Virology Quality Assurance) and for CD4 cell determination (United Kingdom National External Quality Assessment Service [UKNEQAS], United Kingdom).

Determination of Hb level, leucocyte, lymphocyte, and thrombocyte counts was done by a standard hematological analyzer (Coulter ActDiff II). Serum creatinine, alanine aminotransferase, and aspartate aminotransferase were determined by a Cobas Core system (Roche Diagnostics).

Statistical Analysis

The calculation of the 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 6 months from 14% (Turnbull estimate for the breastfeeding population of the arm A in the Petra trial)[11] to 7% in the Mitra Plus study. A significance level of 5% and a power of 80% were used. For comparison with the 222 children remaining in follow-up at 6 months in the breastfeeding population in arm A of the Petra trial, we would then need 324 children in the Mitra Plus study at 6 months. To allow for deaths and loss to follow-up, we planned to enroll at least 450 mothers in the Mitra Plus study.

Data analysis was done using the SPSS software system 15.0 (Statistical Package for Social Sciences; SPSS Inc, Chicago, IL). In case of twins, only the firstborn baby was included in the analyses. HIV-1 transmission, mortality, the combined outcome´HIV infection or death, and breastfeeding were analyzed 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.[32] Univariate analyses with continuous background factors and multivariate analyses were performed with Cox regression. Differences in distributions were tested with the χ^2 test. Differences between means were tested with Student *t* test and differences between medians with the Mann–Whitney test.

Kaplan–Meier survival technique and Cox regression were used to study the relative effectiveness of the preventive measures taken in the Mitra Plus study compared with the Petra trial Arm A (pooled data).

RESULTS

Enrollment of HIV-1–positive mothers into the Mitra Plus study started in April 2004 and ended in June 2006. In total, 16,088 pregnant women were offered HIV counseling and voluntary testing, 14,255 (88.6%) were counseled, 13,637 (95.7 %) were tested for HIV, 1508 (11.1%) were found to be HIV seropositive, and 501 (33.2%) were enrolled into the Mitra Plus study (Fig. 1). Women not eligible for the Mitra Plus study were managed within the National PMTCT program. Of the 501 women enrolled, 9 withdrew or disappeared before delivery and one died undelivered. The remaining 491 women delivered 503 babies including 12 pairs of twins. The second born twins were excluded from this analysis. Among the 491 firstborn babies, there were 18 stillborns and 15 early neonatal deaths (10 infants died within 24 hours after birth and 5 died on days 2–7). There were 3 late neonatal deaths (died between days 8–28). Among the remaining 455 children, 14 were lost to follow-up without

