Human Immunodeficiency Virus Load in Breast Milk, Mastitis, and Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1

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Human immunodeficiency virus (HIV) type 1 load in breast milk and mastitis were examined as risk factors for vertical transmission of HIV-1. Six weeks after delivery, HIV-1 load and sodium (an indicator of mastitis) were measured in breast milk from 334 HIV-1-infected women in Malawi. Median breast milk HIV-1 load was 700 copies/mL among women with HIV-1-infected infants versus undetectable (<200 copies/mL) among those with uninfected infants, respectively (P < .0001). Elevated breast milk sodium levels consistent with mastitis occurred in 16.4% of HIV-1-infected women and were associated with increased vertical transmission of HIV-1 (P < .0001). Median breast milk HIV-1 load was 920 copies/mL among women with versus undetectable among those without elevated breast milk sodium levels, respectively (P < .0001). Mastitis and breast milk HIV-1 load may increase the risk of vertical transmission of HIV-1 through breast-feeding.

An estimated half-million children are infected with human immunodeficiency virus (HIV) type 1 each year through mother-to-child transmission of HIV-1, and the vast majority of new cases occur in sub-Saharan Africa [1]. Although most transmission of HIV-1 occurs during pregnancy and at birth, breast-feeding may account for 5%-15% of infants becoming infected with HIV-1 after delivery [2-4]. In many developing countries where formula preparation is impeded by lack of clean water and proper hygiene, the nutritional and immunologic benefits of breast-feeding are considered to outweigh the risks of mother-to-child transmission of HIV-1. Breast-feeding may also be adopted by HIV-1-infected women who live in situations of limited privacy where formula-feeding could reveal their HIV status to the community. Interventions are urgently needed that could reduce mother-to-child transmission of HIV-1 among breast-feeding populations in developing countries [5].

The risk factors for mother-to-child transmission of HIV-1 in breast-feeding populations are unclear. Breast milk contains HIV-1 and immunologic factors that may protect the infant against becoming infected with HIV [6, 7]. Factors that might influence breast milk HIV-1 load and the relationship between breast milk HIV-1 load and mother-to-child transmission of HIV-1 are unknown. Mastitis is an inflammatory process in the breast in which paracellular pathways between mammary alveolar cells open up, allowing inflammatory cells and extracellular fluid to enter the milk [8-12]. The presence of plasma-derived components and inflammatory cells in breast milk during mastitis, such as HIV-1-infected lymphocytes, could raise HIV-1 load in breast milk and add to the risk of transmission of HIV-1 from mother to child. Normal mature breast milk contains ~5-6 mmol/L of sodium [13-15], whereas extracellular fluid has much higher sodium concentrations. Elevated sodium concentrations in breast milk are considered to be a sensitive indicator of mastitis [10, 12, 13]. Other situations in which breast milk sodium concentrations are elevated are in colostrum and at the termination of weaning [10].

Lactoferrin, an iron-binding glycoprotein with antimicrobial activity, also increases in breast milk during mastitis [16, 17].
We examined breast milk HIV-1 load and mastitis as risk factors for mother-to-child transmission of HIV-1 in a breast-feeding population in Blantyre, Malawi.

Materials and Methods

Study population. Pregnant women of 18-28 weeks' gestation were seen at the antenatal clinic of the Queen Elizabeth Central Hospital in Blantyre, Malawi, from November 1995 through December 1996. The Queen Elizabeth Central Hospital is the main hospital for Blantyre, a city of ~300,000 inhabitants. Women in the study were participants in a clinical trial to determine whether antenatal vitamin A supplementation would improve birth weight, reduce infant mortality, and lower mother-to-child transmission of HIV-1 [18, 19]. Results of this ongoing clinical trial will be reported when the trial has finished.

