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Low and Undetectable Breast Milk Interleukin-7 Concentrations Are Associated With Reduced Risk of Postnatal HIV Transmission

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Abstract

Objective—To investigate if breast milk interleukin [IL]-7 concentrations are associated with postnatal HIV transmission.

Design—A case-control study nested within a cohort of women recruited in Lusaka, Zambia.

Methods—IL-7 breast milk concentrations were measured in samples from 24 HIV-infected breast-feeding women who transmitted HIV to their child after the neonatal period and from 47 women who did not transmit. Samples were frequency-matched by the time of sample collection (1 week and 1 month postpartum). Logistic regression was used to adjust for possible confounders. For comparison, samples from 18 HIV-uninfected women from the same community were included in the analysis, and plasma IL-7 was determined.

Results—Breast milk IL-7 concentrations were significantly higher than plasma IL-7 concentrations in all 3 groups. In contrast to levels among transmitters and HIV-uninfected women, breast milk IL-7 concentrations exhibited a bimodal distribution among nontransmitters. Breast milk IL-7 concentrations undetectable or less than 30 pg/mL were significantly associated with less HIV transmission (odds ratio = 0.13, 95% confidence interval: 0.03 to 0.64). The association remained strong after adjustment for breast milk viral load and sodium, maternal CD4 cell counts, parity, and time of sample collection.

Conclusion—Breast milk IL-7 may be necessary for effective HIV transmission.

Keywords

breast milk; cell activation; interleukin-7; postnatal HIV transmission

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Breast-fed infants of HIV-infected mothers consume large quantities of HIV,[1] but most remain uninfected.[2] The mechanisms that may protect these breast-fed infants are not yet well understood. Risk factors for breast-feeding transmission include high cell-free and cell-associated breast milk viral load,[3–5] maternal immune status,[6] and behavioral factors such as duration of feeding[2] and mixed versus exclusive feeding.[7] Immunomodulating factors in breast milk such as HIV-specific antibodies,[8] cytotoxic T cells,[9] anti-infective factors,[10] and certain chemokines[11] have been found to influence the risk of HIV transmission further in some studies.

Interleukin (IL)-7 is a key regulator of B- and T-cell lymphopoiesis and T-cell homeostasis. Produced by various stromal and epithelial tissues, it binds to specific receptors expressed on most lymphocytes.[12] IL-7 can enhance the number of T cells in lymphopenic hosts by preventing apoptosis, and thus prolonging cell survival,13 and by inducing expansion of naive and mature T cells.14,15 In lymphopenic patients, including HIV-infected persons, circulating IL-7 levels are elevated, which is thought to be a physiologic mechanism to maintain T-cell homeostasis.[16] These immunosalvatory functions have led to considerable interest in IL-7 as therapy to expedite immune reconstitution in HIV-infected individuals receiving antiretroviral therapy.[12,17] However, in vitro IL-7 enhances HIV infection, replication and cytopathicity, and induces HIV expression in latently infected cells,[18–25] raising concern about its use in HIV-infected patients.

IL-7 has been detected in breast milk and has been proposed to have immunostimulatory and protective functions against infectious diseases in breast-fed infants.[26] IL-7 has not yet been investigated in relation to HIV transmission. Based on these previous findings, we initially hypothesized that IL-7 may be beneficial for the prevention of mother-to-child HIV transmission. When breast milk IL-7 concentrations were measured, however, we observed the opposite (ie, low and undetectable concentrations of breast milk IL-7 were associated with reduced postnatal HIV transmission). Our data suggest that rather than playing a protective role, IL-7 may facilitate transmission of HIV through breast-feeding.

