Published in final edited form as:
AIDS Behav. 2012 July ; 16(5): 1260–1264. doi:10.1007/s10461-011-0035-7.

Infant Feeding Practices Were Not Associated with Breast Milk HIV-1 RNA Levels in a Randomized Clinical Trial in Botswana

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Abstract

Exclusive breastfeeding has been associated with a reduced risk of late vertical HIV transmission as compared to an infant diet composed of breast milk mixed with supplemental foods or liquids. Hypothesized mechanisms include increased infectivity of breast milk from mothers who practice mixed breastfeeding (MBF), or mechanisms such as increased gastrointestinal permeability in the infant caused by mixed feeding. It has been proposed that MBF may result in subclinical mastitis and higher breast milk HIV titers. However, little is known about the relationship between feeding strategy and breast milk viral load. We measured the HIV-1 concentration in breast milk in a sub-cohort of women enrolled in a mother-to-child HIV transmission prevention trial (the "Mashi" study). We report no observed relationship between MBF and measured breast milk viral RNA load. Our findings suggest that the increased transmission risk associated with higher breast milk HIV-1 RNA during MBF is unlikely.

Keywords

exclusive; mixed; PMTCT; breast feeding; vertical HIV transmission

Introduction

Breastfeeding is known to improve infant health and survival in the developing world.[1–10] Despite the increased risk of vertical HIV transmission associated with breastfeeding,[11–12] breastfeeding by HIV-infected women continues to be a preferred source of infant nutrition in resource-poor settings because of the established benefits to infant health.[1,7,13] In addition, in the past decade, several studies have demonstrated that exclusive

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breast feeding (EBF) reduces mother-to-child HIV transmission (MTCT) during the breastfeeding period compared with a mixed diet.[4,6,13–14] As a result, the WHO now recommends EBF for six months for the majority of HIV-infected women in resource-poor settings unless 100% replacement feeding is acceptable, feasible, affordable, sustainable, and safe before that time.[15–16] The mechanism of this protection is unclear, but several have been hypothesized. One hypothesized mechanism is that increased mucosal inflammation occurs in the gastrointestinal (GI) tract from early exposure to allergens and pathogens in mixed-fed babies.[11,17] Mixed feeding has been associated with a higher incidence of diarrhea as compared to exclusive breastfeeding,[2] and resultant increased mucosal permeability may increase the risk of trans-mucosal HIV acquisition.

A different hypothesis for the greater risk of a mixed feeding strategy is that increased levels of breast milk HIV-1 RNA might occur in women who practice mixed feeding compared to exclusive breastfeeding. Abrupt weaning of breast feeding has been associated with a significant increase in breast milk HIV-1 RNA,[18] suggesting that re-initiation of breast milk feeding may increase the risk of mother to child transmission (MTCT) of HIV. It has been hypothesized that exclusive breastfeeding might contribute to maintenance of healthy mammary epithelium.[19] Additionally, subclinical mastitis associated with stasis from incomplete emptying of milk ducts is also a hypothesized mechanism for a possible increase in breast milk HIV-1 RNA.[20] Only one previous study has directly evaluated the association between mixed feeding and breast milk HIV-1 RNA.[14] We therefore studied breast milk HIV-1 RNA levels of women who either exclusively breast fed or practiced mixed feeding in the setting of a clinical trial among HIV-infected women in Botswana.

