Veterinary Field Test as Screening Tool for Mastitis and HIV-1 Viral Load in Breastmilk from HIV-Infected Zambian Women

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Abstract

Clinical and subclinical mastitis increase the risk of mother-to-child transmission (MTCT) of HIV-1 through breastfeeding. We hypothesized that a field test for mastitis used for bovine milk, the California Mastitis Test, would detect high cell counts in milk of HIV-infected women. We also investigated whether total milk cell count would positively correlate with viral HIV-1 RNA in the milk of 128 HIV-positive Zambian women. Mean cell counts in each California Mastitis Test scoring category were significantly different ($p < 0.01$, $n = 232$). In a subset of 4-month postpartum milk samples tested for HIV-1 RNA, viral RNA levels did not significantly correlate with total cell count ($r = 0.166$, $p = 244$). The CMT may serve as a screening tool for mastitis in breastmilk, but total cell count does not correlate with HIV-1 RNA levels. Since both cell-free and cell-associated virus are associated with increased risk of MTCT, investigation of the relationship between total milk cell count and HIV-1 proviral DNA is warranted before a conclusive determination is made regarding use of the CMT as a clinical screening tool to detect cases at high risk for breastmilk transmission.

INTRODUCTION

THE HUMAN IMMUNODEFICIENCY (HIV) pandemic presents a dilemma to HIV-infected mothers in developing countries. In resource-limited settings, breastfeeding significantly reduces the risk of death due to diarrhea.[1] However, breastmilk transmission of HIV-1 can account for up to 42% of all mother-to-child transmission (MTCT), depending on the duration of breastfeeding.[2]

Both cell-free[3,4] and cell-associated[5,6] virus contribute to MTCT. In addition, both clinical[4,7] and subclinical[8] mastitis are associated with increased transmission, presumably due to increased breast milk HIV-1 viral load originating from plasma. While clinical mastitis is apparent to a breastfeeding mother, a rapid, easy-to-use, and inexpensive screening test for sub-clinical mastitis could prove useful in both research and clinical settings.

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We hypothesized that the field-based veterinary California Mastitis Test (CMT), developed for use with dairy herds, would also be reliable in estimating breastmilk total cell counts as determined by the direct microscopic somatic cell count (DMSCC). In addition, we anticipated that the total cell counts in human milk would correlate with breastmilk HIV-1 RNA levels in milk from HIV-positive lactating women.

METHODS

Study design and subjects

This pilot study used breastmilk samples from the Zambia Exclusive Breastfeeding Study (ZEBS), in Lusaka, Zambia, which evaluated 4 months of exclusive breastfeeding as a strategy to reduce postnatal HIV transmission and minimize infant mortality. Detailed methodology of the ZEBS study is reported elsewhere.[9]

Data presented here are from breastmilk samples obtained from women who visited the clinic from January 2004 through March 2004. The time frame was chosen as a result of both the financial and logistical limitations of conducting this pilot study. Samples were obtained from each breast and processed separately. Amounts of milk greater than the 5 mL required by the main ZEBS study were made available for this substudy.

The ZEBS trial received ethical approval from the Institutional Review Board (IRB) of Boston University, Columbia University, University of Alabama, Birmingham, University of Southern California and the University of Zambia, Lusaka. Ethical approval for this substudy was obtained from the Boston University and the Tufts University–New England Medical Center Institutional Review Boards.

The CMT and DMSCC were assessed on all 232 samples. Financial limitations of the pilot study constrained the ability to measure HIV RNA to 4-month postpartum samples.

California Mastitis Test (CMT)

The CMT (TechniVet, Brunswick, ME) was used and scored as described elsewhere.[10–12] Briefly, whole milk samples were mixed in a 1:1 ratio with the CMT reagent (2 mL each). The mixture was swirled for 10 seconds in a shallow white container provided by the manufacturer, and assessed and scored as Negative, Trace, 1, 2, or 3, according to the degree of agglutination.

DMSCC

We used the method for somatic cell slide preparation, staining, and cell counting as described for goat milk.[12–14] In addition, in order to ensure adherence of lipid-rich milk to glass slides during staining, 10 μL of 5% chicken egg albumin (Sigma-Aldrich, St. Louis, MO) in isotonic saline was applied and heat-fixed to each 1 cm^2 circular area on the cell count slides (Bellco Glass, Vineland, NJ) prior to applying milk samples. Cells were counted and calculated using the field-wide single strip method.[15]

HIV-1 RNA copies

Samples of milk supernatant and lipid together were used to quantify HIV-1 viral RNA copies using the Amplicor HIV-1 MONITOR Test, Version 1.5 (Roche Molecular Systems, Inc., Branchburg, NJ) ultrasensitive assay, which has a lower limit of detection of 50 copies/mL.

Statistical analyses

Data was analyzed using SPSS version 11.5 (SPSS, Chicago, IL). Cell count and viral load data from breastmilk samples were log-transformed to normalize distribution. For those women who visited the clinic twice during the substudy collection period, only samples from the first visit were included in the analyses. Student's *t*-test for independent samples was used to make pair-wise comparisons of the $\log_{10}$ mean cell count by CMT score. Pearson's correlation coefficient was used to analyze the relationship between cell count and HIV-1 RNA.

RESULTS

Two hundred thirty-two breastmilk samples were obtained from 128 HIV-positive women (from both breasts in 104 women and from a single side in 24 women). The median age of mothers was 26 years (SD 5.14, range 16–41), median parity was two births (SD 1.63, range 0–7), median plasma CD4+ T-cell count was 346 cells/mm^3 (SD 193, range 48–1002), and median baseline plasma viral load at enrollment was 27,355 copies/mL (SD 143,112; range 399–750,001).

Cell counts were performed by DMSCC on all 232 samples. The mean cell counts between adjacent CMT categories were found to be significantly different (Table 1).

No correlation was found between total milk cell count and milk HIV-1 RNA load in the 4-month postpartum samples ($r = 0.166$, $p = .244$, $n = 51$; 24 pairs, three single sides). Viral load results for one pair of the 53 4-month samples were invalid on multiple test runs, most likely due to components in the milk, which made the samples inhibitory to amplification.

DISCUSSION

This study demonstrates that the CMT is a reliable means of qualitatively estimating the total cell count in human breast milk. The CMT is not intended to provide an exact measure of mammary permeability, but rather to serve as a screening tool to indicate a cellular response to inflammation and whether further testing is necessary.

In 4-month postpartum samples, total cell count did not significantly correlate with HIV-1 RNA, perhaps because of the small subset chosen for HIV-1 RNA analysis. In addition, the source of HIV-1 RNA in breastmilk could be twofold: directly from plasma, as well as from HIV replication within a subset of HIV-infected breastmilk cells.

In vitro studies show that cell-associated viral transcytosis across intestinal epithelium to underlying CD4+ T-cells is more efficient than that of cell-free virus.[16] If future studies

demonstrate a correlation between breastmilk total cell count and cell-associated HIV-1, the CMT could be promoted as a useful and inexpensive screening tool for both subclinical mastitis and levels of breastmilk proviral HIV-1 DNA in resource-constrained settings.

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Table 1.

California Mastitis Test (CMT) Score and Mean Breastmilk Total Cell Counts by Direct Microscopic Somatic Cell Count (DMSCC)

CMT score	Number of breastmilk samples	Log$_{10}$ mean total cell count per mL[a]
Negative	68	4.04
Trace	100	4.61
1	45	5.28
2	17	6.26
3	2	7.44

[a]All means significantly different by pairwise analysis of adjacent CMT scores ($p < 0.0001$ except CMT scores 2 and 3, p 0.004).