

Breast-Milk Infectivity in Human Immunodeficiency Virus Type 1–Infected Mothers

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Human immunodeficiency virus type 1 (HIV-1) is transmitted through blood, genital secretions, and breast milk. The probability of heterosexual transmission of HIV-1 per sex act is .0003–.0015, but little is known regarding the risk of transmission per breast-milk exposure. We evaluated the probability of breast-milk transmission of HIV-1 per liter of breast milk ingested and per day of breast-feeding in a study of children born to HIV-1–infected mothers. The probability of breast-milk transmission of HIV-1 was .00064 per liter ingested and .00028 per day of breast-feeding. Breast-milk infectivity was significantly higher for mothers with more-advanced disease, as measured by prenatal HIV-1 RNA plasma levels and CD4 cell counts. The probability of HIV-1 infection per liter of breast milk ingested by an infant is similar in magnitude to the probability of heterosexual transmission of HIV-1 per unprotected sex act in adults.

Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) is a complex process that can occur while the fetus is in utero, during delivery of the infant, or through breast-feeding. An understanding of the risk of mother-to-child transmission of HIV-1 through breast-feeding is particularly important,

since the majority of HIV-1–infected women live in areas of the developing world where breast-feeding is the norm. During 1992–1998, we conducted a randomized clinical trial of breast-feeding versus formula feeding in infants of HIV-1–infected mothers in Nairobi, Kenya, and found the frequency of breast-milk transmission to be 16% [1]. Forty-four percent of mother-to-child transmission of HIV-1 occurred through breast-feeding.

Evaluating the probability of mother-to-child breast-milk transmission of HIV-1 and determining what factors may influence the magnitude of breast-milk infectivity are essential for an understanding of the dynamics of mother-to-child transmission of HIV-1 and for the development of prevention strategies in breast-feeding populations. Both high maternal virus load and compromised maternal immune status increase the risk of breast-milk transmission of HIV-1 [2]. In addition, factors associated with infant feeding, such as mastitis in the mother and duration of breast-feeding, have been shown to influence the risk that an infant will acquire HIV-1 through breast milk [2–5]. Although several stud-

Received 19 September 2002; revised 10 January 2003; electronically published 12 February 2003.

Presented in part: XIIIth International AIDS Meeting, Durban, South Africa, July 2000 (abstract WeOrC492). At that conference, B.A.R. was awarded the International AIDS Society Young Investigator Award for Epidemiology, Prevention, and Public Health Sciences for this work.

The study was approved by the University of Nairobi and University of Washington institutional review boards, and all women provided informed consent. Human experimentation guidelines of the University of Nairobi and University of Washington were followed in the conduct of the clinical research.

Financial support: National Institutes of Health (grants AI-29168 and HD-23412); Elizabeth Glaser Scientist Award (to J.O.).

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The Journal of Infectious Diseases 2003;187:736–40

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ies have demonstrated a relationship between longer duration of breast-feeding and increased mother-to-child transmission of HIV-1, few studies have quantified the probability of breast-milk transmission per duration or volume of exposure [3–7]. To date, no studies have evaluated either the relationship between the quantity of breast milk ingested by an infant and the risk of breast-milk transmission of HIV-1 or the relationship between maternal disease factors and infectivity of breast milk.

Our randomized trial of breast-feeding versus formula feeding provided an opportunity to determine the probability of breast-milk transmission of HIV-1 by using 2 different exposure measurements for breast-feeding: (1) the reported volume of breast milk ingested and (2) the reported number of days of exposure. Because of the follow-up schedule of the infants and mothers, this study also allowed us to investigate, for these 2 different exposure measurements, whether breast-milk infectivity of HIV-1 differs for infants aged <4 or ≥4 months. Finally, because maternal disease- and immune-status variables were collected during the study, we were able to examine how prenatal maternal factors affect breast-milk infectivity of HIV-1-infected mothers.

METHODS

Study protocol. Details of the study protocol have been described elsewhere [1]. In brief, 425 HIV-1-infected pregnant women were randomized, at ~32 weeks gestation, to breast-feed or formula feed their infants. At this enrollment visit, 15 mL of blood were collected for HIV-1 RNA testing and CD4 cell counts. Detailed postnatal interviews with the mother, including questions on infant feeding, were conducted monthly during the infant's first year of life and quarterly during the second year. Five milliliters of anticoagulated infant blood (collected in EDTA tubes), along with 5 drops of blood on filter paper (Schleicher & Schuell), were collected at birth and at 6 weeks, 14 weeks, 6 months, and then quarterly, until 24 months, for HIV-1 testing. This analysis is restricted to the 358 live-born singletons and first-born twins for whom HIV-1 infection status and information regarding breast-feeding were available.