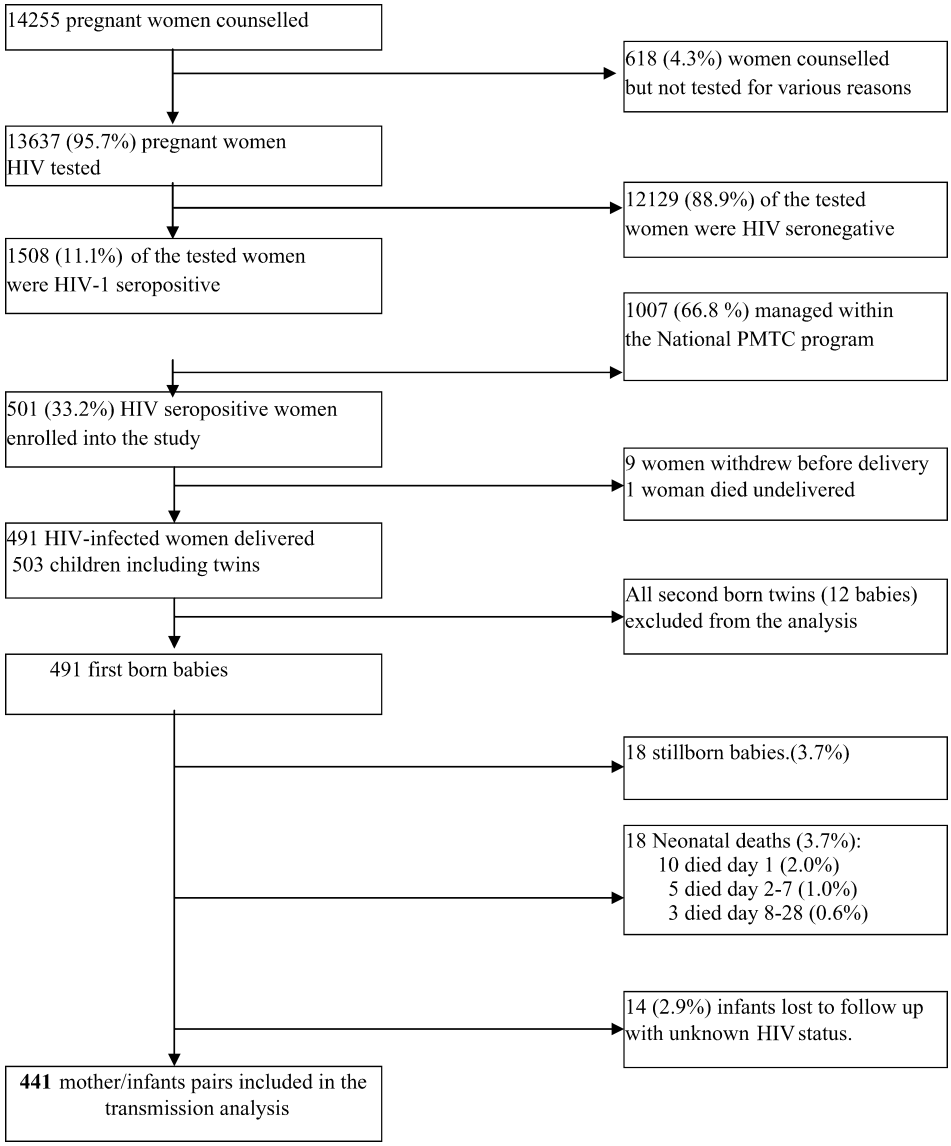

FIGURE 1. Enrollment of the mothers and inclusion of infants in the analysis.

having any HIV result. Thus 441 children were included in the transmission analysis (Fig. 1).

Exclusive breastfeeding and a short weaning period were reported by most of the mothers. Kaplan–Meier estimates of breastfeeding in the Mitra Plus study were 97% at 6 weeks, 90% at 12 weeks, 17% at 26 weeks, 8% at 52 weeks, and 3% at 78 weeks (Table 1).

In total, 26 children in the Mitra Plus study were HIV-1 infected at 18 months, of whom, 18 were HIV-1 infected at 6 weeks (early transmission), 8 were PCR negative at 6 weeks but HIV-1 positive for the first time at 3 months (n = 1), 6 months (n = 3), 9 months (n = 1), 12 months (n = 2), or 15 months (n = 1). Data on the 8 late transmissions are shown in Table 2. All 26 HIV-1–infected infants had at least 2 HIV-1–positive samples collected at different time points. None of the 4 mothers who transmitted HIV infection between 6 months and 18 months were on continued ARV treatment after 6 months.

Thirteen of the 18 infants who were HIV PCR positive at 6 weeks (early transmission) were HIV RNA positive (>1000 copies/mL) at 1 week after birth, corresponding to a transmission rate of 2.9% (13 of 441) at week 1.

The cumulative probability of HIV-1 transmission as analyzed by the Kaplan–Meier technique in the Mitra Plus study is shown in Table 3. Cumulative infection rates were 4.1% [95%confidence interval (CI): 2.2 to 6.0] at 6 weeks, 5.0% (95%CI: 2.9 to 7.1) at 6 months, 5.8% (95% CI: 3.6 to 8.0) at 12 months, and 6.0% (95% CI: 3.7 to 8.3) at 18 months. For the infants who were HIV uninfected at 6 weeks, the cumulative risk of acquisition of infection between 6 weeks and 6 months was 1.0% (95%CI: 0.02 to 1.98) and between 6 weeks and 18 months was 2.1% (95%CI: 0.66 to 3.54). For the children who were HIV PCR negative at 6 months, the cumulative risk of acquisition of HIV infection between 6 months and 18 months was 1.1%.

