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

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Objective: To investigate the possibility of reducing mother-to-child transmission (MTCT) of HIV-1 through breast-feeding by prophylactic antiretroviral (ARV) treatment of the infant during the breast-feeding period.

Design: An open-label, nonrandomized, prospective cohort study in Tanzania (Mitra).

Methods: HIV-1–infected pregnant women were treated according to regimen A of the Petra trial with zidovudine (ZDV) and lamivudine (3TC) from week 36 to 1 week postpartum. Infants were treated with ZDV and 3TC from birth to 1 week of age (Petra arm A) and then with 3TC alone during breast-feeding (maximum of 6 months). Counseling emphasized exclusive breast-feeding. HIV transmission was analyzed using the Kaplan-Meier survival technique. Cox regression was used for comparison with the breast-feeding population in arm A of the Petra trial, taking CD4 cell count and other possible confounders into consideration.

Results: There were 398 infants included in the transmission analysis in the Mitra study. The estimated cumulative proportion of HIV-1–infected infants was 3.8% (95% confidence interval [CI]: 2.0 to 5.6) at week 6 after delivery and 4.9% (95% CI: 2.7 to 7.1) at month 6. The median time of breast-feeding was 18 weeks. High viral load and a low CD4 T-cell count at enrollment were associated with transmission. The Kaplan-Meier estimated risk of HIV-1 infection at 6 months in infants who were HIV-negative at 6 weeks was 1.2% (95% CI: 0.0 to 2.4). The cumulative HIV-1 infection or death rate at 6 months was 8.5% (95% CI: 5.7 to 11.4). No serious adverse events related to the ARV treatment of infants occurred. The HIV-1 transmission rate during breast-feeding in the Mitra study up to 6 months after delivery was more than 50% lower than in the breast-feeding population of Petra arm A (relative hazard = 2.61; $P = 0.001$; adjusted values). The difference in transmission up to 6 months was significant also in the subpopulation of mothers with CD4 counts $\geq$200 cells/μL.

Conclusions: The rates of MTCT of HIV-1 in the Mitra study at 6 weeks and 6 months after delivery are among the lowest reported in a breast-feeding population in sub-Saharan Africa. Prophylactic 3TC treatment of infants to prevent MTCT of HIV during breast-feeding was well tolerated by the infants and could be a useful strategy to prevent breast milk transmission of HIV when mothers do not need ARV treatment for their own health.

Key Words: Africa, breast-feeding, HIV, lamivudine, mother-to-child transmission, prevention

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The United Nations Program on HIV/AIDS (UNAIDS) estimates that, globally, 530,000 children became HIV infected in 2006, with more than 85% of them living in sub-Saharan Africa.[1] Most of the children become HIV infected through mother-to-child transmission (MTCT), which can occur during pregnancy, during delivery, or postnatally through breast-feeding. In the absence of HIV prevention measures, the rates of MTCT of HIV-1 have been estimated to range from 25% to 48% in breast-feeding populations in resource-poor settings.[2] Prolonged breast-feeding is a common practice in the developing world, and more than 40% of MTCT of HIV-1 in developing countries is attributable to breast-feeding.[3,4]

After the report of the AIDS Clinical Trials Group (ACTG) 076 trial showing successful prevention of MTCT

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§See Appendix.

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of HIV-1 by long-course antiretroviral (ARV) treatment in resource-rich settings,[5] a number of trials of short-course ARV therapy to prevent MTCT of HIV-1 have been conducted in resource-poor countries, mostly in breast-feeding populations.[6–15] The use of zidovudine (ZDV) or nevirapine (NVP) alone has been found to reduce early MTCT of HIV-1 by 35% to 50%,[7–9] and the use of a combination of ARV drugs has been shown to be more effective than the use of single-drug therapy in reducing early MTCT.[10–14] Breast-feeding undermines the preventive effect of short-course ARV treatment given around the time of delivery in prevention of MTCT (PMTCT) of HIV programs, however.[10,15,16]

Most HIV-infected mothers in developing countries, including Tanzania, breast-feed their infants by choice or because of a lack of alternative safe, acceptable, or feasible infant feeding options. Morbidity and mortality in infants attributable to infectious diseases, fear of stigmatization, and lack of economic resources are the main obstacles against raising children without breast-feeding in resource-poor settings. According to the latest World Health Organization (WHO) guidelines, exclusive breast-feeding is recommended for HIV-infected women for the first 6 months of life unless replacement feeding is acceptable, feasible, sustainable, and safe for them and their infants before that time.[16]

The objective of this study was to investigate the possibility to reduce MTCT of HIV-1 during breast-feeding by prophylactic ARV treatment of the child during the breast-feeding period. The regimen of the Petra trial arm A (ZDV and lamivudine [3TC] antepartum, intrapartum, and 1 week postpartum)[10] was chosen as the initial treatment, followed by the administration of 3TC to the infants during breast-feeding.