MATERIALS AND METHODS

Study Design

A nested case-control study was conducted within a cohort of mothers and children enrolled in the Zambia Exclusive Breast-Feeding Study (ZEBS) undertaken in Lusaka, Zambia.[27] In brief, the cohort consisted of 1435 HIV-positive women recruited during pregnancy, who were given only single-dose nevirapine to prevent transmission to the child. Antiretroviral treatment was not available at the site when samples were collected. The study participants and their infants were followed for 24 months after delivery. All women planned to breast-feed and received counseling to support exclusive breast-feeding to 4 months. Half of the women were randomized to an intervention to encourage abrupt cessation of breast-feeding at 4 months, and half of the women were encouraged to breast-feed exclusively to 6 months and to wean as per usual practice, with the duration of all breast-feeding determined by the mother's own choice. Heel-stick blood samples were collected from newborns at 1 week of age, and thereafter monthly to 6 months and every 3 months to 24 months. These samples were tested for HIV-1 DNA by polymerase chain reaction (PCR) to determine the infants' infection status and the timing of transmission. Blood samples were collected from mothers at enrollment during pregnancy to measure CD4 cell counts (FACSCount System; BD Immunocytometry Systems, San Jose, CA) and plasma viral load (Roche Amplicor, version 1.5; Roche, Branchburg, NJ). All women signed informed consent forms for participation in the study, and the study was approved by the institutional review boards at the respective institutions of the investigators.

Study Subjects

For the case-control study presented here, we selected as cases 24 HIV-infected women who transmitted HIV to their infants postpartum through breast-feeding. Postpartum infections were defined as a confirmed positive HIV DNA PCR test result at later than 42 days of age with a preceding negative test at the 1-month visit or later. HIV DNA was detected in samples collected before 3 months for 7 infants, from 3 to 6 months for 10 infants, and after 6 months for 7 infants. For comparison, 47 nontransmitting HIV-infected women were selected from the cohort as controls if their infants had only negative PCR test results up to 24 months of age or up to their last available sample if they were lost to follow-up or died. All nontransmitting controls had the opportunity to be followed to 24 months at the time of selection. Eighteen HIV-uninfected women from the same community recruited over the same period were included as negative controls.

Breast Milk Samples and Testing

Breast milk was collected by manual expression at protocol-scheduled visits 1 week and 1 month postpartum. We selected the 1-week sample for testing if it was available; if unavailable, the 1-month sample was tested. We additionally ensured that the same proportions of 1-week and 1-month samples per group were included (frequency matching). Breast milk was processed within 4 hours of collection and was kept cold until processing. The milk was centrifuged at 400*g*, and the cell pellet was removed. The supernatant and lipid portions of the milk were mixed together before aliquoting and were stored at −70°C until use.

The fluid portions of the breast milk samples were tested to quantitate HIV-1 RNA using an ultrasensitive assay with a lower limit of detection of 50 copies/mL (Roche Amplicor, version 1.5) and to quantitate sodium using an anion selective electrode (Beckman-Colter-Synchron LX20; Beckman Coulter, Fullerton, CA). IL-7 in plasma and breast milk was measured using the IL-7 Immunoassay (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Aliquots from selected samples from women with low IL-7 levels were spiked with IL-7 to ensure that the observed low levels were not attributable to the presence of inhibitors. Laboratory personnel performing the assay were blinded to mother's transmission status.

Statistical Methods

For univariate analyses, χ^2 tests were used to compare categoric variables unless expected cell sizes were <5; in such cases, the Fisher exact test was used. For ordered categoric data, we used the Cochrane-Armitage trend test and the exact version of this test if the expected cell sizes were <5. We used *t* tests to compare normally distributed continuous variables, Wilcoxon rank sum tests to compare nonnormal continuous variables, and the Wilcoxon signed rank test to test for difference between paired data such as plasma and breast milk IL-7 levels. Spearman correlation coefficients were calculated to describe correlations between IL-7 and other parameters. Kaplan-Meier methods and log-rank tests were used to compare durations of breast-feeding between groups. Multiple logistic regression was used to examine associations between breast milk IL-7 concentrations and transmission after adjusting for possible confounding. All statistical analyses were performed using SAS software (version 9.1, SAS Institute, Cary, NC).