In the subpopulation of women with CD4 cell counts ≥200 cells per microliter at enrollment (n = 364), the cumulative infection rates were 4.1% (95%CI: 2.0 to 6.2) at 6 weeks, 5.3% (95%CI: 2.9 to 7.6) at 6 months, 6.2% (95%CI: 3.6 to 8.7) at 12 months, and 6.5% (95%CI: 3.9 to 9.1) at 18 months.

The results of a Cox regression analysis of transmission in the Mitra Plus study up to 18 months after delivery are shown in Table 4. Among the baseline characteristics studied (Table 1) at enrollment (age, education, marital status, WHO stage, Hb level, CD4 cell count, and viral load) and at delivery/birth (type of delivery, duration of ARV treatment before delivery, birth weight, and sex of the child), only viral

TABLE 1. Baseline Characteristics for Mothers and Children in the Mitra Plus Study and the Breastfeeding Population in the Petra Trial Arm A

Mother/Child Pairs in Transmission Analysis	Mitra Plus n = 441	Breastfeeding Mothers in Petra Arm A n = 264	*P*
At enrollment			
Age, median (IQR)	26 (24–30)	26 (23–30)	0.700
Hb g/dL, median (IQR)	10.0 (8.9–11)	10.7 (9.8–11.7)	<0.001
CD4 count (cells/μL), median (IQR)	415 (265–577)	459 (295–643)	0.014
CD4%, median (IQR)	21 (15–28)	27 (19.4–35.4)	<0.001
CD4 <200 cells/μL	17.5%	9.4%	<0.001
Viral load, median (IQR)	14621 (2954–59738)	Not available	—
Log_{10} viral load, mean (SD)	4.1595 (0.8775)	Not available	—
WHO stage 1	414 (93.9%)	234 (88.3%)	—
WHO stage 2	20 (4.5%)	18 (6.8%)	—
WHO stage 3	2 (0.5%)	8 (3.4%)	—
WHO stage 4	5 (1.1%)	2 (0.8%)	—
At delivery			
Cesarean section	18.3%	31.1%	<0.001
Birth weight kg, median (IQR)	2.9 (2.5–3.2)	3.1(2.8–3.3)	<0.001
Low birth weight (<2.5 kg)	16.8%	5.8%	<0.001
Female child	49%	51%	0.578
Breastfeeding*			
6 wk	97%	85%†	—
12 wk	90%	77%	—
16 wk	80%	72%	—
20 wk	74%	69%	—
24 wk	51%	68%	—
26 wk	17%	64%	—
28 wk	13%	61%	—

*Kaplan–Meier estimate.
†Breastfeeding in the Petra trial was calculated on the assumption that mothers lacking information on date of stopping breastfeeding went on breastfeeding.

TABLE 2. Late Transmission of HIV-1 in the Mitra Plus Study

Child No.	HIV PCR Results							Maternal CD4 Count at Enrollment (Cells/μL)	Maternal Viral Load at Enrollment (RNA Copies/mL)
	6 wk	3 mo	6 mo	9 mo	12 mo	15 mo	18 mo		
1	−	+	+	+	+	+	+	622	11,700
2	−	−	+	+	+	+	+	324	39,076
3	−	−	+	+	+	+	+	582	151,119
4	−	−	+	+	+	+	+	266	10,670
5	−	−	−	+	+	+	+	502	191,250
6	−	−	−	−	+	+	+	253	213,290
7	−	−	−	−	+	+	+	431	2012
8	−	−	−	−	−	+	+	650	11,829

load ($P < 0.001$), duration of treatment before delivery ($P = 0.02$), and birth weight ($P = 0.009$) were significantly associated with transmission in the univariate analysis. Sex of child had borderline significance ($P = 0.056$). In the multivariate analysis, only viral load ($P < 0.001$), duration of treatment before delivery ($P = 0.02$), and sex of child ($P = 0.04$) were significantly associated with transmission of HIV-1. Mean log viral RNA copies per milliliter at enrollment was 4.75 in transmitting mothers and 4.12 in nontransmitting mothers ($P < 0.001$). A Cox regression analysis was also performed for transmission up to 6 months after delivery (the time period with HAART for all mothers). In this analysis, the association between duration of treatment before delivery and transmission was stronger ($P = 0.003$ in the univariate analysis and $P = 0.004$ in the multivariate analysis) than in the analysis up to 18 months.