METHODS

Study Design

The Mitra study was an open-label, nonrandomized, prospective PMTCT study. The study was performed at the Dar es Salaam site used for the Petra trial.[10] The research clinic and research organization built for the Petra trial were used also for the Mitra study. Enrollment into the Mitra study started while the follow-up of mothers and children enrolled in the Petra trial was still going on in the same clinic. At the time of planning of the Mitra study (1999), it was not considered possible from an ethical point of view to conduct a randomized study in this setting to evaluate the efficacy of prophylactic ARV treatment of children during breast-feeding. Neither breast-feeding without any intervention nor formula feeding was considered ethically acceptable at that time at our site in Dar es Salaam. Exclusive breast-feeding as an intervention to prevent MTCT of HIV had not yet been recommended. ARV treatment of mothers during breast-feeding was not considered possible in Dar es Salaam at that time.

Study Population and Setting

The study population consisted of pregnant women enrolled at the study clinic in Dar es Salaam, Tanzania. Women were recruited from 3 primary health care antenatal clinics, 1 each from the 3 municipal districts of Dar es Salaam and from the antenatal clinic at the Muhimbili National Hospital in Dar es Salaam. Voluntary counseling and HIV testing were offered to all pregnant women before 34 weeks of gestation. Trained midwives gave pretest and posttest counseling. The HIV screening was conducted at the antenatal clinics by the midwife counselors or by health laboratory technicians using rapid HIV antibody tests. All HIV-seropositive women were then introduced to the study and offered participation if they met the eligibility criteria. Mothers who accepted participation in the study were bled again, and the blood samples were tested at the research laboratory in the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS).

The eligibility criteria included HIV-1 seropositivity determined by testing of 2 blood samples, intention to breast-feed, hemoglobin (Hb) level not <7 g/dL, being 18 years or older, willingness to take drugs and to give drugs to the newborn 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-positive women who were not eligible or did not want to participate in the Mitra study were enrolled into the National PMTCT Program, in which mother and child received single-dose NVP at labor/birth.

Enrollment into the Mitra study was at 36 weeks of gestation. Women who enrolled received normal antenatal, labor, and delivery care. In addition, they received ARV treatment according to the Petra arm A regimen: 300 mg of ZDV plus 150 mg of 3TC administered twice daily from 36 weeks of gestation, intrapartum, and for 1 week postpartum.[10]

Infants were treated with ZDV (4 mg/kg given twice daily) and 3TC (2 mg/kg given twice daily) from birth to 1 week of age (as in Petra arm A) and then with 3TC alone (2 mg/kg given twice daily from weeks 2 to 4 and 4 mg/kg given twice daily after week 4) during breast-feeding (maximum of 6 months) and 2 weeks after stopping breast-feeding. GlaxoSmithKline provided the drugs. All mothers were advised to deliver at Muhimbili National Hospital. Postnatally, mothers and infants received free medical care within the study. A special postnatal clinic was set up near a pediatric clinic, and follow-up appointments were given at weeks 1, 3, and 6 and at months 3, 6, 9, 12, 15, 18, 21, and 24 after delivery. At each visit, a clinical examination of the child was performed, adverse events since the last visit were registered, and detailed information on feeding practices and changes since the last visit was collected. Blood samples were drawn from the infants at each planned visit, except the visit at week 3. Determination of Hb level; leukocyte, lymphocyte, and thrombocyte counts; and serum creatinine and liver enzymes was done at birth; weeks 1 and 6; and months 3, 6, and 9. Counseling on infant feeding was done at every visit to the clinic. Home tracing of study subjects was conducted if the children missed 2 consecutive appointments. 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 information was obtained from relatives and friends for those who moved up country.

The study did not provide replacement feeding for infants, except in case of failure to thrive after stopping breast-feeding. Counseling on infant feeding emphasized exclusive breast-feeding and weaning of the child between 5 and 6 months. All infants were given prophylactic treatment with cotrimoxazole from 6 weeks of age to the time when they were shown to be HIV-negative after having stopped breast-feeding. Children diagnosed as HIV infected continued with cotrimoxazole prophylaxis after cessation of breast-feeding. The 3TC treatment of HIV-infected children was withdrawn when the HIV diagnosis was confirmed on a second sample.

ARV treatment was not available apart from the short regimen given within the study period around delivery and postnatally to the infant during breast-feeding to prevent MTCT of HIV.

The main study protocol was approved by the Ethical Committee of the Tanzania National Institute for Medical Research, MUHAS Research and Publications, and Institutional Review Board of the Karolinska Institute (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, 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 antiHIV 1 + 2 Plus ELISA (Behring, Marburg, Germany) and Wellcozyme HIV-1 recombinant ELISA (Murex, Dartford, United Kingdom). Sera reactive on both ELISAs were considered to be HIV-1 antibody–positive. Those with repeatedly discordant results on ELISA were tested by Western blot assay; if positive on the Western blot test, they were considered to be HIV-1 antibody–positive.