Laboratory methods. HIV-1 infection status of the children was determined by use of a nested HIV-1 DNA polymerase chain reaction (PCR) assay performed on infant peripheral blood mononuclear cell samples and/or filter-paper samples [1, 8–10]. In addition, for some children aged >15 months, HIV-1 serologic testing was used to determine HIV-1 infection status. This testing was performed by use of an HIV ELISA (Behring), for screening, and a second ELISA (Cambridge Biotech), for confirmation. Maternal prenatal CD4 lymphocyte counts and HIV-1 RNA levels were determined as described elsewhere [9, 11].

Classification of HIV-1 infection status. The method for

classification of infant HIV-1 infection status in this study has been described in detail elsewhere [1]. A child was classified as HIV-1 infected if (1) samples from 2 consecutive visits were positive, by polymerase chain reaction (PCR), for HIV-1 DNA, (2) a single blood sample had a positive test result for HIV-1 DNA if that sample had been obtained at the last visit seen, or (3) a serum sample had a positive HIV-1 ELISA result if that sample had been obtained at the last visit of a child ≥15 months old for whom no sample from that date was available for PCR testing. A child was classified as HIV-1 uninfected if (1) none of the above criteria were met and a blood sample from the last visit for that child tested HIV-1 DNA negative by PCR or (2) a serum sample obtained at the last visit of a child aged ≥15 months tested negative by ELISA and no sample from that date was available for PCR.

Since mother-to-child transmission of HIV-1 can take place either in utero, during delivery, or during early or late breast-feeding, it was necessary to classify children into a variety of infection categories on the basis of their HIV-1-testing history, to obtain estimates of breast-milk infectivity. Infants were classified into 9 different categories (table 1). These categories were extrapolated from a published working definition of in utero and intrapartum transmission, a definition based on HIV-1 DNA PCR testing, as described elsewhere [12]. For these definitions, infants definitively infected through early breast-milk transmission must have had a negative HIV-1 test at their 6-week visit and subsequently had to test positive for HIV-1. Because not all infants were tested at exactly the scheduled testing times, we allowed an ~3-week window for the 14-week HIV-1 test to occur, to distinguish between early and late breast-milk infectivity. Hence, the cutoff of 4 months allowed us to define early infectivity at as young an age as possible, while allowing us a window to distinguish between intrapartum transmission and early breast-milk transmission among breast-feeding infants.

Estimates of breast-milk exposure. At each follow-up visit, the mother was asked whether her child had been breast-fed since the last visit. Mothers who indicated that they had ceased breast-feeding were asked at what age their child had stopped breast-feeding. For the analyses of breast-milk infectivity, the cumulative number of days of exposure was estimated on the basis of the mother's responses to these questions. Randomization status was not used to define breast-milk exposure, because not all women were compliant with their assigned feeding modality.

If a child was still breast-feeding, the mother was asked to estimate what percentage of total nutritional intake for the infant was breast milk. Estimates of the volume of breast milk ingested per day, which were based on the volume of breast milk ingested per day by fully breast-fed children (i.e., those for whom 100% of nutritional intake was breast milk), were

Table 1. Classification of timing of infant human immunodeficiency virus type 1 (HIV-1) infection.

Infection category or timing of infection	No. of live-born singletons and first-born twins			
	Total	Never breast-fed	Age at last negative HIV-1 test	Age at first positive HIV-1 test
In utero	10	NA	No negative tests	Birth
In utero, intrapartum, or early breast milk	20	7	No negative tests	>Birth and <4 months
In utero, intrapartum, or early or late breast milk	0	0	No negative tests	≥4 months
Intrapartum or early breast milk	26	6	<6 weeks	<4 months
Intrapartum or early or late breast milk	3	0	<6 weeks	≥4 months
Early breast milk	1	0	≥6 weeks	<4 months
Early or late breast milk	5	0	≥6 weeks	≥4 months
Late breast milk	10	0	≥4 months	≥4 months
Uninfected at last visit	283	115	At or after birth	No positive tests

NOTE. NA, not applicable.

combined with these maternal self-report data, to estimate the cumulative volume ingested by each child. These estimated volumes for fully breast-fed children were 335 mL per day during the first week of life, 670 mL per day from day 8 through month 2, 750 mL per day from month 3 through month 5, and 900 mL per day after month 5 [13].

