Association between Breast Milk Erythropoietin and Reduced Risk of Mother#to#Child Transmission of HIV

Published in final edited form as:
J Infect Dis. 2010 August 15; 202(3): 370–373. doi:10.1086/653706.

Breast milk erythropoietin is associated with reduced risk of mother-to-child transmission of HIV

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Abstract

We examined the prospective associations between breast milk concentrations of erythropoietin, a factor with trophic effects on infant gut epithelia, and risk of MTCT through breastfeeding in a study of 59 MTCT cases and 116 controls nested within a cohort of antiretroviral-naïve HIV-infected Tanzanian women. Controls were matched to cases on the time from birth when the breast milk sample was collected. The risk of MTCT was inversely related to breast milk EPO concentration (adjusted OR for highest vs. lowest EPO tertiles=0.34; 95% CI=0.14, 0.82; P for trend=0.02). These results suggest a protective effect of breast milk EPO against MTCT.

Keywords

Erythropoietin; mother-to-child transmission; HIV; breast milk

Introduction

In the absence of interventions, transmission of HIV occurs through breastfeeding in about 5–20% of children born to HIV-positive women; it accounts for an estimated one-third to one-half of all HIV infections in African children [1]. Although avoiding breastfeeding would eliminate this risk, replacement feeding during the first 6 months is not recommended in developing countries where cost is prohibitive and lack of sanitary conditions for preparation of replacement foods introduce risks for other infections. Not all infants who are breastfed by HIV-infected mothers become infected; thus, identifying potential factors in breast milk that are protective against mother-to-child transmission (MTCT) is an important area of research to prevent MTCT in resource-poor settings.

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None of the authors have commercial or other associations that might pose a conflict of interest in relation to this paper.

It has been hypothesized that erythropoietin (EPO) in human milk may be protective against MTCT [2]. EPO is a protein secreted by the kidney that stimulates red blood cell production and that is found in breast milk [3]. The cytoprotective properties of EPO [4] might promote the integrity of the mammary epithelium which is necessary to prevent leakage of HIV particles from blood into milk. Mammary gland leakiness associated with subclinical breast inflammation is a risk factor for MTCT [5,6]. In addition, EPO may promote gut integrity in infants, as premature infants given recombinant human EPO had a lower incidence of necrotizing enterocolitis [7].

Using a nested case-control study design, we prospectively examined the association between breast milk EPO concentrations and the risk of MTCT among infants born to HIV-infected women in Tanzania.

Methods

Study Design

We conducted a case-control study nested in a cohort of 1078 HIV-1-infected Tanzanian women who participated in a randomized, double-blind, placebo-controlled trial of vitamins. Women were recruited during pregnancy and followed-up with their infants throughout the lactation period [8]. Baseline information was obtained on sociodemographic characteristics, clinical and immunological HIV disease stage, and potential risk factors for transmission of HIV. Vertical transmission of HIV was determined by testing blood samples collected from the children at birth, 6 weeks of age, and every 3 months thereafter by use of the enzyme-linked immunosorbent assay (ELISA) and/or Western blot analysis, or polymerase chain reaction (PCR) in children younger than 18 months (Amplicor HIV-1 DNA assay version 1.5, Roche Diagnostics, Branchburg, NJ). Children who tested HIV-negative by 6 weeks and positive thereafter were presumed to have been infected via breastfeeding. About 10 mL of breast milk were collected by manual expression from either breast approximately every 3 months, and stored at −70° C.

Cases were women who transmitted HIV-1 to their infants through breast milk (n=61). We aimed to select samples that had been collected before the child's first HIV-positive test, or at the same time as the first HIV-positive test when an earlier sample was not available. Two cases did not have a suitable sample and so the final sample size was 59 cases. In 27 cases, a sample was available between 4 and 24 weeks before the first positive test. In the remaining 32 cases, the only breast milk sample available was >24 weeks before the first positive test (n=22), at the same time as the child's first positive test (n=8), or within the 4 weeks prior to the first positive test (n=2). We selected two controls per case from the pool of mothers whose child's final HIV status was negative after the end of breastfeeding. Controls were individually matched to cases on the time since delivery at which the breast milk sample was collected ± 1 week. For three cases, control samples were only available within ± 2 weeks, and in five cases within 3–4 weeks. Two of the control samples had insufficient volume for EPO analyses and suitable replacements could not be found; thus, there were two case-control sets with only one control per case and the total number of controls was 116.