Children were tested for HIV-1 infection at week 6 and months 3 and 6 by means of the Amplicor HIV-1 DNA v1.5 qualitative polymerase chain reaction (PCR) assay (donated by Roche Diagnostics, Branchburg, NJ). Children with a positive PCR test result were retested at the next scheduled visit. Children with 2 positive HIV test results were diagnosed as being HIV-1 infected. Children who died or were lost to follow-up after a single positive PCR test result were considered to be HIV-1 positive in the analyses.

Plasma HIV-1 RNA was quantified by the Amplicor HIV-1 RNA Monitor v1.5 assay (Roche Diagnostics, Randburg, South Africa.).

Determination of T-lymphocyte subsets was done using the SimulSET flow cytometry method (Immunocytometry System; Becton Dickinson, San Jose, CA) as described previously.[17]

Determination of Hb level and leukocyte, lymphocyte, and thrombocyte counts was conducted by means of a standard hematologic analyzer (Coulter ActDiff II, Miami, FL). Serum creatinine, alanine aminotransferase (SGPT), and aspartate aminotransferase (SGOT) were determined by a Cobas Core system (Roche, Basel, Switzerland).

Statistical Analyses

The calculation of the sample size for the Mitra study was based on the assumption that prophylactic 3TC treatment of infants during breast-feeding would decrease the HIV-1 transmission rate at 6 months from 14% (Turnbull estimate for the breast-feeding population of the Petra trial arm A) to 7% in the Mitra 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 breast-feeding population in arm A in the Petra trial, we would then need 324 children in the Mitra study at 6 months. To allow for deaths and loss to follow-up (mainly because mothers move from Dar es Salaam), we planned to enroll at least 450 mothers in the Mitra study.

Data analysis was done using the SPSS software system 14.0 (Statistical Package for Social Sciences; SPSS, Inc., Chicago, IL). In case of twins, only the firstborn baby was included in the analyses. The definition of HIV-1 positivity is described in the section on laboratory methods. HIV-1 transmission, mortality, the combined outcome "HIV infection or death," and breast-feeding were analyzed using the Kaplan-Meier survival technique. In the transmission analyses, time for HIV-1 infection was considered to be the midpoint between the date for the last negative sample and the date for the first positive sample.[18] Multivariate analyses and univariate analyses with continuous background factors were performed with Cox regression. Differences in distributions were tested with the χ^2 statistic. Differences between means were tested with the Student *t* test, and differences between medians were tested with the Mann-Whitney test. The analysis of transmission of HIV-1 in the Mitra study in relation to mothers' viral load at enrollment was performed as a case-control study in which all transmitting mothers and 3 nontransmitting controls for each transmitting mother were included (viral load at enrollment was not determined in the other mothers). To enable a direct comparison of HIV-1 transmission in the Mitra study and the breast-feeding population of arm A in the Petra trial, individual data on the breast-feeding population in Petra arm A were provided by the data management center at the International Antiviral Therapy Evaluation Center (IATEC; Amsterdam, The Netherlands). Cox regression was used to study the relative effectiveness of the preventive measures taken in the Mitra study compared with the Petra trial arm A (pooled data). In the analysis of transmission, uninfected children were regarded as being at risk only as long as they were breast-fed (censoring was done at the day breast-feeding stopped or at the date of the last visit to the clinic if the child was still breast-fed at that time).[18] All HIV infections up to 6 months after delivery were considered as events. In the analysis of HIV infection or death, censoring at cessation of breast-feeding was not done.

RESULTS

Enrollment of HIV-1–positive mothers into the Mitra study started in August 2001 and ended in August 2003. In

total, 9378 women were tested, 1029 (11.0%) women were found to be HIV-1–seropositive, and 468 women were enrolled into the study (Fig. 1). Of the 468 HIV-1–infected women enrolled, 13 did not want to continue or disappeared before delivery. The remaining 455 women delivered 470 babies, including 15 pairs of twins. The second-born twins were excluded from this analysis. There were 16 stillborn babies and 6 neonatal deaths; all 6 were early neonatal deaths (<7 days). Among the remaining 433 children, 31 were lost to follow-up without having any HIV result. Four mothers were excluded from the study because they decided not to breast-feed their children at all. Thus, 398 children were included in the analyses with regard to HIV-1 transmission. There were no statistically significant differences regarding age, CD4 cell counts, and WHO stage at enrollment between the Mitra study mothers included in the analysis and the 31 Mitra study mothers whose babies were lost to follow-up without HIV results (data not shown).