Statistical methods. To estimate breast-milk infectivity, a model that used the children's serial HIV-1 DNA PCR test results to categorize the timing of infection of the children was developed to determine the contribution of each infant to a likelihood function (table 1) [14]. This model allowed for random variation in infectivity of women. Maximum-likelihood methods were used to obtain estimates of infectivity. Likelihood-ratio tests were used for hypothesis testing, and bootstrap methods were used to calculate 95% confidence intervals (CIs). Goodness of fit of the model was assessed by visually comparing the expected cumulative probability of infection (calculated by the model) to the observed cumulative probability of infection at each testing time. S-Plus 2000 (Insightful) was used for all analyses.

RESULTS

Study population. The study population of the randomized clinical trial has been described in detail elsewhere [1]. Of the 358 live-born singletons and first-born twins for whom HIV-1 infection status and information regarding breast-feeding were available, 75 were HIV-1 infected. On the basis of (1) the classification of infection status described both above and in table 1 and (2) information regarding breast-milk exposure, 52 children were possibly infected through breast milk.

The median prenatal maternal HIV-1 RNA plasma level in the 358 mothers was 43,120 copies/mL, and the median prenatal CD4 cell count in these mothers was $400 \times 10^6/L$. Among

breast-fed children, the median duration of breast-feeding was 17 months (range, 1 day to >24 months), and the median age at introduction of weaning foods was 3.8 months. Among the 220 children who had ever breast-fed before 4 months of age, the average number of days of breast-feeding before either HIV-1 infection or weaning (whichever came first) was 93 days, compared with 301 days for the 157 children aged ≥4 months who breast-fed at or after 4 months of age.

Breast-milk infectivity. The overall probability of breast-milk transmission of HIV-1 per liter of breast milk ingested was .00064 (95% CI, .00035–.00093). This corresponds to ~1 infection/1500 liters of breast milk ingested. The average breast-feeding infant in this cohort consumed ~150 liters of breast milk during the course of breast-feeding. The overall probability of breast-milk transmission of HIV-1 per day of exposure was .00028 (95% CI, .00013–.00042). This corresponds to ~10 infections/100 child-years of breast-feeding. Analyses restricted to women randomized to the breast-feeding arm of the study resulted in similar estimates of infectivity (data not shown). The curve for observed cumulative time to HIV-1 infection and the curves generated by the two models of infectivity per exposure were very comparable, demonstrating that the models fit the observed data well (data not shown).

Breast-milk infectivity per liter of breast milk ingested was not statistically significantly different for children <4 months old versus children ≥4 months old (.00023 vs. .00077; $P = .2$). In addition, the probability of breast-milk infection per day of exposure was not statistically significantly different for children <4 months old versus children ≥4 months old (.00015 vs. .00031; $P = .4$).

Maternal disease and immune status, as measured by CD4 cell count and HIV-1 RNA plasma level, were both predictive of breast-milk infectivity (table 2). Mothers with prenatal HIV-1 RNA plasma levels above the median in the cohort (43,120

Table 2. Breast-milk infectivity, by maternal disease and immune status.

Measure of breast-milk infectivity	Breast-milk infectivity					
	Maternal HIV-1 RNA plasma virus load			Maternal CD4 cell count		
	≥43,120 copies/mL (n = 160)	<43,120 copies/mL (n = 160)	P	<400 × 10 ⁶ /L (n = 174)	≥400 × 10 ⁶ /L (n = 160)	P
Per liter ingested	.00104 (.00044–.00171)	.00025 (.00005–.00055)	.01	.00095 (.00045–.00156)	.00036 (.00008–.00067)	.06
Per day of exposure	.00044 (.00019–.00075)	.00011 (.00003–.00025)	.01	.00043 (.00020–.00074)	.00015 (.00003–.00030)	.04

NOTE. Data are median (95% confidence interval).

copies/mL) had 4-fold-higher breast-milk infectivity, both per liter of breast milk ingested by the infant ($P = .01$) and per day of breast-feeding by the infant ($P = .01$), compared with mothers with virus loads at or below the median. In addition, mothers with prenatal CD4 cell counts $<400 \times 10^6/L$ (the median in the cohort) had almost 3-fold-higher breast-milk infectivity, both per liter of breast milk ingested ($P = .06$) and per day of breast-feeding ($P = .04$), compared with mothers with CD4 cell counts $\geq 400 \times 10^6/L$.