Methods

Participants and Approvals

This study of breast milk HIV-1 RNA was conducted as a sub-study to the Mashi study, the design of which has been described previously.[21] The Mashi trial was conducted in Botswana, and completed enrollment of 1200 HIV-infected pregnant women in October 2003. It employed a factorial design to determine (1) if single dose nevirapine (NVP) given to mothers and infants provides additional MTCT prevention in the setting of short-course maternal and infant ZDV,[22] and (2) if prophylactic ZDV given to breastfeeding infants for 6 months prevents breastfeeding-related MTCT.[21] Mothers received antenatal ZDV from 34 weeks gestation, and intrapartum ZDV plus either single-dose NVP or placebo. Infants initially received single-dose NVP or placebo at birth and ZDV prophylaxis for 1 month (formula arm) or 6 months (breastfeeding arm). After 17 months of enrollment, the study was modified after a Data Safety and Monitoring Board review of external data and all infants received single-dose NVP. In October 2002, highly active antiretroviral therapy (HAART) became available through the Botswana Government Antiretroviral Treatment Program, and was offered to women with CD4 counts < 200 cells/mm^3 or AIDS either in the antenatal or postnatal period. Only women who were not receiving HAART were included in this sub-study of breast milk HIV-1 RNA. Informed consent was obtained from all participants; approval for the study was granted by human subjects committees in Botswana and at the Harvard School of Public Health; and human experimentation guidelines of the US Department of Health and Human Services were followed in conducting this trial.

Specimen Collection and Laboratory Analysis

Breast milk was collected at birth and 2 weeks, 2 months, and 5 months postpartum from women in the breastfeeding arm of the study. Birth samples were excluded from this analysis because of limited availability.

Breast milk was centrifuged at 400 *g* for 20 min. The fat/lipid layer was removed and discarded. The breast milk fluid (supernatant) layer was aspirated, aliquoted, and stored at −70°C. Breast milk viral RNA was extracted using the High Pure System Viral Nucleic Acid Kit (Roche) for manual specimen preparation according to manufacturer's instructions. Cell-free HIV-1 viral load was measured in breast milk samples using the COBAS® TaqMan Analyzer (Roche Diagnostics Corporation, Indianapolis, USA). The lower limit of detection was 40 copies/ml HIV-1 RNA.

Classification of Feeding Strategies

Infants born to mothers randomized to the breast feeding arm were categorized as exclusively breast-fed (EBF) or mixed-breast-fed (MBF) based on information reported by the mother at birth and at subsequent monthly follow-up visits. For a given 1-month interval, if women self-reported EBF with no formula, solids (fruit or cereal), milk (cow's milk or other), or liquids (water or juice), then the infant was classified as EBF. All other infants with any non-breast milk consumption reported were classified as MBF. One reported consumption of non-milk liquid (water or juice) was allowed for the infant to remain classified as EBF. However, once this allowance was exceeded, the infant would be subsequently classified as MBF. The feeding strategies for the individual time intervals were then used to derive the cumulative feeding strategies over time.

Statistical Analyses

Statistical analyses were performed using SAS V9.1 (SAS Institute Inc., Cary, NC) and SPSS version 13 (SPSS Inc. Chicago Illinois). HIV-1 RNA levels in plasma were transformed by log10 for analyses. Wilcoxon sum-rank tests were used to compare the distributions of age, CD4 counts, and breast milk HIV-1 RNA levels at baseline. Mean BM HIV-1 RNA levels at each time point (2 weeks, 2 months, or 5 months) were compared using a one-way analysis of variance test. The Wilcoxon signed rank test was used to examine changes in BM HIV-1 RNA levels over time. Comparison of mastitis occurrence between the feeding arms was done using Fisher's exact test.

Results

A subset of 261 women with quantified HIV-1 RNA in breast milk and feeding data available were included in this study from the breastfeeding arm of the Mashi Study. At two weeks, 85% of women were exclusively breastfeeding, but at later time points there was a transition to mixed feeding, it being difficult in this population, as in many, to change prevailing mixed feeding practices to exclusive breastfeeding. There were no baseline differences in age, CD4, HIV-1 RNA levels or Mashi drug randomization between women who were EBF or MBF (Table 1). No significant differences in breast milk HIV-1 RNA levels were observed between EBF and MBF groups at either 2 weeks, 2 months, or 5 months postpartum (p=0.57, p=0.35, p=0.88, respectively) (Table 2). Among both feeding groups, the mean breast milk HIV-1 RNA decreased slightly between 2 weeks and 5 months postpartum but the difference did not reach statistical significance (p=0.59). There were no significant differences in the proportions of women experiencing clinical mastitis at any of the visits. The receipt of AZT/SD NVP or AZT alone (placebo) did not affect RNA levels in EBF and MBF groups at the 2-week, 2-month or 5-month time points (p=0.89, 0.22, 0.81, respectively).