RESULTS

Study Population

Characteristics of the 24 HIV-infected mothers who transmitted HIV through breast-feeding and the 47 HIV-infected mothers who did not transmit are displayed in Table 1. As expected,

postnatal transmitters were more likely to have higher breast milk viral loads, lower CD4 T-cell counts, and more low-birth-weight infants than nontransmitters. There was also a significant difference between postnatal transmitters and nontransmitters in parity and a tendency toward a higher maternal age of the transmitters. Both of these differences were explained by maternal CD4 cell count, which inversely correlated with older maternal age and higher parity.

A previous study observed lower breast milk IL-7 levels during seasons of food shortages compared with the harvest season.[26] Given the urban economy of Lusaka, we used the price of the staple food maize as a related indicator. Samples from transmitters were more often collected during a period of high maize prices in Lusaka. This is most likely explained by the sampling chronology during the ongoing trial. Because the food shortage occurred during a later period of the study and the transmitters could be identified as soon as they occurred but nontransmitters could only be identified once they had had the opportunity to complete their 24-month visit, our study population overrepresented nontransmitters born before the period of food shortage. We examined the consequence of this imbalance between the groups in all analyses.

Breast Milk Interleukin-7 and HIV Transmission

Distributions of breast milk IL-7 concentrations extended over a wide range of values, covering several orders of magnitude (<0.25 pg/mL up to 0.16 μg/mL). There was a distinct difference in the distribution of breast milk IL-7 among nontransmitters compared with postnatal transmitters and uninfected control women. Although all 3 groups exhibited a peak at relatively high IL-7 concentrations (~150 pg/mL), nontransmitters showed a second accumulation at low and undetectable levels (Fig. 1). To take this distribution into account, a cutoff value of 30 pg/mL was chosen to separate the 2 peaks.

Significantly more nontransmitters (40%) had low breast milk IL-7 concentrations (<30 pg/mL) than transmitters (8%; $P = 0.005$). Low IL-7 concentrations were associated with less breast-feeding HIV transmission (odds ratio [OR] = 0.13, 95% confidence interval [CI]: 0.03 to 0.64; Table 2). The distribution of breast milk IL-7 concentrations observed among the transmitters was similar to that observed among the HIV-uninfected control women. The nontransmitting HIV-infected women differed from the uninfected control women, however, because they had significantly more samples with low IL-7 concentrations ($P = 0.006$).

When the analysis was restricted to IL-7 concentrations greater than 30 pg/mL, there was no longer a significant association with transmission (OR = 0.63, 95% CI: 0.28 to 1.43 per $\log_{10}$-increase of breast milk IL-7).

Plasma Interleukin-7 Concentrations

For a subset of 74 women, plasma IL-7 concentrations were also measured. IL-7 was detectable in plasma among all women (range: 1.3 to >51.2 pg/mL) and was normally distributed after $\log_{10}$ transformation. There were no significant differences in mean plasma IL-7 concentrations between the 3 categories of women. Plasma IL-7 concentrations were, however, significantly lower than breast milk IL-7 concentrations in all 3 groups of women (see Table 2). Plasma IL-7 concentrations were not significantly correlated with breast milk IL-7 concentrations in any of the 3 groups.

Predictors of Low Breast Milk Interleukin-7 Among Nontransmitters

To understand the anomalous pattern of breast milk IL-7 among the nontransmitters better, we investigated characteristics associated with low IL-7 concentrations (<30 pg/mL) in this group. Low IL-7 concentrations were more common in 1-month samples than in 1-week samples.

Women with low breast milk IL-7 concentrations had more previous live births (median of 3 compared with 1.5; $P = 0.03$) than women with high breast milk IL-7 concentrations. There were no significant differences in breast milk IL-7 concentration by maternal age, infant birth weight, postpregnancy body mass index (BMI), time of high maize prices, wet versus dry season, CD4 T-cell count, or plasma viral load. There was a nonsignificant trend toward more low breast milk IL-7 concentrations if milk sodium was also less than 13 mmol ($P = 0.08$), but there was no association with breast milk viral load (Table 3).

Is the Association Between Breast Milk Interleukin-7 and Transmission Explained by Other Factors?