In addition to the 18 neonatal deaths, 32 children died between day 29 and month 18. All but 6 were HIV negative at their last HIV test before death. The 6 HIV-positive children who died were HIV positive at week 6. Causes of death were malaria, pneumonia, fever and convulsions, diarrhea, sudden death, marasmus, and heart disease (Table 5). Kaplan-Meier–estimated mortality in the Mitra Plus study is shown in Table 3 together with transmission and HIV-free survival. The 10 neonates who died within 24 hours after birth were excluded from the analysis (never breastfed).

A comparison of the baseline characteristics of mothers and infants in the Mitra Plus study and in the breastfeeding population in the Petra trial arm A (all Petra sites, n = 264) is shown in Table 1. The Mitra Plus mothers had significantly lower Hb value and CD4 cell counts at enrollment than the Petra mothers. The percentage of mothers who delivered by cesarean section was significantly lower in the Mitra Plus study. The birth weight for the babies was significantly lower in the Mitra Plus study than in the Petra trial. The proportion of mothers breastfeeding was higher in the Mitra Plus study than in the Petra trial up to 5 months.

Table 6 shows the Kaplan-Meier–estimated HIV-1 transmission, mortality, and HIV-free survival in the Mitra Plus study and in the breastfeeding population of the Petra trial arm A.

To take differences in background factors into account in the comparison of transmission of HIV-1 in the Mitra Plus study and the breastfeeding population of the Petra trial arm A, we performed Cox (stepwise forward) regression analyses of transmission on the pooled data. In the analysis of transmission up to 6 months after delivery, the cohort factor (Mitra Plus/Petra) and CD4 absolute cell counts were the only significant factors. Competing nonsignificant factors were age, Hb, CD4%, and WHO stage of mother at enrollment, type of delivery, sex, and birth weight of child. Transmission up to 6 months after delivery in the Mitra Plus study was about half of the transmission up to 6 months in the breastfeeding population of the Petra trial arm A [adjusted relative hazard (RH) = 0.49 (95% CI: 0.33 to 0.73), $P < 0.001$] (Table 7). In the analysis of transmission up to 18 months after delivery, the cohort factor (Mitra Plus/Petra) was the only significant factor in the analysis. Competing nonsignificant factors were the same as in the analysis of transmission up to 6 months (data not shown). The cumulative transmission at 18 months in the Mitra Plus study was about one third of the cumulative transmission at 18 months in the breastfeeding population of the Petra trial arm A [adjusted RH = 0.33 (95% CI: 0.21 to

TABLE 3. Kaplan-Meier–Estimated Transmission of HIV-1, Mortality, and HIV-free Survival in the Mitra Plus Study

Age	HIV-1 Infection			Mortality			HIV-1 Infection or Death		
	No. At Risk	Cumulative No. Infected	Cumulative Infection Rate % (95% CI)	No. At Risk	Cumulative No. Dead	Cumulative Death 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 to 6.0)	440	9	2.0 (0.7 to 3.3)	425	27	5.9 (3.7 to 8.1)
3 mo	418	19	4.3 (2.4 to 6.2)	434	12	2.7 (1.2 to 4.2)	419	30	6.6 (4.3 to 8.9)
6 mo	397	22	5.0 (2.9 to 7.1)	417	19	4.3 (2.4 to 6.2)	400	39	8.6 (6.0 to 11.2)
9 mo	387	23	5.3 (3.2 to 7.4)	407	27	7.1 (4.7 to 9.5)	388	50	11.2 (8.2 to 14.2)
12 mo	368	25	5.8 (3.6 to 8.0)	386	37	8.5 (5.8 to 11.2)	369	57	12.8 (9.6 to 16.0)
18 mo	333	26	6.0 (3.7 to 8.3)	350	40	9.2 (6.4 to 12.0)	334	60	13.6 (10.3 to 16.9)