Discussion

This study compared breast milk HIV-1 RNA levels among EBF (exclusive breast feeding) and MBF (mixed breast feeding) women and found no significant difference between

groups. Although prior studies have shown increased breast milk viral load after abrupt weaning and increased indicators of mastitis (a potential risk factor for MTCT) in MBF women,[7,23] our study findings did not support the hypothesis that MBF might be associated with higher maternal breast milk HIV-1 RNA levels.

Our findings are consistent with one other recently published study[14] which also demonstrated no association between MBF and HIV-1 RNA or mastitis among women in Zimbabwe. In contrast with several other studies,[4,8,18] no excess risk for late MTCT was detected among MBF women in the Mashi Study. This may have been related to the small number of late transmissions in Mashi compared with other trials, since Mashi had a short overall duration of breastfeeding (6 months), and extensive maternal and infant prophylaxis (including 6 months of infant ZDV prophylaxis). However, the Mashi study was similar to other reports[24–26] associating MTCT risk with higher HIV-1 RNA in maternal blood and in breast milk.[27]

Short-course antiretroviral prophylaxis has been shown to decrease breast milk viral load during the early postpartum period.[28–32] Although we did not observe any effect of NVP/AZT or AZT alone on breast milk HIV-1 RNA levels in EBF and MBF groups at the 2 week, 2 month and 5 month time -points studied, this could have been due to the small sample size, ARV regimen or analyzed timepoints. Previous studies have shown a statistically significant decrease in breast milk viral load from NVP at early timepoints between 1 and 3 weeks postpartum.[28,32]

Our study did have limitations. First, sample availability was limited, and we evaluated only 3 timepoints in the postpartum period, which could have missed differences at other times. Second, the extent of MBF was not quantified, and it is possible that – similar to previous findings following abrupt weaning[7,18] – higher HIV-1 RNA levels could be associated with extremely intermittent breast feeding. Third, we evaluated exclusively HIV-1 RNA and cannot exclude other mechanisms within the breast milk of MBF women that might facilitate MTCT. Results from DNA would also have been valuable as data from several studies suggested that cell associated viral DNA was predictive of transmission.[2425,33] Unfortunately, sufficient paired DNA samples were not available for analysis. Finally, because our main MTCT results did not demonstrate an increased transmission risk from MBF,[27] it is not possible to draw direct conclusions regarding the relationship of breast milk HIV-1 RNA and MTCT risk caused by MBF practices. It is possible that other factors in our study setting masked the mechanisms of risk that have been associated with MBF in other settings.

In conclusion, we found no association between MBF and breast milk HIV-1 RNA levels among breastfeeding women in the Mashi Study. We agree with WHO recommendations that HIV-infected women who choose to breastfeed should do so exclusively because of its important health benefits, including the prevention of late MTCT.

Acknowledgments

This study was supported in part by a grant from the Belgian Technical Corporation (RR), by NIH grant D43 TW000004 (RR), and by the Fogarty International Clinical Research Scholars and Fellows Program (RR, JEH). We are grateful to the patients who participated in the *Mashi* study in Botswana. We thank R. Madison, L. Melton, and M. Pretorius Holme for administrative assistance.