Because there seemed to be differences in breast milk IL-7 concentration by postnatal age when the milk was collected, we repeated the analysis restricted to the 1-week samples. The association remained strong within the 1-week samples alone (OR = 0.15, 95% CI: 0.02 to 1.29). Breast milk IL-7 concentration <30 pg/mL was associated with significantly less HIV postnatal transmission (OR = 0.10, 95% CI: 0.02 to 0.62) after adjustment for breast milk viral load and maternal CD4 T-cell counts in a logistic regression model. Both of these other factors were significantly associated with postnatal transmission (Table 4). The univariate effects of maternal age, parity, birth weight, and plasma viral load on HIV transmission (see Table 1) were explained by low maternal CD4 cell counts. None of these factors remained significantly associated with HIV transmission after adjustment for maternal CD4 cell counts, nor did they appreciably change the magnitude of the association between breast milk IL-7 and postnatal HIV transmission entered alone or in a model that included breast milk viral load and CD4 cell counts. Further adjustment for plasma IL-7, breast milk sodium concentrations, and time of high maize prices did not markedly change the association between breast milk IL-7 and postnatal transmission.

Finally, we tested if calendar time might influence the results. We restricted the analysis to samples collected before March 2002, which corrected the temporal differences of sample collection between transmitters and nontransmitters (median of October 31, 2001 for 44 nontransmitters and November 12, 2001, for 11 transmitters; $P = 0.18$). The association with low IL-7 concentration remained strong (OR = 0.13, 95% CI: 0.02 to 1.12) within this subgroup. Similarly, it did not appreciably change when adjusted for the period of high maize prices (OR = 0.16, 95% CI: 0.03 to 0.79).

DISCUSSION

Breast milk IL-7 concentration less than 30 pg/mL at 1 week or 1 month postpartum was strongly associated with less HIV transmission through breast-feeding. This finding was unexpected and contrary to our initial hypothesis. Our data suggest that rather than conferring protection, medium and high concentrations of IL-7 in breast milk may promote HIV transmission through this route.

Circulating IL-7 levels have been shown to be up-regulated in response to low CD4 cell counts, [16] which is a strong risk factor for postnatal HIV transmission. One may therefore speculate that low circulating CD4 cell counts may also translate into high breast milk IL-7 levels, and thus explain the observed association between high breast milk IL-7 levels and enhanced risk of HIV transmission. However, we did not find any correlation of breast milk IL-7 with blood CD4 cell counts or any attenuation of the association between IL-7 and HIV transmission when adjusted for CD4 cell counts, however. Because breast milk IL-7 levels were approximately 10 times higher than plasma levels, they probably are not regulated by means of the same mechanisms that influence plasma IL-7. More importantly, our data indicate that high breast milk IL-7 levels are independently associated with postnatal HIV transmission.

There are several mechanisms by which IL-7 could enhance HIV transmission. First, IL-7 could influence breast milk viral parameters. For example, IL-7 has been shown to enhance HIV replication,[29,30] reactivate latent HIV from resting cells,[18,23,31–33] and influence the type of expressed provirus.[18] Although we did not observe any correlation between IL-7 and HIV RNA levels in breast milk or an attenuation of the IL-7 effect on postnatal HIV transmission when adjusted for viral load, we cannot exclude the possibility that IL-7 may influence other parameters in breast milk, such as viral infectivity or the quantity of cell-associated virus. An influence of IL-7 on breast milk CD4 T cells may seem especially attractive, given their greater capacity to enter viral replication as compared with blood CD4 T cells.[34]

Second, IL-7 may enhance productive infection of target cells within the breast-feeding infant. Most T cells are quiescent, and therefore less susceptible to productive HIV infection.[35] IL-7 exposure renders quiescent cells vulnerable to productive HIV infection, an effect that is more pronounced in neonatal compared with adult T cells.[19–22,24,25]

Additionally, exogenous IL-7 has been associated with colitis in murine models, and IL-7 and its receptor have been implicated in intestinal inflammatory immune responses.[36–38] Inflammation is associated with increased transmission and replication of HIV.[39,40]