TABLE 4. Cox Univariate and Multivariate (Stepwise Forward) Regression Analysis With Respect to HIV-1 Transmission Up to 18 Months in the Mitra Plus Study

	Univariate Analysis			Stepwise Analysis		
Factor	**RH**	***df***	***P***	**RH (Multiple)**	**95% CI**	***P***
Viral load	2.28	1	<0.001	2.58	1.57 to 4.23	<0.001
Length of treatment before delivery	0.39	1	0.02	0.39	0.18 to 0.88	0.023
Female child	2. 27	1	0.06	2.36	1.01 to 5.47	0.046
Birth weight	0.61	1	0.01	0.72	0.44 to 1.17	0.185
Low birth weight	0.44	1	0.05	—	—	—
CD4 abs	1.00	1	0.74	—	—	—
CD4%	0.98	1	0.40	—	—	—
WHO stage		3	0.86	—	—	—
Hb	0.86	1	0.20	—	—	—
Age	0.99	1	0.88	—	—	—
Caesarean section	0.71	1	0.58	—	—	—

CD4abs indicates CD4 absolute cell count.
df indicates degrees of freedom.

0.54), $P < 0.001$]. Viral load was not available on the Petra mothers and could thus not be included in these analyses.

Another Cox regression analysis on the pooled data was performed to compare the combined outcome HIV infection or death in the Mitra Plus study and in the breastfeeding population of the Petra trial arm A at 18 months after delivery. The cohort factor (Mitra Plus/Petra) was the only significant factor in the analysis. Competing nonsignificant factors were the same as in the transmission analysis above (data not shown). HIV infection or death was significantly lower in the Mitra Plus study than in the breastfeeding population of the Petra trial arm A (adjusted RH = 0.61 (95% CI: 0.42 to 0.87), $P = 0.007$).

Among the 433 women exposed to NVP-containing regimen, 4 withdrew from the study for reasons other than drug-related side effects. The incidence of skin reactions considered to be drug related and the incidence of laboratory hepatotoxicity in the remaining 429 women exposed to NVP-containing HAART are shown in Table 8. All women with drug-related rash had CD4 cell counts >200 cells per microliter. None of the NVP-associated reactions were fatal. Of the 62 women started on NFV-containing regimen, 7 (11.3%) had mild diarrhea in the first 10 days of treatment. In none of these 7 cases, NFV was suspended.

TABLE 5. Child Deaths at 1–18 Months in the Mitra Plus Study

Cause of Death	1–6 mo	7–18 mo	Total	HIV-positive
Malaria	2	4	6	1
Pneumonia	3	4	7	3
Fever and convulsions	1	5	6	1
Diarrhoea	2	3	5	1
Sudden death	—	2	2	—
Marasmus	—	1	1	—
Heart disease	1	—	1	—
Unknown	1	3	4	—
Total	10	22	32	6

DISCUSSION

The aim of the Mitra Plus study was to prevent postnatal MTCT of HIV-1 through HAART prophylaxis during 6 months of breastfeeding and at the same time provide treatment and care of mothers who needed HAART for their own health. Treatment of the women during late pregnancy and for 6 months after delivery irrespective of their stage of HIV disease followed by continued treatment of women eligible for HAART resulted in low transmission during breastfeeding (1.0% between 6 weeks and 6 months) and low cumulative HIV-1 transmission rates at 6 months and at 18 months after delivery (5.0% and 6.0%, respectively).

In an ongoing PMTCT study of HIV-1 among breastfeeding mothers in Kisumu, Kenya, women were given HAART irrespective of their stage of HIV infection from 34 weeks gestation to 6 months postpartum as in the Mitra Plus study. The reported cumulative HIV-1 transmission rates at 6 weeks to 12 months in that study (3.9% at 6 weeks, 5.0% at 6 months, and 5.9% at 12 months)[33] are very similar to those in the Mitra Plus study. In the Mitra Plus study, there was a significant association between viral load at enrollment and transmission but no significant association between CD4 cell level at enrollment and transmission. Lack of association between maternal CD4 cell count and transmission was also reported in the Kisumu study.