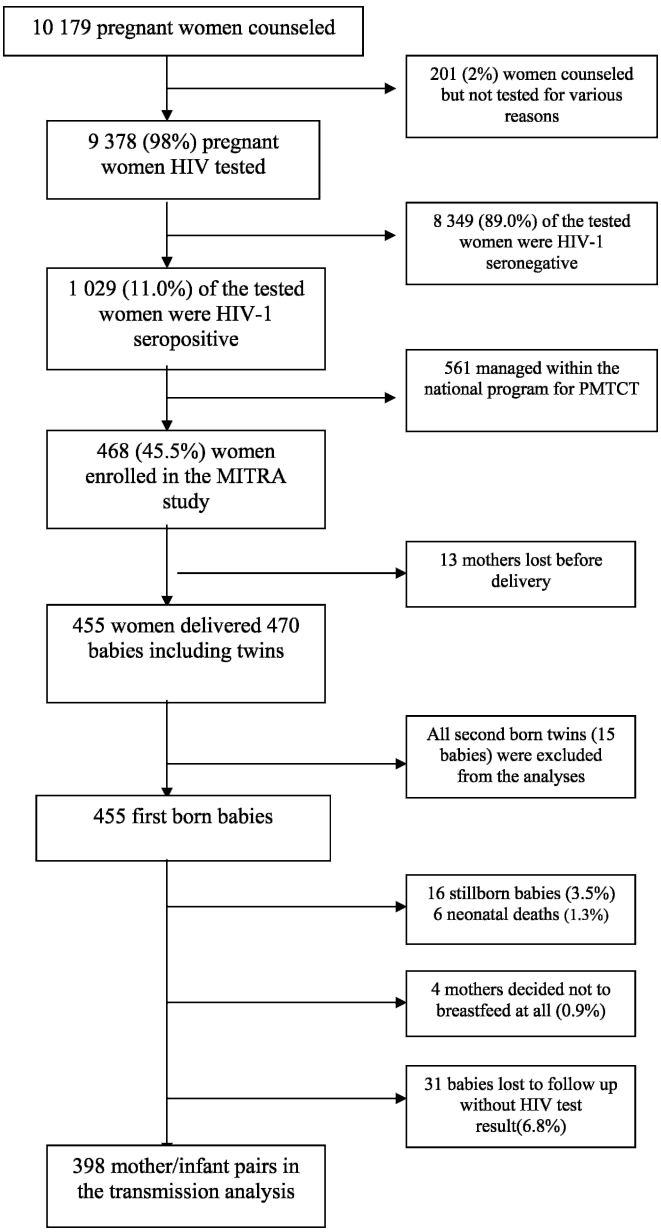

FIGURE 1. Enrollment, follow-up, and inclusion of infants in analysis.

Exclusive breast-feeding and a short weaning period were reported by most of the mothers, and most of the mothers gave an exact date for stopping breast-feeding. Kaplan-Meier estimates of breast-feeding in Mitra study were 95% breast-feeding at 6 weeks after delivery, 86% at 12 weeks, and 18% at 26 weeks (Table 4).

In total, 19 children in the Mitra study were HIV-1 infected at 6 months, of whom 15 were infected at 6 weeks (early transmission) and 4 were PCR-negative at 6 weeks but PCR-positive at month 3 (n = 1) or month 6 (n = 3); (late transmission). Of the 15 early transmissions, 13 were confirmed on a second sample, whereas 2 children who were positive at 6 weeks were lost to follow-up before a second sample could be taken. Data on the 4 late transmissions are shown in Table 2. Only 1 of the 4 children with late transmission had been given ARV treatment without any interruption during the whole breast-feeding period.

The cumulative probability of HIV-1 transmission as analyzed by the Kaplan-Meier technique in the Mitra study is shown in Figure 2. Cumulative infection rates were 3.8% (95% confidence interval [CI]: 2.0 to 5.6) at 6 weeks and 4.9% (95% CI: 2.7 to 7.1) at 6 months after delivery. The results of a Cox regression analysis of transmission in the Mitra study are shown in Table 3. Among the baseline characteristics studied at enrollment (age, WHO stage, Hb level, and CD4 cell count) and at delivery/birth (type of delivery and birth weight), only CD4 percentage (CD4%) showed a significant association with transmission ($P = 0.046$) and the absolute CD4 cell count showed a borderline significant association with transmission ($P = 0.051$) in the univariate analyses. Absolute CD4 cell counts grouped into 3 classes (<200, 200 to 500, and >500 cells/μL) were not significantly associated with transmission ($P = 0.25$). No factor (viral load not included in the analysis) could add prognostic information in the multivariate analysis when the CD4% was already considered (see Table 3). Viral load at enrollment was determined in all transmitting mothers and in 3 matched controls for each case. Mean log viral RNA copies/mL was 4.54 (n = 19) in the transmitting group and 4.06 (n = 57) in the nontransmitting group. The viral load, expressed as log RNA copies/mL, was significantly associated with transmission ($P = 0.012$).

For 380 infants who were not infected with HIV at 6 weeks, the cumulated risk of acquisition of infection between 6 weeks and 6 months of age was 1.2% (95% CI: 0.0 to 2.4).