The study was approved by the Institutional Review Board of the Harvard School of Public Health and the Research and Publications Committee of Muhimbili University of Health and Allied Sciences.

Laboratory Procedures

Erythropoietin was quantified in whole milk samples by ELISA (Human Erythropoietin Quantikine IVD, R & D Systems, Minneapolis, MN). One aliquot of milk was centrifuged and separated into aqueous and cell fractions prior to freezing. HIV-1 viral RNA was

isolated from the cell-free aqueous milk fraction using the High Pure Viral RNA Kit (Roche Diagnostics, Indianapolis, IN). Cell-free viral load was quantified using the ultrasensitive protocol of the Amplicor HIV-1 Monitor Test, v 1.5 (Roche Diagnostics), as detailed previously [9].

Statistical Analyses

We compared baseline characteristics of cases and controls with the Wilcoxon matched-pairs rank-sum test for continuous variables and McNemar test for categorical variables. The association between breast milk erythropoietin concentrations and transmission of HIV was examined by fitting conditional logistic regression models with case-control status as the outcome and ordinal categories representing tertiles of EPO as the exposure. Adjusted odds ratios and 95% CI were estimated from models that included risk factors for MTCT such as maternal CD4 cell counts, clinical stage of HIV disease at baseline, and assignment to the vitamin A supplementation arm of the trial [8,10]. To test for dose-response associations between the concentration of EPO in breast milk and MTCT, we introduced an ordinal variable representing the tertiles as continuous into the regression models and tested its statistical significance with the use of the Wald test. To examine whether relations between EPO and MTCT could be mediated by changes in breast milk viral load, we evaluated cross-sectional associations between EPO and cell-free virus concentrations in milk, using cases and controls as a single sample. High cell-free virus concentrations in breast milk were defined dichotomously, using the median among the cases as the cutoff point [9]. The proportions of women with high cell-free virus were compared across EPO tertiles with use of the Cochran-Armitage test for trend.

Results

Maternal age, body mass index, and hemoglobin concentration did not differ between cases and controls (Table 1). As expected, cases were more likely to have lower CD4 cell counts, more advanced disease stage, and higher cell-free viral load in milk than controls.

Median EPO concentrations were 0.8 mIU/mL in cases and 4.1 mIU/mL in controls (p=0.03). Forty-seven percent of cases and 35% of controls had EPO concentrations below the detection limit of 0.6 mIU/mL. The risk of MTCT was inversely related to EPO concentration (Table 2). After adjusting for potential confounders, women with EPO concentrations in the highest tertile had a 66% reduced risk of transmitting HIV to their children compared to those in the lowest tertile (p, test for trend=0.02). After additional adjustment for breast milk viral load, the odds ratio for MTCT between extreme tertiles of EPO was attenuated to 0.45 (95% CI=0.17, 1.22), possibly suggesting that viral load might be in the causal pathway between EPO and MTCT. Nevertheless, the attenuation was mild and the proportion of women with high cell-free viral load was not significantly different across tertiles of EPO (data not shown).

Discussion

In this nested case-control study, breastfeeding transmission of HIV was inversely related to breast milk erythropoietin concentrations. While we are unable to attribute causality to the findings given the observational nature of the study, the results are suggestive of a protective effect of EPO against MTCT.

It has been hypothesized that erythropoietin in breast milk may prevent MTCT due to its potential effects on the integrity of mammary or infant intestinal epithelia [2]. In newborn rats, recombinant human EPO administration increased small bowel length and villus surface area [11], inhibited apoptosis, and stimulated intestinal cell proliferation [12].

Supplementation of premature infants with recombinant human EPO decreased the incidence of necrotizing enterocolitis [7]. Endocytes lining the mammary gland are similar morphologically to intestinal enterocytes, and may similarly be affected by EPO. Human mammary epithelial cells express EPO and its receptor [3], indicating mammary production of EPO and suggesting that EPO may have specific functions within the breast. An intact mammary mucosa may be less permeable to free or cell-associated virus. Since we did not find associations between EPO concentrations and viral load in breast milk, a potential effect of EPO on the infant intestinal mucosa may be more likely.