References

1. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Lancet. 2000; 355:451–455. [PubMed: 10841125]
2. Ahiadeke C. Breast-feeding, diarrhoea and sanitation as components of infant and child health: a study of large scale survey data from Ghana and Nigeria. J Biosoc Sci. 2000; 32:47–61. [PubMed: 10676059]
3. Becquet R, Bequet L, Ekouevi DK, et al. Two-year morbidity-mortality and alternatives to prolonged breast-feeding among children born to HIV-infected mothers in Cote d'Ivoire. PLoS Med. 2007; 4:e17. [PubMed: 17227132]
4. Coutsoudis A, Pillay K, Spooner E, Kuhn L, Coovadia HM. Influence of infant-feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa: a prospective cohort study. South African Vitamin A Study Group. Lancet. 1999; 354:471–476. [PubMed: 10465172]
5. Creek, T.; Arvelo, W.; Kim, A., et al. Role of infant feeding and HIV in a severe outbreak of diarrhea and malnutrition among young children, Botswana 2006. CROI 2007: 14th Conference of Retroviruses and Opportunistic Infections; 2007 Feb 25–28; Los Angeles, California, US. Abstract 770.
6. Iliff PJ, Piwoz EG, Tavengwa NV, et al. Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. AIDS. 2005; 19:699–708. [PubMed: 15821396]
7. Kuhn L, Aldrovandi GM, Sinkala M, et al. Effects of early, abrupt weaning on HIV-free survival of children in Zambia. N Engl J Med. 2008; 359:130–141. [PubMed: 18525036]
8. Kuhn L, Sinkala M, Kankasa C, et al. High uptake of exclusive breastfeeding and reduced early post-natal HIV transmission. PLoS ONE. 2007; 2:e1363. [PubMed: 18159246]
9. Smith MM, Kuhn L. Exclusive breast-feeding: does it have the potential to reduce breast-feeding transmission of HIV-1? Nutr Rev. 2000; 58:333–340. [PubMed: 11140904]
10. Thomas, T.; Masaba, R.; van Eijk, A., et al. Rates of diarrhea associated with early weaning among infants in Kisumu, Kenya. CROI 2007: 14th Conference of Retroviruses and Opportunistic Infections; 2007 Feb 25–28; Los Angeles, California, US. Abstract 774.
11. Dunn DT, Newell ML, Ades AE, Peckham CS. Risk of human immunodeficiency virus type 1 transmission through breastfeeding. Lancet. 1992; 340:585–588. [PubMed: 1355163]
12. Miotti PG, Taha TE, Kumwenda NI, et al. HIV transmission through breastfeeding: a study in Malawi. JAMA. 1999; 282:744–749. [PubMed: 10463709]
13. Coovadia HM, Rollins NC, Bland RM, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet. 2007; 369:1107–1116. [PubMed: 17398310]
14. Lunney KM, Iliff P, Mutasa K, et al. Associations between breast milk viral load, mastitis, exclusive breast-feeding, and postnatal transmission of HIV. Clin Infect Dis. 2010; 50:762–769. [PubMed: 20121424]
15. World Health Organization. Geneva: World Health Organization; 2010. HIV and infant feeding. http://www.who.int/child_adolescent_health/topics/prevention_care/child/nutrition/hivif/en/index.html
16. World Health Organization. Geneva: World Health Organization; 2009. Rapid advice: revised WHO principles and recommendations on infant feeding in the context of HIV. http://whqlibdoc.who.int/publications/2009/9789241598873_eng.pdf
17. Rollins NC, Filteau SM, Coutsoudis A, Tomkins AM. Feeding mode, intestinal permeability, and neopterin excretion: a longitudinal study in infants of HIV-infected South African women. J Acquir Immune Defic Syndr. 2001; 28:132–139. [PubMed: 11588506]
18. Thea DM, Aldrovandi G, Kankasa C, et al. Post-weaning breast milk HIV-1 viral load, blood prolactin levels and breast milk volume. AIDS. 2006; 20:1539–1547. [PubMed: 16847409]
19. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis. 1999; 180:93–98. [PubMed: 10353866]