Study protocol. Pregnant women received instructions in prenatal care, AIDS education, HIV-1 testing, pre- and posttest HIV counseling, and counseling and testing for sexually transmitted diseases (STDs). During each visit to the antenatal clinic, participants received physical examination and were treated for STDs, malaria, and iron-deficiency anemia. A detailed questionnaire was administered seeking information on medical history, previous pregnancies, and level of education. Height and weight were recorded, and gestational age was estimated by using the last reported menstrual period. After delivery, women and their infants were seen in 6 weeks and then every 3 months for a year. Women continued to receive counseling and testing for STDs, physical examination, and treatment of STDs, malaria, and iron-deficiency anemia at each visit. Antibiotic treatment was given to women with clinically apparent mastitis. In Malawi, formula-feeding is not recommended by Ministry of Health guidelines, given the lack of access to clean water, hygiene problems, and issues regarding limited privacy and confidentiality. Thus, universal breast-feeding is encouraged for all women, regardless of HIV status.

Laboratory analysis. At enrollment, a blood sample was drawn by venipuncture and allowed to clot. Serum was separated and used for the presence of HIV-1 antibody by EIA (Wellcome; Wellcome Diagnostics, Dartford, UK, and Genetic Systems EIA, Seattle). Both EIAs had to be positive for a woman to be considered HIV-1-positive. Immunoblotting (Bio-Rad Laboratories, Hercules, CA) was used to confirm HIV-1 status in women with equivocal HIV-1 EIA test results. After follow-up involving HIV counseling, further AIDS education, and enrollment, a second blood sample was drawn by venipuncture, and plasma was separated immediately and frozen at ~70°C. Plasma HIV-1 load was measured by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) (AmpliCor Monitor; Roche, Branchberg, NJ), and these assays were run and validated in the AIDS Clinical Trials Group reference laboratory at Johns Hopkins Hospital (B. Jackson). A complete blood count was done by an automated cell counter (Coulter, Hialeah, FL). Percentage of CD4+ and CD8+ lymphocytes was determined by standard flow cytometry methods [20].

Six weeks after delivery, heelstick blood samples were obtained from the infants, and qualitative DNA PCR was used to detect HIV-1 DNA on a blood spot on filter paper [21]. PCR for HIV-1 DNA and validation studies were conducted at the virology laboratories of the National Cancer Institute, Fort Detrick, Maryland. A sample of milk was obtained from either breast by manual expression, and breast milk was immediately aliquoted and stored in a sample archive at -70°C until analysis for sodium, lactoferrin, and HIV-1 load in January 1998. Breast milk samples were centrifuged at 1300 g for 7 min, and the lipid and aqueous portions were separated and removed. The aqueous portion was analyzed for sodium concentration by use of ion-selective electrodes (Boehringer Mannheim/Hitachi 747 analyzer; Roche/Boehringer Mannheim, Indianapolis) in the Department of Pathology, Johns Hopkins Hospital. Breast milk sodium was defined as elevated at >12 mmol/L, because this concentration of sodium is >3 SD above the mean for normal human milk at 1-month lactation as determined by ion-selective electrodes on fat-free samples [13, 22].

We measured breast milk lactoferrin by sandwich ELISA on plates coated with rabbit anti-human lactoferrin (Organon Teknika, Durham, NC), dilutions of human breast milk, peroxidase-conjugated rabbit IgG to human lactoferrin (Organon Teknika), and substrate development with o-phenylenediamine dihydrochloride. Absorbances were read at 490 nm with wavelength correction at 630 nm. Purified human lactoferrin (Organon Teknika) was used as a standard to estimate the amount of breast milk lactoferrin.

Among HIV-1-infected women with breast milk sodium concentrations consistent with mastitis, breast milk HIV-1 load was measured in 34 women who had sufficient breast milk sample volume. Among HIV-1-infected women with normal breast milk sodium concentrations, breast milk HIV-1 load was obtained in a simple random sample of 100 women. We measured HIV-1 load using 400 μL of the aqueous portion of breast milk after centrifugation, as described above, by quantitative RT-PCR (Roche AmpliCor Monitor). The lower limit for detectability of HIV-1 RNA for the assay was 200 copies/mL. Previous experiments in which known quantities of HIV-1 RNA were added to the aqueous portion of breast milk demonstrated excellent correlation and the absence of inhibitors.