Some research suggests that HIV-infected patients have a blunted EPO response to anemia [13]. The normal response to anemia is increased production of EPO by the kidney and increased serum EPO concentrations. HIV-infected patients classified as CDC stage III had a significantly more impaired EPO response to anemia than patients in stages I or II [13]. Another study found no difference in serum EPO concentrations according to HIV disease stage [14]. It is unclear if EPO in breast milk may be affected by HIV status or disease stage. However, we controlled for indicators of HIV disease stage in the analysis to minimize potential confounding by this factor.

The EPO concentrations in breast milk from the control subjects in our study (median of 4.1 mIU/mL) are in agreement with those reported previously by Juul et al [3] (median of 4.7 mIU/mL). In their study, EPO concentrations increased during lactation. Because we matched cases to controls by the time since delivery when the sample was collected, fluctuations in EPO concentrations during lactation is not a likely source of bias in our study. One potential limitation of the study is that, for some of the samples, the time between assessment of the exposure and the outcome was relatively long (>24 weeks). The interpretability of results could be affected if EPO concentrations were different in samples closer to the estimated time of transmission; nevertheless, results were not substantially different in supplemental analyses limited to cases with the exposure assessed ≤24 weeks from the first positive HIV test.

The results from our case-control study suggest a possible protective effect of breast milk EPO against MTCT. The gut-trophic and anti-inflammatory properties of EPO may contribute to the prevention of MTCT. Breast milk EPO concentrations can be increased with administration of recombinant human EPO [15]. Further research should be considered to elucidate potential mechanisms and to examine whether breast milk EPO concentrations can be influenced by dietary or other modifiable factors.

Acknowledgments

The study was funded by a New Researcher Award from the Thrasher Research Fund (NR-0031) and grant R01HD045134 from the National Institutes of Health. JEA was supported by NIH training grant T32DK07703.

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Table 1

Characteristics of cases and controls

	Cases n=59	Controls n=116	*P*-value [a]
Maternal characteristics at recruitment:			
Age (y)	25.6 ± 5.1 [b]	25.1 ± 4.5	0.41
Lacks primary schooling [n (%)]	8 (13.6)	16 (13.8)	0.99
BMI (kg/m^2)	23.8 ± 3.2	23.3 ± 2.8	0.13
Symptomatic HIV disease [n (%)]	15 (25.4)	16 (14.0)	0.09
CD4 count (cells/mm^3)	386 ± 233	435 ± 185	0.04
Hemoglobin (g/L)	94 ± 15	95 ± 17	0.83
Assigned to vitamin A [n (%)]	35 (59.3)	53 (45.7)	0.11
Assigned to multivitamins [n (%)]	30 (50.9)	55 (47.4)	0.67
Pregnancy outcomes			
Low birth weight (<2500 g) [n (%)]	5 (9.3)	7 (6.2)	0.54
Preterm delivery (<37 wk) [n (%)]	16 (27.1)	25 (21.6)	0.38
Breast milk sample characteristics			
Age of child at time of breast milk sample (d)	250 ± 152	253 ± 150	0.83
Cell-free viral load (copies/mL)	2428 ± 4889	186 ± 624	<0.0001

[a] From Wilcoxon matched-pairs rank-sum test for continuous variables and the McNemar test for categorical variables.

[b] Values are mean ± SD.

Table 2

Odds ratios for mother-to-child transmission of HIV by tertile of erythropoietin (EPO) concentrations in breast milk

	Tertile 1	Tertile 2	Tertile 3	*P* for trend [a]
Median EPO, mIU/mL (Range)	0.60 (≤0.60 [b])	2.98 (0.61–6.79)	22.41 (6.80–218.76)	
Ratio of the number of controls:cases	41:28	29:18	46:13	0.03
Adjusted OR (95% CI) [c]	1.00	0.71 (0.30, 1.66)	0.34 (0.14, 0.82)	0.02

[a] Wald test for an ordinal variable representing the tertiles of EPO that was introduced as a continuous predictor in univariate (for the unadjusted ratio of controls to cases) and multivariate conditional logistic regression models (for the adjusted odds ratios).

[b] The level of detection of EPO concentrations is 0.6 mIU/mL.

[c] Odds ratios (OR) and 95% CI were derived from conditional logistic regression models with case-control status as the outcome and predictors that included indicator variables for the 2nd and 3rd tertiles of EPO, maternal CD4 cell counts and clinical stage of HIV disease at recruitment, and assignment to vitamin A supplementation.