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Human Milk Galectin-3 Binding Protein and Breastfeeding-Associated HIV Transmission

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Abstract

Analysis of milk from 247 HIV-infected Zambian mothers showed that Galectin-3 Binding Protein (Gal3BP) concentrations were significantly higher among HIV-infected mothers who transmitted HIV through breastfeeding (6.51±2.12 ug/mL) than among non-transmitters but were also correlated with higher milk and plasma HIV RNA copies/ml and lower CD4+ cell counts. The association between Gal3BP and postnatal transmission was attenuated after adjustment for milk and plasma HIV load and CD4+ cell counts. This suggests that although milk Gal3BP is a marker of advanced maternal disease, it does not independently modify transmission risk.

Keywords

HIV transmission; breastfeeding; Galectin-3 Binding Protein; oral transmission

Infants who are breast-fed by their HIV-infected mothers are at risk of acquiring HIV infection through their mother's milk but ~85% do not acquire post-partum HIV infection despite on-going exposure to the virus in milk over many months [1]. Mother-to-child transmission of HIV via breastfeeding provides a unique *in vivo* model system to study associations between components of human milk and HIV transmission risk [2]. One of the

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potentially immunomodulatory milk components is Galectin-3 binding protein (Gal3BP), a glycoprotein that has been implicated in macrophage activation, cell signaling and cell migration [3]. Gal3BP concentrations in mature human milk are comparable to corresponding maternal serum concentrations, but are 8-10-fold higher in colostrum [4]. Gal3BP in human milk has been postulated as one of the potential mechanisms that may explain benefits of breastfeeding in protection against gastrointestinal and acute respiratory infections [5]. Here, we analyzed Gal3BP concentrations in milk samples collected as part of a unique study cohort of HIV-infected women and their infants in Lusaka, Zambia, to determine whether Gal3BP is associated with HIV transmission risk via breastfeeding [6].

Methods

A nested case-control study was conducted within the context of a randomized trial to test the safety and efficacy of abrupt cessation of breastfeeding at 4 months on postnatal HIV transmission and child mortality (Clinical trials.gov NCT00310726) [6]. In brief, 958 HIV-infected pregnant women were recruited at two antenatal clinics in Lusaka, Zambia between May 2001 and September 2004 and followed with their infants to 24 months post-partum. Women received single-dose nevirapine as prophylaxis to prevent transmission to the child. Uninfected breastfeeding women and their infants were recruited in parallel to serve as controls. All women provided written informed consent for their participation and all the investigators' Institutional Review Boards approved the study.

Blood samples were collected from pregnant women at the time of enrollment and tested for CD4 and CD8 cell counts (FACSCount system, BD Immunocytometry Systems, San Jose, CA), hemoglobin (Hemocue® system, Lake Forest, CA) and plasma viral load (Roche Amplicor® 1.5, Branchburg, NJ). Heel stick blood samples were collected from all infants at birth, monthly to 6 months and every 3 months to 24 months of age. DNA was extracted from each infant's blood sample and tested for HIV by real-time polymerase chain reaction (PCR). Transmission was assumed to have occurred through breastfeeding if the first positive PCR result was obtained after 6 weeks of age. The median of the estimated age of postnatal infection was 4 ½ months. Breast milk samples were collected by manual expression, kept cold until processed within 4 hours, centrifuged and the aqueous portion stored at -80°C until further analysis. After storage at -80°C, breast milk samples were thawed to room temperature, heat-inactivated, and Gal3BP concentrations determined by ELISA (eBioscience Platinum ELISA). HIV-1 RNA in breast milk (viral load) was measured with the Amplicor Ultrasensitive HIV-1 1.5 kit (Roche Molecular Systems, Inc., Branchburg, NJ).

We selected all available one-month milk samples from women who transmitted HIV to their infants through breastfeeding (n=77) as well as a sample of approximately twice as many HIV-infected women who did not transmit despite breastfeeding (n=134) and a group of HIV-uninfected breastfeeding women as uninfected controls (n=34). We selected one-month samples as they would be collected prior to postnatal HIV acquisition for all infants and are from a period when lactation is fully established.

Continuous and categorical variables were compared by t-tests or nonparametric Wilcoxon tests and chi-squared statistics, respectively. Logistic regression was conducted and unadjusted and adjusted odds ratios (OR) were reported with 95% confidence intervals. Correlations were described using Spearman's correlation coefficients. All statistical analyses were performed using SAS (version 9.2).

Results

Mean breast milk Gal3BP concentrations were similar between HIV-infected mothers who did not transmit (5.4 ± 2.23 ug/mL) and HIV-uninfected control mothers (4.94 ± 1.33 ug/mL) ($p=0.13$). However, Gal3BP concentrations were significantly higher among HIV-infected mothers who transmitted HIV through breastfeeding (6.51 ± 2.12 ug/mL) than among non-transmitters ($p=0.0005$) (Table 1).

In univariable analysis, for each ug/mL increase in Gal3BP the odds of postnatal HIV transmission increased by 26% (Odds Ratio [OR]=1.26, 95% CI: 1.10, 1.44). A Gal3BP concentration above the median (5.26 ug/mL) was associated with a 2.5-fold increased (95% CI: 1.37, 4.55) risk of postpartum HIV transmission. A dose-response relationship was also observed. Gal3BP concentrations above the 75th percentile (6.31 ug/mL) were strongly associated with an increased risk of transmission relative to <25th percentile (<4.15 ug/mL) [OR=4.37; 95% CI: 1.77, 10.78].

Higher breast milk Gal3BP concentrations were significantly associated with higher breast milk HIV RNA copies/ml (Spearman's $r=0.44$, $p<0.0001$), higher maternal plasma HIV RNA copies/ml (Spearman's $r=0.28$, $p<0.0001$) and lower CD4⁺ cell counts (Spearman's $r=-0.43$, $p<0.0001$). Breast milk and maternal plasma HIV-1 RNA concentrations were predictors of postnatal transmission along with maternal CD4⁺ cell counts in univariable analysis (Table 1). After adjustment for these known risk factors for HIV transmission, the association between milk Gal3BP concentration and transmission was attenuated (OR= 1.05, 95% CI: 0.88, 1.24) (Table 2).

Discussion

The analyzed milk samples stem from a unique cohort of mother-infant dyads that were recruited and followed prior to the availability of effective antiretroviral (ARV) therapy [6], providing a unique opportunity to investigate mucosal correlates of postnatal HIV infection independent of ARV [2]. Our data demonstrated that higher Gal3BP concentrations in breast milk were associated with higher concentrations of HIV-1 RNA in breast milk and plasma as well as with lower CD4 counts. Our data are in line with previous reports in HIV-infected adults and children, where Gal3BP serum level is suggested as an important marker of disease progression [7,8] and correlates with lower CD4⁺ cell counts and higher plasma viral load [9-10]. This association with markers of disease progression in the mother explained the association we observed between *higher* Gal3BP concentrations and *increased* risk of postnatal HIV transmission. We infer that Gal3BP in breast milk is a marker of advanced maternal disease and may be modulated by progression of maternal HIV infection but its presence in milk does not appear to independently modify the risk of HIV transmission. Our conclusions are tempered by several limitations of our study, including testing milk samples only at a single time point, neglect of developmental changes in milk composition and failure to take into consideration other potential immunomodulatory components of human milk. More research on the role of mucosal correlates of postnatal HIV-1 transmission via breastfeeding is needed.

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