Third, IL-7 may have effects on cells other than T cells. IL-7 induces the development of thymic and monocyte dendritic cells[12,41,42] and is involved in dendritic cell–mediated T-cell activation.[43] Dendritic cells are believed to modulate HIV transfer through the mucosal membranes and further to lymph nodes;[44] thus, a higher number of activated dendritic cells may also positively influence HIV transmission. Monocytes also express IL-7 receptors and produce inflammatory cytokines in response to high levels of IL-7.[45]

Finally, we cannot exclude the possibility that IL-7 is not a causal agent in our study but is simply a correlate. For example, transforming growth factor-β (TGFβ) is generally inversely related to levels of IL-7, and high levels of TGFβ could inhibit HIV transmission.[16,46] Nevertheless, given the magnitude of the association, and IL-7's potent immunomodulatory and virologic effects, it is possible that IL-7 plays a causal role in HIV transmission.

In contrast to an earlier study in The Gambia that found seasonal variations in breast milk IL-7 levels, possibly attributable to variation in food supply,26 we did not observe consistent seasonal patterns in IL-7 or an association with periods of famine. It is possible that HIV infection over-whelmed any other exogenous factor or that nutrition is generally poor in this community year round. In concordance with well-established temporal changes in the concentration of milk constituents,47 we found higher levels of IL-7 in 1-week samples than in 1-month samples. Additionally, we found low IL-7 levels to be associated with higher parity. Parity has been shown to modulate risks of breast cancer and, in rodents, to change the expression of certain cytokines in mammary tissue.[48] Genetic rearrangements and irreversible differentiation of mammary tissue may additionally influence breast milk IL-7 concentrations. Finally, maternal HIV infection per se might result in deregulation of breast milk IL-7 (albeit deregulation that seems to be beneficial), because HIV-uninfected women did not exhibit a second accumulation at low IL-7 concentrations.

Breast milk IL-7 concentrations have been reported to be influenced by freeze-thaw cycles.[26] There were no differences in handling of samples from nontransmitters and transmitters, and all testing was undertaken blinded to transmission status. Because of the sequence of events, samples of transmitting mothers were generally collected later during the course of the study than samples of nontransmitting mothers. The protective association remained, however, even if adjusted for the time of sampling.

Whether local levels of IL-7 may also play a role in facilitating other forms of HIV remains to be determined. IL-7 is highly expressed in intestinal epithelium, and this may facilitate infection by means of rectal transmission. Also, endocervical but not ectocervical tissues can produce IL-7 in vitro,[49] and the endocervix is believed to be the major site of HIV infection in the female genital tract.[50] Among young women, and also among pregnant and postpartum women, endocervical columnar epithelium everts onto the ectocervix.[51] If the endocervix is found in vivo to produce high levels of IL-7, this may explain the biologic vulnerability of pregnant and young women to acquire HIV infection.

In summary, our data suggest that mothers who have unusually low and undetectable breast milk IL-7 concentrations seem to be at reduced risk of transmitting HIV by means of breast-feeding. The underlying mechanisms should be investigated further, and a potential role of IL-7 in other routes of HIV transmission should be explored.