No serious adverse events during the follow-up of the infants were considered to be related to the study medication. There were 6 early neonatal deaths: 3 of these newborns died within a few hours, and the other 3 died between days 1 and 7. There were no late neonatal deaths (days 8 to 28). Eight children died between day 29 and month 6, all of whom were HIV-negative at their last HIV test. Another 4 children died between 6 and 7 months, of whom 1 was HIV-1 positive. The main causes of death were malaria (6 children), pneumonia/septicemia (3 children), fever and convulsions

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

	HIV-1 Infection % (95% CI)		Mortality % (95% CI)		HIV-1 Infection or Death % (95% CI)	
	Mitra	Petra*	Mitra	Petra*	Mitra	Petra*
6 wk	3.8% (2.0 to 5.6)	5.4% (2.7 to 8.1)	0.8% (0 to 1.6)	0.4% (0 to 1.1)	4.5% (2.4 to 6.5)	8.7% (5.4 to 11.9)
6 mo	4.9% (2.7 to 7.1)	11.9% (7.9 to 15.8)	3.7% (1.9 to 5.6)	4.7% (2.1 to 7.3)	8.5% (5.7 to 11.4)	15.5% (11.1 to 19.9)

*Indicates the breast-feeding population in arm A of the Petra trial.

(1 child), diarrhea and dehydration (1 child), and severe malnutrition/encephalitis (1 child). Kaplan-Meier estimated mortality and HIV infection or death in the Mitra study are shown in Table 1. The three newborns who died within a few hours after birth were excluded from the analysis because they were never breast-fed.

A comparison of the baseline characteristics of mothers and infants in the Mitra study and in the breast-feeding population in the Petra trial arm A (all 5 Petra sites, n = 264) is shown in Table 4. There were no statistically significant differences at enrollment between the Mitra and Petra study mothers with regard to age and WHO clinical stage, but the Mitra study mothers had significantly lower Hb and CD4 cell values than the Petra study mothers. There were fewer cesarean sections done on the Mitra study mothers. There was no difference in gender of the child, but the Mitra study children had significantly lower birth weight. Breast-feeding in the Petra arm A was 85% at 6 weeks, 77% at 12 weeks, and 64% at 26 weeks, assuming that all mothers lacking information on the date of stopping breast-feeding went on breast-feeding during the whole follow-up period. There was no information on type of breast-feeding (exclusive or mixed) in the Petra trial.

Kaplan-Meier estimated HIV transmission, mortality, and HIV infection or death rates in the Mitra study and in the breast-feeding population in the Petra trial arm A are shown in Table 1.

To take differences in background factors into account, a Cox regression analysis of transmission in the Mitra study and the breast-feeding population in Petra arm A was performed on the pooled data. The time variable used was number of days from birth to cessation of breast-feeding, to HIV infection, or to last visit at the clinic within the first 6 months if breast-feeding continued for uninfected children. All HIV infections up to 6 months were treated as events. According to this analysis, the transmission of HIV up to 6 months (26 weeks) after delivery was more than 50% lower in the Mitra study compared with the Petra trial (adjusted relative hazard [RH] =2.61; $P = 0.001$; Table 5). The only factor that showed a significant relation to transmission and was differently distributed in the Mitra and Petra studies was CD4 cell count. Adjusting for absolute CD4 cell count gave a slightly increased RH in favor of the Mitra study (from 2.37 to 2.61). Competing nonsignificant factors were WHO stage and Hb level at enrollment, type of delivery, and gender and birth weight of the child. Viral load was not available on the Petra trial mothers and only on a sample of the Mitra study mothers, and thus could not be included in this analysis. The Cox regression analysis of transmission was also performed separately on the subpopulation of mothers with CD4 counts $\geq$200 cells/μL. There was a significantly lower transmission of HIV-1 during breast-feeding in the Mitra study compared with the Petra trial arm A also in this subpopulation (cumulative transmission at 6 months = 4.0% [95% CI: 1.6% to 6.2%] and 9.9% [95% CI: 5.9% to 13.8%], respectively; adjusted RH = 2.40 [95% CI: 1.20 to 4.78]; $P = 0.013$).

Another Cox regression analysis on pooled data was performed to compare HIV infection or death rates in the Mitra study and in the breast-feeding population of the Petra trial arm A. The results are shown in Table 6. HIV infection or death at 6 months was significantly lower in the Mitra study than in the breast-feeding population of the Petra trial arm A (adjusted RH = 2.75; $P < 0.001$).

TABLE 2. Late Transmission in the Mitra Study

Child No.	HIV PCR Results 6 Wk	3 Mo	6 Mo	9 Mo	Maternal CD4 Count at Enrollment (Cells/μL)
1	−	+	+	+	574
2	−	−	+	+	410
3	−	−	+	No sample*	150
4	−	No sample	+	No sample (infant died)	75

*Serology positive at 12 months.