Statistical analysis. Comparisons between groups were made by use of Student's t test for continuous variables with a normal distribution. Plasma HIV-1 load (copies/milliliter) and breast milk lactoferrin (micrograms/milliliter) were transformed by log, to achieve a normal distribution. The Wilcoxon rank sum test was used for nonparametric comparison between groups of variables that could not be transformed into a normal distribution, such as breast milk HIV-1 load. x² tests or Fisher’s exact tests were used to compare categorical variables between groups. Spearman correlation was used to examine the relationship between breast milk HIV-1 load and plasma HIV-1 load. Multivariate logistic regression models were used to examine the relationship between maternal risk factors and mother-to-child transmission of HIV-1 and to examine the relationship between maternal risk factors and HIV-1 load in breast milk.

Results

Mother-to-child transmission of HIV-1. In total, 900 women enrolled in the study in the antenatal period (697 HIV-1-positive and 203 HIV-1-negative). At 6 weeks after delivery, a sufficient sample of breast milk was obtained from 430 lactating women.
women (334 HIV-1-positive, 96 HIV-1-negative) for analysis of sodium and lactoferrin concentrations. The overall mother-to-child transmission of HIV-1 was 26.8% at age 6 weeks among 328 of the 334 woman-infant pairs who had infant PCR results available. Six infants were missing heelstick blood samples at age 6 weeks. PCR identified significant differences in maternal plasma HIV-1 load, CD4/CD8 ratio, and breast milk lactoferrin between women with HIV-1-infected versus -uninfected infants at age 6 weeks (table 1). The cumulative mother-to-child transmission rate of HIV-1 was 30.2% at 12 months of age. A comparison of maternal characteristics between mothers with HIV-1-infected versus uninfected infants at age 12 months was similar to the results in table 1 (data not shown). Vitamin A supplementation had no significant effect on mother-to-child transmission of HIV-1 at 6 weeks and 12 months, breast milk HIV-1 load, or breast milk lactoferrin levels.

**Breast milk HIV-1 load.** Breast milk HIV-1 load in the subsample of 134 women ranged from undetectable (<200 copies/mL) to 40,627 copies/mL. Spearman correlation between breast milk HIV-1 load and plasma HIV-1 load was 0.47 (P < .001). The subsample of 134 HIV-1-infected women who had breast milk HIV-1 load measured did not differ from the remaining 200 HIV-1-infected women by age, plasma HIV-1 load, CD4+ lymphocyte count, body mass index, or other variables (data not shown). Median breast milk HIV-1 load was 700 copies/mL among women with HIV-1-infected infants, compared with undetectable (<200 copies/mL) among women with HIV-1-negative infants at 6 weeks of age (P < .0001; table 1). In the subsample of 134 women, a multivariate logistic regression model was fitted to examine maternal plasma HIV-1 load and mastitis as predictors of detectable HIV-1 in breast milk (≥ and <200 copies/mL). Both maternal plasma HIV-1 load (odds ratio [OR], 8.25; 95% confidence interval [CI], 3.41–19.9; P < .0001) and mastitis (OR, 9.69; 95% CI, 3.49–26.80; P < .0001) were predictors of detectable breast milk HIV-1 load.