The risk of acquisition of infection between 6 weeks and 6 months in the Mitra Plus study, and in the Kisumu study, was similar to that between 1 month and 6 months in the Drug Resource Enhancement against AIDS and Malnutrition (DREAM) study in Mozambique, in which breastfeeding women irrespective of stage of HIV infection were given HAART from 25 weeks of gestation to 6 months after delivery.[34] However, the cumulative HIV-1 transmission rate at 1 month (1.2%) and 6 months (2.2%) was lower in the DREAM study than at 6 weeks and 6 months in the Mitra Plus study and the Kisumu study. In a PMTCT study in Abidjan, Cote d'Ivoire, in which women requiring HAART received treatment from 24 weeks of gestation, the cumulative transmission rates at

TABLE 6. Kaplan-Meier–Estimated Transmission of HIV-1, Mortality, and HIV-Free Survival in the Mitra Plus Study and in the Breastfeeding Population in the Petra Trial Arm A

	HIV-1 Infection % (95% CI)		Mortality % (95% CI)		HIV-1 Infection or Death % (95% CI)	
	Mitra Plus	Petra*	Mitra Plus	Petra*	Mitra Plus	Petra*
6 wk	4.1 (2.2 to 6.0)	5.4 (2.7 to 8.1)	2.0 (0.7 to 3.3)	0.4 (0 to 1.1)	5.9 (3.7 to 8.1)	5.7 (2.8 to 8.6)
6 mo	5.0 (2.9 to 7.1)	11.9 (7.9 to 15.8)	4.3 (2.4 to 6.2)	4.7 (2.1 to 7.3)	8.6 (6.0 to 11.2)	15.5 (11.1 to 19.9)
12 mo	5.8 (3.6 to 8.0)	16.4 (11.7 to 21.1)	8.5 (5.8 to 11.2)	7.6 (4.2 to 11.0)	12.8 (9.6 to 16.0)	19.7 (14.7 to 24.7)
18 mo	6.0 (3.7 to 8.3)	17.7 (12.8 to 22.6)	9.2 (6.4 to 12.0)	10.3 (6.4 to 14.2)	13.6 (10.3 to 16.9)	21.9 (16.7 to 27.1)

*Indicates the breastfeeding population in the Petra trial arm A.

1 month and 6 months were 1.0% and 3.3% (n = 96). However, only 65% of these women breastfed.[35] The most likely explanation for the lower early HIV transmission rates in these 2 studies compared with the early transmission rates in the Mitra Plus study and the Kisumu study is the difference in the duration of treatment before delivery. In the Mitra Plus study, most women started HAART at 34 weeks of gestation, and the median duration of treatment before delivery was 5.4 weeks. A Cox regression analysis of transmission in the Mitra Plus study showed that duration of treatment before delivery was significantly associated with transmission. Furthermore, the HIV transmission rate at 1 week after birth was 2.9% indicating that a high proportion of the early transmission had occurred already in utero or at birth.

The transmission in the Mitra Plus study was analyzed using the breastfeeding population in the Petra trial arm A as historical controls. The transmission rate at 6 months was about 50% lower in the Mitra Plus study than in the Petra trial arm A in which only short-course treatment with ZDV + 3TC was given to the women for 2 weeks before and 1 week after delivery and to the infants for 1 week after birth. (adjusted RH = 0.49). The proportion of breastfeeding mothers was higher in the Mitra Plus study during the first 20 weeks after delivery but higher in the Petra trial arm A from 24 weeks after delivery. Most mothers in the Mitra Plus study reported exclusive breastfeeding which has been shown to reduce breast-milk HIV-1 transmission,[14–17] whereas there was no information about the type of breastfeeding in the Petra trial. Exclusive breastfeeding could have contributed to the lower 6-month infection rate in the Mitra Plus study compared with the Petra trial arm A. However, the risk of acquisition of HIV-1 infection between 6 weeks and 6 months in the Mitra Plus study (1.0%) was lower than that reported in a recent large study of exclusively breastfed infants in Kwa Zulu Natal, South Africa (4.04%).[15] The low transmission in the Mitra Plus study compared with the breastfeeding population in the Petra trial arm A from 6 to 18 months was achieved by counseling mothers to stop breastfeeding at 6 months. However, in the Mitra Plus study, there were cases of malnutrition and failure to thrive after weaning which needed nutritional support in the clinic. There are data from recent HIV PMTCT studies in African countries indicating that weaning at 6 months or earlier may increase the risk of malnutrition and infectious disease in the infants.[36–39]