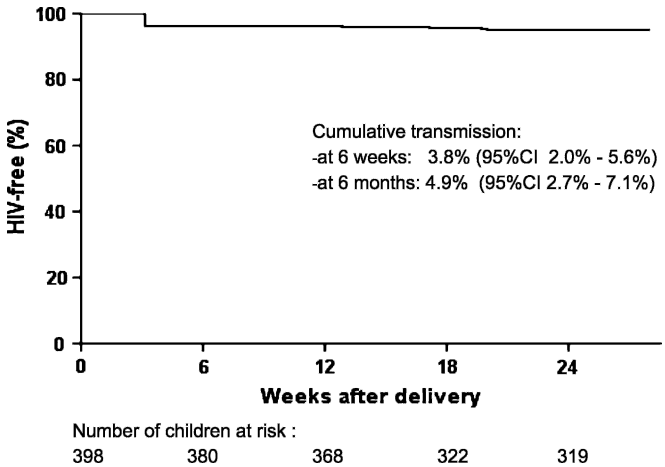

FIGURE 2. Kaplan-Meier estimated MTCT of HIV-1 in the Mitra study.

TABLE 3. Cox Regression Analysis With Respect to HIV-1 Transmission in the Mitra Study

Univariate Analysis Factor	*df*	RH	95% CI	*P*
CD4%	1	0.94	0.90 to 1.00	0.046
CD4abs*	1	1.00	0.99 to 1.00	0.051
WHO stage	3			0.93
Hb	1	0.95	0.76 to 1.19	0.64
Age	1	0.94	0.85 to 1.04	0.21
Cesarean section	1	1.15	0.38 to 3.45	0.81
Birth weight	1	0.72	0.44 to 1.17	0.18

*CD4abs indicates CD4 absolute cell count.
No factor could add prognostic information in the multivariate analysis when the CD4% was already considered.
df indicates degrees of freedom.

DISCUSSION

This study showed low cumulative HIV-1 transmission rates at 6 weeks and 6 months of age in infants treated prophylactically with 3TC during breast-feeding. The HIV-1 transmission rate at 6 weeks in the Mitra study (3.8%) is one of the lowest reported early transmission rates in a PMTCT study in a breast-feeding population in a sub-Saharan African country. In a clinical trial in Botswana (the Mashi trial part I), low transmission rates (4.3% and 3.7%, respectively) were demonstrated at 1 month of age after short-course maternal treatment with ZDV with or without single-dose NVP and infant treatment with ZDV for 1 month plus single-dose NVP.[19] In an MTCT study in Côte d'Ivoire, short-course treatment with ZDV plus 3TC plus single-dose NVP resulted in a 6-week HIV-1 transmission rate of 4.7%.[13] As in the Mitra study, mothers were counseled to practice exclusive breast-feeding in both of these studies.

The use of extended prophylactic ARV treatment of breast-fed infants for the prevention of postnatal HIV-1 transmission has been evaluated in 2 other studies: the Mashi trial in Botswana[20] and the Simba trial in Rwanda and Uganda.[21] The results from the Simba trial, in which infants of HIV-1–infected women received 3TC or NVP during the breast-feeding period, have been presented at conferences but are not yet published. The Mashi trial (part II) showed a significantly lower 7-month HIV transmission rate in infants of HIV-1–infected mothers who were formula fed and given 1 month of prophylactic ZDV compared with infants who were breast-fed and given prophylactic ZDV for 6 months (5.6% and 9.0%, respectively).[20] The 6-month HIV transmission rate in the Mitra study was lower than the 7-month transmission rate in the breast-fed ZDV-treated infants in the Mashi trial and similar to the infection rate in the formula-fed infants in the Mashi trial. The cumulative mortality rate at 7 months in the Mitra study (4.2%) was similar to that of the breast-fed ZDV-treated infants in the Mashi trial (4.9%), however.

TABLE 4. Baseline and Follow-Up Characteristics for Mothers and Children in the Mitra Study and in the Breast-Feeding Population in the Petra Trial Arm A

	Mitra	Breast-Feeding Mothers in Petra Arm A	*P*
Mother/child pairs in transmission analysis	n = 398	n = 264	
Age, y, median (IQR)	26 (23 to 30)	26 (23 to 30)	0.13
Hb, g/dL, median (IQR)	9.6 (8.6 to 10.6)	10.7 (9.8 to 11.7)	<0.001
CD4 count, cells/μL, median (IQR)	411 (269 to 611)	459 (295 to 643)	0.05
CD4%, median (IQR)	21 (14 to 27)	27 (19.4 to 35.4)	<0.001
CD4 count <200 cells/μL	15.4%	9.4%	0.03
WHO stage			
1	353 (88.7%)	234 (88.3%)	
2	28 (7%)	18 (6.8%)	
3	15 (3.8%)	8 (3.4%)	
4	2 (0.5%)	2 (0.8%)	0.88*
Cesarean section	18.6%	31.1%	<0.001
Female child	53%	51%	0.70
Birth weight, kg, median (IQR)	3.0 (2.7 to 3.3)	3.1 (2.8 to 3.3)	0.01
Low birth weight (<2.5 kg)	14.6%	5.8%	0.001
Breast-feeding†			
6 wk	95%	85%‡	
12 wk	86%	77%	
16 wk	61%	72%	
20 wk	44%	69%	
24 wk	30%	68%	
26 wk	18%	64%	
28 wk	15%	61%	

*χ^2 test for trend.
†Kaplan-Meier estimate.
‡Breast-feeding in the Petra trial was calculated on the assumption that mothers lacking information on the date of stopping breast-feeding went on breast-feeding to 6 months or longer.