**Breast milk sodium concentrations.** Fifty-five (16.4%) of 334 HIV-1-positive women and 15 (15.6%) of 96 HIV-1-negative women had elevated sodium (>12 mmol/L) consistent with mastitis 6 weeks after delivery (P = .84). The char-

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**Table 1.** Characteristics of mothers with human immunodeficiency virus (HIV)-infected and HIV-negative infants at 6 weeks.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-positive infant (n = 88)</th>
<th>HIV-negative infant (n = 240)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.7 (0.5)</td>
<td>24.4 (0.3)</td>
<td>.33</td>
</tr>
<tr>
<td>Plasma log HIV load</td>
<td>4.61 (0.07)</td>
<td>4.25 (0.04)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4 cells/μL</td>
<td>397 (27)</td>
<td>457 (16)</td>
<td>0.07</td>
</tr>
<tr>
<td>CD8 cells/μL</td>
<td>959 (69)</td>
<td>830 (28)</td>
<td>0.09</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.45 (0.02)</td>
<td>0.61 (0.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal body mass index (wt/ht')b</td>
<td>22.0 (0.2)</td>
<td>22.4 (0.1)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median breast milk HIV load (copies/mL)c</td>
<td>700</td>
<td>&lt;200f</td>
<td>0.001</td>
</tr>
<tr>
<td>Undetectable breast milk HIV load (%)</td>
<td>34.2</td>
<td>73.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Log breast milk lactoferrin</td>
<td>2.90 (0.02)</td>
<td>2.82 (0.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis (% with breast milk Na &gt;12 mmol/L)</td>
<td>28.4</td>
<td>12.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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**Table 2.** Characteristics of human immunodeficiency virus (HIV)-infected women with and without elevated breast milk sodium and their infants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Elevated sodiuma (n = 55)</th>
<th>Normal sodiuma (n = 279)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>23.8 (0.6)</td>
<td>24.3 (0.3)</td>
<td>.44</td>
</tr>
<tr>
<td>Maternal plasma log HIV load</td>
<td>4.61 (0.07)</td>
<td>4.30 (0.05)</td>
<td>.007</td>
</tr>
<tr>
<td>Maternal CD4 cells/μL</td>
<td>376 (31)</td>
<td>450 (16)</td>
<td>.05</td>
</tr>
<tr>
<td>Maternal CD8 cells/μL</td>
<td>885 (83)</td>
<td>864 (29)</td>
<td>.81</td>
</tr>
<tr>
<td>Maternal CD4/CD8 ratio</td>
<td>0.51 (0.04)</td>
<td>0.57 (0.02)</td>
<td>.26</td>
</tr>
<tr>
<td>Maternal body mass index (wt/ht')b</td>
<td>22.4 (0.2)</td>
<td>22.3 (0.1)</td>
<td>.61</td>
</tr>
<tr>
<td>Median breast milk HIV load (copies/mL)c</td>
<td>920</td>
<td>&lt;200f</td>
<td>0.001</td>
</tr>
<tr>
<td>Undetectable breast milk HIV load (%)</td>
<td>26.5</td>
<td>75.0</td>
<td>.0001</td>
</tr>
<tr>
<td>Log breast milk lactoferrin</td>
<td>3.00 (0.03)</td>
<td>2.81 (0.01)</td>
<td>.0001</td>
</tr>
<tr>
<td>HIV-infected infants, 6 weeks</td>
<td>2555 (45.4%)</td>
<td>63273 (23.0%)</td>
<td>.001</td>
</tr>
<tr>
<td>HIV-infected infants, 12 months, cumulative</td>
<td>2855 (50.9%)</td>
<td>71273 (26.1%)</td>
<td>.0001</td>
</tr>
<tr>
<td>Infant HIV negative at 6 weeks and HIV-infected at 12 months by PCR</td>
<td>3/20 (15.0%)</td>
<td>7/167 (4.1%)</td>
<td>.08</td>
</tr>
</tbody>
</table>

NOTE. For continuous variables, data are mean (SE).

a Elevated breast milk sodium level >12 mmol/L consistent with mastitis

b Body mass index at enrollment (18–28 weeks' gestation).

c Breast milk HIV load measured in subsample of 134 subjects.