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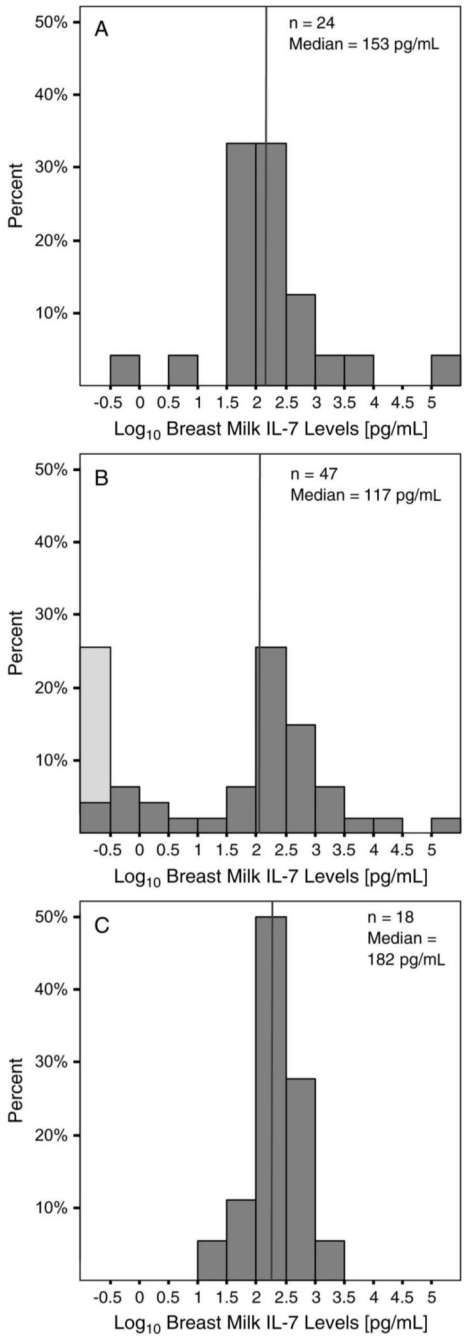

FIGURE 1.

Distribution of breast milk IL-7 concentrations among HIV-infected mothers who transmitted HIV to their infants through breast-feeding (A), HIV-infected mothers who did not transmit HIV to their infants (B), and HIV-uninfected mothers (C). Undetectable samples (<0.25 pg/mL) are marked in yellow. The red line indicates the median.

TABLE 1

Characteristics of 71 HIV-Infected and 18 HIV-Uninfected Women in Lusaka, Zambia

	Mother HIV-Infected			Mother HIV-Uninfected
	Postnatal Transmitters	Nontransmitters	*P*	
N	24	47		18
Breast milk				
n (%) 1-wk samples	17 (71)	34 (72)	0.89	13 (72)
n (%) sample collected during a period of high maize prices*	16 (67)	18 (38)	0.02	15 (83)
n (%) HIV RNA >50 copies/mL†	16 (70)	19 (41)	0.03	—
Median (IQR) $\log_{10}$ HIV RNA	2.5 (<1.7, 3.9)	<1.7 (<1.7, 2.2)	0.007	—
n (%) sodium ≥13 mmol	9 (38)	17 (36)	0.91	7 (39)
Maternal parameters during pregnancy				
Mean (SD) $CD4^+$ cells/μL	215 (107)	390 (197)	<0.001	845 (227)
Mean (SD) $\log_{10}$ plasma HIV RNA copies/mL	4.9 (0.6)	4.5 (0.9)	0.08	—
Clinical factors				
Mean (SD) age in years at enrollment	27.9 (4.5)	25.9 (4.3)	0.07	26.2 (7.1)
n (%) randomized to early weaning group	12 (50)	27 (57)	0.55	0 (0)
% (SE) still breast-feeding at 4 mo‡	91 (6.0)	91 (4.3)	0.91	94 (5.7)
% (SE) still breast-feeding at 12 mo‡	35 (10.4)	49 (7.9)	0.34	94 (5.7)
Parity, n (%)			0.01	
Primipara	1 (4)	7 (15)		5 (28)
1 to 2 previous live births	8 (33)	25 (53)		5 (28)
3+ previous live births	15 (63)	15 (32)		8 (44)
Mean (SD) birth weight (g)	2672 (612)	3028 (497)	0.01	3004 (616)
n (%) birth weight <2500 g	7 (29)	5 (11)	0.09	3 (17)
n (%) preterm births <35 wk	4 (17)	8 (17)	1.0	3 (17)
Mean (SD) maternal BMI 1 mo postpartum	20.5 (2.2)	20.9 (3.0)	0.58	22.8 (3.8)

IQR indicates interquartile range.

*Real maize price >2000 ZMK per 15 kg from December 2001 to March 2003.[28]

†Data are available for 23 of 24 transmitters and for 46 of 47 nontransmitters.

‡Kaplan-Meier estimates for the duration of breast-feeding.