TABLE 5. Cox Univariate and Multivariate (Stepwise Forward) Regression Analysis With Respect to HIV-1 Transmission During Breast-Feeding in the Mitra Study and the Petra Trial Arm A

Univariate Analysis Factor	RH	*df*	*P*	Stepwise Analysis RH (Multiple)	95% CI	*P* (Multiple)
Mitra Petra	2.37	1	0.003	2.61	1.44 to 4.73	0.001
CD4abs*	0.83	1	0.01	0.82	0.72 to 0.94	0.004
CD4%	0.97	1	0.04			
WHO stage		2	0.78			
Hb	1.12	1	0.15			
Age	1.01	1	0.70			
Cesaren section	1.74	1	0.07			
Gender	0.11	1	0.11			
Birth weight	0.98	1	0.46			

*CD4abs indicates CD4 absolute cell count.
Viral load not in analysis (not available).
df indicates degrees of freedom.

Among the 380 Mitra study infants who were HIV uninfected at 6 weeks, only 4 had acquired HIV infection at 6 months, corresponding to a Kaplan-Meier estimated risk of acquisition of infection of 1.2%, which is lower than the transmission risk of 4.5% between 4 weeks and 7 months in the ZDV-treated infants in the Mashi trial.[20] It should be noted that in the Mitra study, only 1 of the 4 infants who became infected between 6 weeks and 6 months had been given 3TC prophylaxis without any interruption during the whole breast-feeding period. The overall adherence to treatment in the study has not been analyzed.

The finding that high maternal viral loads and low CD4 cell counts were associated with transmission in the Mitra study confirms earlier reports, which have shown that these parameters are risk factors for perinatal and postnatal MTCT of HIV-1.[4,22–28]

Prophylactic infant treatment with 3TC in the Mitra study was not associated with any serious adverse events considered to be related to the study medication. Prophylactic infant treatment with ZDV in the Mashi trial resulted in toxicity, however, leading to cessation of ZDV treatment in 9.2% of the infants.[20]

The infants with early HIV-1 infection in the present study were exposed to 3TC monotherapy for several weeks, which is known to involve a high risk of emergence of viral resistance to 3TC as a result of the M184V mutation in the reverse transcriptase enzyme.[29,30] The M184V mutation is associated with impaired viral fitness, however, and 3TC may still contribute to the effectiveness of combination ARV therapy even after the appearance of the M184V mutation.[29,30] HIV resistance testing was carried out in 4 HIV-infected infants at 3 months of age when they were still being treated with 3TC. Three of these infants displayed the M184V mutation, and the fourth displayed the M184I mutation. The M184I mutation is also associated with 3TC resistance and is usually seen during transition toward the M184V mutation. At 9 months of age, however, when they were no longer on 3TC therapy, the M184V mutation had reverted in 2 of the 4 infants (J. Albert et al, Karolinska Institutet, Stockholm, Sweden, unpublished data, 2007). In our opinion, the benefit of reducing the risk of breast milk transmission of HIV-1 by prophylactic 3TC treatment of infants of HIV-1–infected mothers during breast-feeding outweighs the disadvantage of the appearance of 3TC resistance in the low proportion of

TABLE 6. Cox Univariate and Multivariate (Stepwise Forward) Regression Analysis With Respect To HIV-1 Infection or Death in the Mitra Study and the Breast-Feeding Population of the Petra Trial Arm A

Univariate Analysis Factor	RH	*df*	*P*	Stepwise Analysis RH (Multiple)	95% CI	*P* (Multiple)
Mitra Petra	2.47	1	<0.001	2.75	1.68 to 4.50	<0.001
CD4abs*	0.88	1	0.03	0.88	0.79 to 0.97	0.012
CD4%	0.99	1	0.43			
WHO stage		2	0.32			
Hb	1.16	1	0.43			
Age	1.02	1	0.25			
Cesaren section	1.17	1	0.56			
Gender	1.22	1	0.22			
Birth weight	0.99	1	0.34			

*CD4abs indicates CD4 absolute cell count.
Viral load not in analysis (not available).
df indicates degrees of freedom.

infants who become infected. Previous exposure to 3TC monotherapy should be taken into consideration when choosing ARV drugs for subsequent therapy of these children, however.