d Not detected by RNA polymerase chain reaction (<200 copies/mL).
Risk factors for mother-to-child transmission of HIV-1. Univariate logistic regression models were fitted among the 328 HIV-1-positive women to examine the relationship between maternal factors and mother-to-child transmission of HIV-1 at 6 weeks (table 3) and 12 months of age (table 4). Elevated breast milk sodium concentration, maternal plasma HIV-1 load, and breast milk lactoferrin level were associated with significantly elevated risk of mother-to-child transmission of HIV-1 by 6 weeks and 12 months of age. In a multivariate logistic regression analysis, maternal plasma HIV-1 load and elevated breast milk sodium were independently associated with significantly increased risk for mother-to-child transmission of HIV-1 to 6 weeks of age (table 3).

In the subsample of 134 women who had breast milk HIV-1 load measurements, multivariate logistic regression models were fitted to examine plasma and breast milk HIV-1 load as risk factors for mother-to-child transmission of HIV-1. Both plasma HIV-1 load (OR, 3.53; 95% CI, 1.57–7.87; P < .003) and detectable breast milk HIV-1 load (OR, 2.97; 95% CI, 1.23–7.18; P < .016) were independently associated with mother-to-child transmission of HIV-1 to 6 weeks of age. Both plasma HIV-1 load (OR, 3.44; 95% CI, 1.56–7.49; P < .002) and detectable breast milk HIV-1 load (OR, 2.97; 95% CI, 1.25–7.04; P < .01) were also associated with mother-to-child transmission of HIV-1 to 12 months of age.

Discussion

To our knowledge, this is the first study to demonstrate an association between breast milk HIV-1 load and mother-to-child transmission of HIV-1. Both plasma HIV-1 load and breast milk HIV-1 load were predictors of mother-to-child transmission of HIV-1 in univariate and multivariate analyses. Mother-to-child transmission can occur in utero, during delivery, and during breast-feeding [23], and this study is limited in that it cannot be precisely known when HIV-1 transmission occurred in these HIV-1-infected infants. Therefore, it cannot be directly inferred from this study that high breast milk HIV-1 load is associated solely with transmission of HIV-1 through breast milk.

Mastitis, as indicated by elevated breast milk sodium concentrations, was fairly common, occurring among ∼16% of HIV-1-positive and ∼16% of HIV-1-negative lactating women 6 weeks postpartum in Malawi. These data are consistent with recent epidemiologic studies that suggest that clinically apparent mastitis occurs in 20%–33% of women some time during lactation [24–27]. One-third of women who breast-feed long-term may develop mastitis [24]. A recent prospective cohort study of 306 breast-feeding women showed that 27.1% developed clinically apparent mastitis within 3 months of delivery among women with normal breast milk sodium levels (P < .001; figure 1).
HIV Load in Breast Milk and Transmission

Table 3. Univariate and multivariate models for mother-to-child transmission of human immunodeficiency virus (HIV) at 6 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate OR (95% CI), P</th>
<th>Multivariate OR (95% CI), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastitis (breast milk Na &gt;12 mmol/L)</td>
<td>2.77 (1.52-5.04), &lt;.0009</td>
<td>2.06 (1.41-2.98), &lt;.0002</td>
</tr>
<tr>
<td>Maternal plasma log HIV load</td>
<td>2.06 (1.41-2.98), &lt;.0002</td>
<td>0.12 (0.04-0.37), &lt;.008</td>
</tr>
<tr>
<td>Maternal CD4/CD8 ratio</td>
<td>5.46 (2.43-12.09), &lt;.0001</td>
<td>3.92 (1.28-11.82), &lt;.02</td>
</tr>
<tr>
<td>Detectable breast milk HIV load*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log breast milk lactoferrin*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; OR, odds ratio.
* Breast milk HIV load measured on subsample of 134 subjects. Breast milk HIV load by RNA polymerase chain reaction was undetectable at <200 copies/mL. Breast milk HIV load not included in final model because of colinearity with mastitis and limited size of subsample (n = 134).

transmission of HIV-1, given that mastitis is associated with an opening of paracellular pathways with an increase in sodium, inflammatory mediators, and inflammatory cells, such as neutrophils and lymphocytes, and plasma-derived components that could contain HIV-1 [16, 17, 29]. This is borne out in the observation that elevated breast milk sodium concentrations were associated with higher HIV-1 load in breast milk. It seems unlikely that the presence of HIV-1 itself caused mastitis, since in this study ~16% of both HIV-1-infected and HIV-1-negative women had mastitis.