An alternative to giving HAART to mothers irrespective of their stage of HIV infection to prevent breast-milk HIV-1 transmission is prophylactic ARV treatment of breastfed infants of HIV-1–infected mothers who are not eligible for HAART. This approach was investigated in the Mitra study,[19] which was performed at the same site in Dar es Salaam as the Mitra Plus study and in other recently reported studies from sub-Saharan Africa.[20–23] In the Mitra study, HIV-1–infected women were given short-course treatment with ZDV and 3TC from 36 weeks gestation to 1 week postpartum. Infants were

TABLE 7. Cox Univariate and Multivariate (Stepwise Forward) Regression Analysis With Respect to HIV-1 Transmission Up to 6 months in the Mitra Plus Study and the Breastfeeding Population in the Petra Trial Arm A

	Univariate Analysis			Stepwise Analysis		
Factor	RH	*df*	*P*	RH (Multiple)	95% CI	*P*
Mitra Plus/Petra	0.50	1	0.001	0.49	0.33 to 0.73	<0.001
CD4abs	1.00	1	0.04	1.00	0.99 to 1.00	0.023
Female child	1.41	1	0.09	—	—	—
Low birth weight	0.86	1	0.59	—	—	—
CD4 %	0.98	1	0.09	—	—	—
Weight	0.89	1	0.37	—	—	—
Age	0.97	1	0.12	—	—	—
Stage	—	3	0.83	—	—	—
Hb	1.06	1	0.38	—	—	—
Cesarean section	1.15	1	0.57	—	—	—

CD4abs indicates CD4 absolute cell counts.
Viral load was not in analysis (not available in the Petra trial).
df indicates degrees of freedom.

TABLE 8. Drug-Related Skin Reactions and Laboratory Hepatotoxicity in 429 HIV-Infected Women Exposed to NVP-Containing HAART

	Grade 1, n (%)	Grade 2, n (%)	Grade 3 or 4, n (%)	Total, n (%)
Drug-related skin reactions	6 (1.4)	15 (3.5)	7 (1.6)*	28 (6.5)
Laboratory hepatotoxicity	50 (11.7)	16 (3.7)	2 (0.5)	68 (15.9)

*Steven Johnson syndrome.

treated with ZDV and 3TC for 1 week and then with 3TC alone during breastfeeding for a maximum of 6 months. The cumulative infection rate was 3.8% at 6 weeks and 4.9% at 6 months, which is very similar to the infection rate at these time points in the Mitra Plus study. The postnatal transmission rate between 6 weeks and 6 months was also similar in the Mitra Plus study (1.0%) and the Mitra study (1.2%). The median duration of breastfeeding was longer in the Mitra Plus study (24 weeks) compared with the Mitra study (18 weeks). A randomized controlled trial in Malawi showed that extended infant ARV prophylaxis with NVP or NVP plus ZDV in the first 14 weeks of life significantly reduced the cumulative risk of acquisition of HIV-1 infection from birth to 9 months of age from 10.6 % in the control group given single-dose maternal/infant NVP combined with 1 week infant ZDV to 5.2% in the extended NVP group and 6.4% in the extended NVP/ZDV group.[20] However, in randomized trials in Ethiopia, India, and Uganda, extended infant prophylaxis given only during the first 6 weeks of life did not significantly reduce the risk of HIV infection at 6 months of age.[23]