20. Willumsen JF, Filteau SM, Coutsoudis A, et al. Breastmilk RNA viral load in HIV-infected South African women: effects of subclinical mastitis and infant feeding. AIDS. 2003; 17:407–414. [PubMed: 12556695]
21. Thior I, Lockman S, Smeaton LM, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. JAMA. 2006; 296:794–805. [PubMed: 16905785]
22. Shapiro RL, Thior I, Gilbert PB, et al. Maternal single-dose nevirapine versus placebo as part of an antiretroviral strategy to prevent mother-to-child HIV transmission in Botswana. AIDS. 2006; 20:1281–1288. [PubMed: 16816557]
23. Sinkala, M.; Kuhn, L.; Kankasa, C., et al. No benefit of early cessation of breastfeeding at 4 months on HIV-free survival of infants born to HIV-infected mothers in Zambia: the Zambia Exclusive Breastfeeding Study. CROI 2007: 14th Conference of Retroviruses and Opportunistic Infections; 2007 Feb 25–28; Los Angeles, California, US. Oral abstract LB 74.
24. Koulinska IN, Villamor E, Chaplin B, et al. Transmission of cell-free and cell-associated HIV-1 through breast-feeding. J Acquir Immune Defic Syndr. 2006; 41:93–99. [PubMed: 16340480]
25. Rousseau CM, Nduati RW, Richardson BA, et al. Association of levels of HIV-1-infected breast milk cells and risk of mother-to-child transmission. J Infect Dis. 2004; 190:1880–1888. [PubMed: 15499546]
26. Richardson BA, Nduati R, Mbori-Ngacha D, Overbaugh J, John-Stewart GC. Acute HIV infection among Kenyan infants. Clin Infect Dis. 2008; 46:289–295. [PubMed: 18171265]
27. Shapiro RL, Smeaton L, Lockman S, et al. Risk factors for early and late transmission of HIV via breast-feeding among infants born to HIV-infected women in a randomized clinical trial in Botswana. J Infect Dis. 2009; 199:414–418. [PubMed: 19090775]
28. Chung MH, Kiarie JN, Richardson BA, Lehman DA, Overbaugh J, John-Stewart GC. Breast milk HIV-1 suppression and decreased transmission: a randomized trial comparing HIVNET 012 nevirapine versus short-course zidovudine. AIDS. 2005; 19:1415–1422. [PubMed: 16103773]
29. Chung MH, Kiarie JN, Richardson BA, et al. Highly active antiretroviral therapy versus zidovudine/nevirapine effects on early breast milk HIV type-1 Rna: a phase II randomized clinical trial. Antivir Ther. 2008; 13:799–807. [PubMed: 18839781]
30. Chung MH, Kiarie JN, Richardson BA, et al. Independent effects of nevirapine prophylaxis and HIV-1 RNA suppression in breast milk on early perinatal HIV-1 transmission. J Acquir Immune Defic Syndr. 2007; 46:472–478. [PubMed: 18077838]
31. Manigart O, Crepin M, Leroy V, et al. Effect of perinatal zidovudine prophylaxis on the evolution of cell-free HIV-1 RNA in breast milk and on postnatal transmission. J Infect Dis. 2004; 190:1422–1428. [PubMed: 15378434]
32. Rossenkhan R, Ndung'u T, Sebunya TK, et al. Temporal reduction of HIV type 1 viral load in breast milk by single-dose nevirapine during prevention of MTCT. AIDS Res Hum Retroviruses. 2009; 25:1261–1264. [PubMed: 20001515]
33. Tovanabutra S, de Souza M, Sittisombut N, et al. HIV-1 genetic diversity and compartmentalization in mother/infant pairs infected with CRF01_AE. AIDS. 2007; 21:1050–1053. [PubMed: 17457103]

Table 1

Baseline characteristics by overall feeding strategy

Randomization arm	EBF	MBF	P-value
AZT/NVP	29	103	<0.001[a]
AZT/Placebo	28	101	<0.001[a]
P-value	0.8875[a]	0.9211[a]	
Median Age	27 (19–41)	25 (19–42)	0.330[b]
Median baseline CD4	424	387	0.332[b]
Median baseline plasma VL	4.44	4.32	0.153[b]

[a] z-statistic (comparing proportions)

[b] Wilcoxon Rank Sum Test

Table 2

Mean breast milk HIV-1 RNA levels

	EBF			MBF			
	n	**Mean (SD)**	**Range**	**n**	**Mean (SD)**	**Range**	**P-value[a]**
Log BM VL 2W	30	2.05 (0.62)	(1.6–3.4)	125	2.14 (0.78)	(1.6–4.5)	0.57
Log BM VL 2M	35	2.04 (0.65)	(1.6–4.0)	135	2.17 (0.72)	(1.6–4.1)	0.35
Log BM VL 5M	38	1.94 (0.62)	(1.6–4.0)	98	1.92 (0.53)	(1.6–3.7)	0.88

[a]One-way ANOVA