TABLE 2

Breast Milk and Plasma IL-7 Concentrations

	Mother HIV-Infected			
	Postnatal Transmitters	Nontransmitters	*P*	Mother HIV-Uninfected
Breast milk				
N	24	47		18
Median (IQR)	153 (70, 325)	117 (0.31, 373)	0.25 182	(109, 416)
n (%) <30 pg/mL	2 (8)	19 (40)	0.005	1 (6)
Maternal plasma				
N	13	43		18
Median (IQR)	11.7 (8.8, 12.9)	9.8 (4.4, 21.1)	0.52	11.1 (8.6, 15.0)
Mean (log_{10}-transformed pg/mL) (SD)	1.1 (0.2)	1.0 (0.4)	0.45	1.0 (0.2)
Relations: milk and plasma				
N	13	43		18
Spearman rank order coefficient	0.36	−0.08		−0.33
P	0.22	0.63		0.18
Median difference: milk minus plasma (range)*	135 (−12, 711)	90 (−51, 13250)		172 (10, 1043)
P (rank sign test)	0.0005	0.0002		<0.0001

IQR indicates interquartile range.

*Undetectable IL-7 concentrations were imputed with the detection limit of the assay of 0.25 pg/mL.

TABLE 3

Predictors of Breast Milk IL-7 Among 47 Nontransmitters

	Low Breast Milk IL-7 <30 pg/mL	Normal breast milk IL-7 ≥30 pg/mL	*P*
N	19	28	
Breast milk			
n (%) 1-wk samples	10 (53)	24 (86)	0.01
n (%) sample collected during a period of high maize prices*	5 (26)	13 (46)	0.16
n (%) HIV RNA >50 copies/mL†	10 (53)	9 (33)	0.19
Median (IQR) $\log_{10}$ HIV RNA	1.8 (<1.7, 2.6)	<1.7 (<1.7, 2.2)	0.41
n (%) sodium ≥13 mmol	4 (21)	13 (46)	0.08
Maternal blood			
Mean (SD) $CD4^+$ cells/μL	394 (208)	387 (193)	0.90
Mean (SD) $\log_{10}$ HIV RNA copies/mL	4.5 (0.9)	4.6 (0.8)	0.63
Mean (SD) $\log_{10}$ IL-7‡	1.1 (0.3)	0.9 (0.4)	0.17
Clinical factors			
Mean (SD) age in years	26.9 (4.2)	25.2 (4.4)	0.19
Parity, n (%)			0.04
Primipara	2 (11)	5 (18)	
1 to 2 previous live births	7 (37)	18 (64)	
3+ previous live births	10 (53)	5 (18)	
Mean (SD) birth weight (g)	3153 (381)	2944 (554)	0.16
n (%) birth weight <2500 g	1 (5)	4 (14)	0.63
Mean (SD) maternal BMI 1 mo postpartum	21.2 (3.0)	20.6 (3.0)	0.50

IQR indicates interquartile range.

*Real maize price >2000 ZMK per 15 kg from December 2001 to March 2003.[28]

†Data are available for 19 of 19 women with low IL-7 levels and for 27 of 28 women with normal IL-7 levels.

‡Data are available for 19 of 19 women with low IL-7 levels and for 24 of 28 women with normal IL-7 levels.

TABLE 4

Risk Factors for Postnatal HIV Transmission Through Breast-Feeding (47 Nontransmitters and 24 Transmitters)

	Unadjusted OR	95% CI	Adjusted OR*	95% CI
Undetectable or low (<30 pg/mL) breast milk IL-7	0.13	0.03 to 0.64	0.10	0.02 to 0.62
Breast milk viral load per $\log_{10}$ increase†	2.32	1.27 to 4.24	2.18	1.06 to 4.46
Blood $CD4^+$ T cells per 10-cell increase	0.93	0.89 to 0.97	0.92	0.88 to 0.97

*Adjusting for all 3 variables shown.

†Breast milk viral loads are available for 23 of 24 transmitters and for 46 of 47 nontransmitters.