Although breast milk HIV load seems low compared with plasma HIV load, oral exposures of the infant to HIV may reach high levels through breast-feeding during mastitis. Median breast milk HIV load in women with mastitis was 920 copies/mL. Infants may consume >700 mL of breast milk per day [8], and if we assume that an infant consumes 350 mL of breast milk from each breast, the oral exposure to HIV from breast milk would be 322,000 copies of HIV RNA per day from a breast with mastitis (350 mL/day x 920 copies/mL). The breast milk HIV load in this study was >20,000 copies/mL for some mothers, which could result in an oral exposure to HIV in breast milk >7 million copies per day. High levels of breast milk HIV load may represent a substantial exposure even if only a small proportion of these virus particles are infectious virions [6]. Although there are immunologic factors in breast

[26]. Most cases of clinically apparent mastitis occur within the first several weeks after delivery [26-28]. In The Gambia, Prentice et al. [16] observed a mean monthly incidence of clinically apparent mastitis of 2.6% among lactating women seen in a community health clinic, and repeat episodes of mastitis were common.

In this study, elevated breast milk sodium concentrations were used to make a laboratory a posteriori diagnosis of mastitis, and it may be possible that this method of detection would include women with subclinical or chronic mastitis. A limitation of this study is that it was not possible to make the clinical correlation of elevated breast milk sodium concentrations with clinically apparent mastitis; however, correlations between mastitis and breast milk sodium concentrations have been made in other clinical studies [12, 16]. Clinically apparent mastitis in industrialized countries is mostly due to Staphylococcus aureus, isolated from the breast milk of ~40%–50% of women [28-30]. Further investigation is needed to characterize the microbiology and treatment of mastitis in developing countries.

A striking finding was that women with elevated breast milk sodium concentrations consistent with mastitis had almost twice the rate of mother-to-child transmission of HIV-1 at both 6 weeks and 12 months compared with women who had normal breast milk sodium concentrations. It is biologically plausible that mastitis would be associated with higher mother-to-child transmission of HIV-1, given that mastitis is associated with an opening of paracellular pathways with an increase in sodium, inflammatory mediators, and inflammatory cells, such as neutrophils and lymphocytes, and plasma-derived components that could contain HIV-1 [16, 17, 29]. This is borne out in the observation that elevated breast milk sodium concentrations were associated with higher HIV-1 load in breast milk. It seems unlikely that the presence of HIV-1 itself caused mastitis, since in this study ~16% of both HIV-1-infected and HIV-1-negative women had mastitis.

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milk that may protect the infant from HIV infection [7], an extremely high number of virions may possibly overwhelm these immunological mechanisms.

This study suggests that mastitis, a condition with generally low morbidity that can be treated with antibiotics, might possibly have serious consequences for infants in the setting of HIV infection. The issue of breast- versus formula-feeding for HIV-infected women remains a major issue of debate, especially in Africa. There are settings in many developing countries, such as Malawi, in which breast-feeding may be most appropriate and safe for HIV-infected women and their infants, given lack of potable water, poor hygiene, and lack of privacy in housing [31]. Mastitis is usually unilateral, and whether the contralateral, unaffected breast has lower breast milk HIV load than the affected breast is not yet known. Further studies are needed to confirm these observations and to elucidate the relationship between breast milk HIV load, mastitis, and transmission of HIV through breast-feeding.

Acknowledgments

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References