DISCUSSION

In this study of mother-to-child transmission of HIV-1, we found that the overall probability of breast-milk transmission of HIV-1 was .00064 per liter of breast milk ingested and .00028 per day of exposure. These estimates correspond to ~1 infection/1500 liters of breast milk ingested and to 10 infections/100 child-years of breast-feeding.

We also found that the probability of breast-milk infection per day of exposure and per liter ingested was not statistically significantly different between children aged <4 months and those aged ≥ 4 months. Previous studies have speculated that the risk of breast-milk transmission may be higher for younger infants, because of the immaturity of their immune systems, the permeability of their guts, or a higher concentration of cells in colostrum [1, 15]. Because of infrequent HIV-1 testing of infants during the first few weeks after birth, it was not possible to compare the relative infectivity of breast milk during the first month of life versus that later in life, a comparison that would likely show the largest difference in breast-milk infectivity; rather, by comparing children aged <4 months versus those aged ≥ 4 months, we may have diluted any effect that young age has on breast-milk infectivity. However, the results found here are important in that they suggest that efforts to prevent mother-to-child transmission of HIV-1 through breast-feeding should concentrate on the entire breast-feeding period, rather than on the first few months postpartum, since breast-milk infectivity remains substantial for older infants.

Finally, an important finding in this study is that both high prenatal maternal HIV-1 RNA levels and low prenatal maternal CD4 cell counts significantly increase breast-milk infectivity of

HIV-1-infected mothers—and that the magnitude of increase for these maternal factors is similar per liter of breast milk ingested and per day of exposure. Potential strategies for prevention of breast-milk transmission of HIV-1 would be to lower HIV-1 breast-milk virus loads in the mother during the breast-feeding period, perhaps through antiretroviral treatment, and to encourage women with advanced disease to avoid breast-feeding.

The major strengths of this study are its prospective design; frequent sampling schedule for infant blood, to allow for timing of the infection to be determined; and careful collection of infant feeding data, at frequent intervals, from mothers. The main limitation of this study is that both the number of days of breast-milk exposure for each child and the estimated volume of breast-milk ingested per day were based on maternal self-report, which may not be completely accurate; however, it is unlikely that accuracy would vary by maternal-disease-status variables, so the comparisons between groups are likely to be unbiased. An additional limitation, described above, is the inability to distinguish between breast-milk infectivity during the first weeks of life and later breast-milk infectivity. Finally, because the data used to obtain estimates of breast-milk infectivity are from a randomized trial of breast-feeding versus formula feeding, it is possible that women in the formula-feeding group underreported breast-milk exposure of their infants. This underreporting could result in either inflated estimates of breast-milk infectivity (from women reporting some breast-feeding—but less than that which actually occurred) or deflated estimates of breast-milk infectivity (from women reporting no breast-feeding and from breast-milk infections being attributed to intrapartum infections); however, the fact that analyses limited only to the breast-feeding group gave similar estimates of breast-milk infectivity, both per day exposed and per liter ingested, suggest that this potential underreporting is not a large problem.

The issue of breast-milk transmission of HIV-1 continues to be an important one for sub-Saharan Africa and other parts of the developing world, and consensus regarding the best feeding modality for children of HIV-1-infected mothers in these settings has not been achieved. Recommendations range from complete avoidance of breast-feeding to exclusive breast-feeding [1, 16].

Our results add to this discussion by providing the first quantitative estimates of breast-milk infectivity per liter of breast milk ingested. Studies investigating infectivity of HIV-1 in the setting of sexual transmission have shown that the probability of transmission of HIV-1 per unprotected sexual act is .0003–.0015 [17–23]. Our estimates indicate that ingestion of 1 liter of breast milk from an HIV-1–infected mother confers similar risk as does 1 unprotected sex act with an HIV-1–infected partner.

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