A nonrandomized design was chosen for the Mitra study because that was considered to be the only possible alternative for a study on prevention of HIV-1 transmission from mother to child in Dar es Salaam at the time of planning the study. In such situations, a cohort approach is acceptable even if there are difficulties in interpretation of results.[18] The Kaplan-Meier estimated cumulative infection rate at 6 months was significantly lower in the Mitra study compared with that in the breast-feeding population in the Petra trial arm A, in which infants did not receive prophylactic ARV treatment during breast-feeding. The Kaplan-Meier estimated cumulative HIV infection or death rate at 6 months was also significantly lower in the Mitra study than in the breast-fed infants in the Petra trial arm A. As always, however, when using historical controls, the comparison of HIV transmission rates between the Mitra study and the Petra trial must be interpreted with caution. The Mitra mothers were in an immunologically more advanced stage of HIV infection than the Petra mothers at enrollment (had lower CD4 cell counts) and had more other known risk factors (fewer cesarean sections and lower birth weight of the infants). Adjusting for absolute CD4 cell count at enrollment led to a slightly larger reduction in the risk of transmission up to 6 months in Mitra study compared with the unadjusted values (from RH = 2.37 to adjusted RH = 2.61). The other factors in the analyses (CD4%, WHO stage, and Hb level at enrollment; type of delivery; gender of child; and birth weight of child) did not significantly affect the difference in transmission up to 6 months. Viral load could not be considered, because data were not available on the Petra trial mothers. Because of differences in counseling on breast-feeding in the 2 studies, the proportion of breast-feeding mothers was higher in the Mitra study during the first 12 weeks but higher in the Petra trial at 16 to 26 weeks after delivery. By censoring the infants at cessation of breast-feeding, these differences in length of breast-feeding are taken into account in the transmission analyses. There was no information about the type of breast-feeding in the Petra trial, however. Counseling on exclusive breast-feeding was not included in the original Mitra study protocol but was added for ethical reasons, because in 2001, the WHO recommended exclusive breast-feeding for HIV-infected women who could not avoid breast-feeding,[31] with reference mainly to the findings in a study on exclusive breast-feeding and HIV transmission in South Africa.[32] In the latest guidelines agreed on in October 2006, the WHO recommends exclusive breast-feeding for HIV-infected women for the first 6 months of life unless replacement feeding is acceptable, feasible, affordable, sustainable, and safe for them and their infants before that time.[16] It is not clear to what extent exclusive breast-feeding contributed to the lower cumulative HIV transmission rate at 6 months in the Mitra study compared with the Petra trial arm A, but differences in the type of breast-feeding could probably not explain the entire difference in transmission between the 2 studies. The Kaplan-Meier estimated risk of HIV-1 transmission between 6 weeks and 6 months among infants who were HIV-negative at 6 weeks in the Mitra study (1.2%) is lower than that reported in a recent large study of exclusively breast-fed infants in Kwa Zulu Natal, South Africa (4.04%).[33] The results from the study in Kwa Zulu Natal are close to the figures presented in a previous study in Durban, where the difference in cumulative transmission between 6 weeks and 6 months among infants who were exclusively breast-fed for at least 3 months (n = 118) was 4.4%.[34] Yet, in a study in Harare, Zimbabwe, the estimated risk of transmission between 6 weeks and 6 months for the whole cohort (n = 2060) was 3.9%, and for the small group of children classified as exclusively breast-fed in that study (n = 156), the estimated risk was as low as 1.3%.[35]

Another approach to prevent HIV-1 transmission during breast-feeding that is now under evaluation is to give highly active antiretroviral treatment (HAART) to HIV-1–infected mothers during the breast-feeding period even if they do not need HAART for their own health. A number of studies assessing this approach are ongoing, including the Mitra PLUS study in Dar es Salaam.[36] The WHO guidelines from 2006 on ARV drugs for treating pregnant women and preventing HIV infection in resource-limited settings recommend that HIV-infected pregnant women who need ARV treatment for their own health should be treated with triple-ARV drugs.[37] There is probably no need to add prophylactic 3TC treatment of the infant during breast-feeding if the mother is already on HAART; however, for HIV-infected mothers who do not require ARV treatment for their own benefit, the strategy used in the Mitra study could still be a choice. MTCT of HIV-1 during breast-feeding was also significantly lower in the Mitra study than in the Petra trial arm A when we compared the subpopulation of mothers with CD4 counts $\geq$200 cells/μL.

In conclusion, the present study showed that prophylactic 3TC treatment of breast-fed infants of HIV-1–infected mothers was well tolerated by the infants and that the transmission of HIV-1 up to 6 months after delivery was low and significantly lower than in the Petra trial arm A, in which infants received ARV treatment for only 1 week. The prophylactic infant treatment strategy used in the Mitra study could be useful to prevent breast milk transmission when the mothers do not need ARV treatment for their own health.

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APPENDIX

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