There is a risk that giving HAART for 6 months postnatally to HIV-1–infected mothers who do not require treatment for their own health could lead to development of ARV drug resistance in infected mothers and in infants who become infected in spite of maternal HAART. In the Kisumu breastfeeding study, drug-resistant virus was demonstrated in a high proportion of infants who became HIV-1 infected postnatally despite maternal prophylactic HAART.[40] HIV resistance testing will be carried out in infected infants and in mothers in the Mitra Plus study. Infant ARV prophylaxis to prevent breast-milk transmission of HIV-1 also entails a risk of development of drug resistance in infants who become infected. Testing for HIV resistance to 3TC in 4 infants in the Mitra study who became infected despite infant 3TC prophylaxis showed resistance mutations in all 4 infants, which reverted in 2 of them when they were no longer on 3TC treatment.[19] However, the benefit of reducing postnatal HIV-1 transmission by prophylactic ARV treatment of mothers or infants during breastfeeding could outweigh the disadvantage of development of ARV drug resistance.

Another area of concern is the safety of HAART given for prevention of breast-milk HIV transmission to mothers who do not need ARV treatment for their own health. The NVP-containing regimen used in this study is one of the recommended first-line ARV treatment regimens for HAART-eligible HIV-infected pregnant women in resource-limited countries.[25] However, because of documented cases of severe hepatotoxicity in NVP-treated HIV-infected women with CD4 cell counts >250 cells per microliter, international guidelines from 2005 recommend that NVP should only be used in HIV-infected women with CD4 counts above 250 cells per microliter if benefits clearly outweigh the risks.[24–27] Therefore, toward the end of the Mitra plus study, NVP was replaced by NFV in women with CD4 cell counts above 200 cells per microliter.

Rash is the most commonly reported and treatment-limiting toxicity associated with NVP treatment which may occur alone or in combination with hepatotoxicity. In the Mitra Plus cohort, the incidence of rash as confirmed by the clinic physicians was 6.5% and the incidence of grades 3 and 4 mucocutaneous rash (Steven Johnson syndrome) was 1.6% which is similar to what has been reported in other studies.[41–45] All affected mothers in the Mitra Plus study responded well to symptomatic treatment and withdrawal of NVP with no reported case of fatality. The incidence of severe hepatotoxicity was low, only 0.5. A similar low incidence of severe hepatotoxicity has been reported in a study in Brazil.[41] The incidence of severe NVP-associated hepatotoxicity in studies in Kenya, Uganda, and Mozambique has varied from 1.7% to 6.5%.[42–45]

The cost for postnatal ARV treatment with AZT plus 3TC plus NVP for 6 months, which was used in most of the mothers in the Mitra Plus study, is US $71 in Tanzania in 2009. However, the need to replace NVP with another ARV drug, for example, a protease inhibitor such as Kaletra, in breastfeeding mothers who are not eligible for HAART will increase the cost for 6 months of postnatal prophylactic treatment to US $327. This cost may not be affordable in many resource-limited settings. Prophylactic 3TC treatment of infants during breastfeeding which resulted in a low postnatal HIV transmission rate in the Mitra study similar to that after maternal postnatal prophylaxis in the present study is a cheaper alternative to prevent breast-milk HIV transmission (a cost of US $11 for 6 months of treatment in 2009).

In conclusion, this study showed low MTCT of HIV-1 during breastfeeding and improved HIV-free survival of children at 18 months when HIV-1–infected mothers were given HAART in late pregnancy and during 6 months of breastfeeding in an African population. Ideally, the ARV treatment of pregnant women should start earlier than was done in this study (week 34 of pregnancy) to further reduce prenatal MTCT of HIV. According to present guidelines from WHO, all pregnant women who need ARV treatment for there own health should be started on HAART as soon as possible during pregnancy and the treatment should be life long. For breastfeeding mothers who do not need HAART for their own health, the strategy to use maternal HAART for prevention of HIV transmission during breastfeeding should be further evaluated and compared with the use of infant postnatal ARV prophylaxis with regard to safety and cost effectiveness. Further research is also required to find out the appropriate duration of postnatal prophylaxis and the impact on mothers health of stopping HAART after cessation of breastfeeding.

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