Temporal Reduction of HIV Type 1 Viral Load in Breast Milk by Single-Dose Nevirapine during Prevention of MTCT

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Abstract

Short-course zidovudine (ZDV) with or without a single dose of nevirapine (sdNVP) is widely used to prevent mother-to-child HIV transmission (PMTCT). However, more data on viral load in breast milk following PMTCT regimens are needed. In a randomized PMTCT study in Botswana, in which half of the women received sdNVP in labor, stored samples from mothers assigned to breastfeed were analyzed for HIV-1 RNA in breast milk supernatant. A total of 527 samples from 282 women, collected at delivery, 2 weeks, 2 months, and 5 months postpartum were available for testing. Cell-free breast milk HIV-1 RNA was detectable (>40 copies/ml) in 44.8% (236/527) of samples analyzed. Women randomized to sdNVP + ZDV were more likely to have undetectable breast milk viral loads at 2 weeks postpartum compared with those who received ZDV alone (67.8% vs. 38.5%, $p = 0.002$). By 2 months postpartum the difference between study arms disappeared, and 43.8% of women who received sdNVP + ZDV had undetectable HIV-1 RNA compared to 53.8% of the ZDV alone group ($p = 0.19$) and 60.5% vs. 64.5%, respectively, at month 5 ($p = 0.61$.) The addition of sdNVP to antenatal short-course AZT resulted in significantly reduced breast milk viral loads at 2 weeks postpartum suggesting a reduced risk of MTCT during the early postpartum period. However, viral loads in both study arms were comparable at 2 and 5 months postpartum, suggesting that the receipt of sdNVP in labor may defer rather than blunt the postpartum viral load rebound seen in breast milk after the discontinuation of ZDV.

IT IS ESTIMATED THAT ONE-THIRD TO ONE-HALF of HIV-infected children in Africa acquire their infection from breastfeeding.¹ However, complete avoidance of breastfeeding by HIV-1-infected women is not always possible in settings such as the rural areas of Africa, where formula feeding may not be safe or culturally acceptable. The use of extended maternal or infant prophylaxis may reduce mother-to-child HIV transmission (MTCT) during breastfeeding² but more data are needed about the risks and timing of breastfeeding transmission to appropriately apply these interventions.

The concentration of cell-free HIV-1 RNA in breast milk has been associated with breastfeeding MTCT.^{3–6} Antiretroviral therapy has been shown to effectively reduce cell-free HIV-1 RNA,⁷ and one recent study described an association between receipt of maternal single dose nevirapine (sdNVP) given in labor and lower breast milk HIV-1 RNA during the first month of life.⁸ No previous study has extended the evaluation

of the effect of maternal sdNVP on breast milk HIV-1 RNA to the later period in breastfeeding. The aim of this study was to determine, at three time points during breastfeeding, the concentration of cell-free HIV-1 RNA in the breast milk of women who received either sdNVP or placebo in the setting of antenatal ZDV for prevention of MTCT (PMTCT) in Botswana.

The “Mashi” (“milk” in Setswana) clinical trial began enrolling participants in March 2001 and completed the enrollment of 1200 HIV-infected women in October 2003 and aimed to evaluate perinatal and postnatal interventions in reducing mother-to-child transmission of HIV in Botswana. The detailed trial design is described elsewhere.^{9,10} Briefly, the trial made use of a 2×2 factorial design to determine whether (1) a single dose of NVP given to mothers and infants provides additional protection against MTCT in the setting of maternal and infant ZDV therapy and (2) extended prophylactic ZDV given to breastfeeding infants prevents MTCT, compared

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with formula feeding. For this study, women from the breastfeeding arm of *Mashi* were selected via simple random sampling. Stored breast milk samples from 282 women who had not initiated HAART were analyzed. A total of 527 milk samples were available for analysis. Ethical approval for this research was obtained from the Human Research Development Committee of the Botswana Ministry of Health and the Human Subjects Committee at Harvard School of Public Health.

Blood and breast milk samples were collected at study visits in the *Mashi* study. Whole breast milk samples were obtained by manual expression into sterile plastic containers at birth, 2 weeks, 2 months, and 5 months postpartum. The breast milk samples were processed within 6 h of collection by centrifugation at 400×g for 20 min. The lipid layer was removed and the 0.5–1.8 ml of supernatant was aliquoted and stored at –70°C.

Viral RNA was extracted using the High Pure System Viral Nucleic Acid Kit (Roche) for manual specimen preparation according to the manufacturer's instructions. Cell-free HIV-1 viral load was measured in breast milk samples using the COBAS TaqMan Analyzer (Roche Diagnostics Corporation, Indianapolis, IN). A lower limit of detection was 40 copies/ml HIV-1 RNA.

HIV-1 RNA copy number in plasma was quantified using the automated standard protocol with a lower detection limit of 400 copies/ml by COBAS Amplicor/Ampli Prep HIV-1 Monitor Test V1.5 (Roche Molecular Systems Inc., Branchburg, NJ).

Data analysis was performed with Statistical Analysis Software 9.1 (SAS Institute, Cary, NC). Summary statistics and comparisons assume those samples with undetectable HIV-1 RNA levels are equivalent to those for which the RNA concentration was below the standard and therefore also uninterpretable. Medians and interquartile ranges (IQR) were used to summarize continuous data unless otherwise specified. Medians across groups were compared using the Wilcoxon rank sum test. Differences in viral loads were compared between weeks 2 and month 2 or month 5 using the signed rank test, and correlations were quantified using Spearman's method. All statistical tests were two-sided at the 0.05 level and are not adjusted for multiple testing.

A total of 527 samples from 282 HAART-naïve women were analyzed. From the AZT/NVP arm, there were 281 samples

from 143 women and 246 samples from 139 women in the placebo arm, representing a range of one to four samples per patient.

HIV-1 was detected at levels ≥ 40 copies/ml in 44.8% (236/527) of breast milk samples (range 42–30,000 copies/ml). Table 1 compares the viral loads for the two study arms at each time point. At birth, proportions of samples with viral loads < 40 copies/ml did not differ between the active (ZDV/NVP) and placebo (ZDV/placebo) arms (73.7% vs. 53.8%, $p = 0.28$). However, at week 2, the ZDV/NVP arm had a higher percentage of samples with viral loads < 40 copies/ml (67.8% vs. 38.5%, $p < 0.001$, Fisher's exact test). This difference was not seen at subsequent time points. Absolute HIV-1 RNA levels in breast milk were similar at birth, but significantly lower in the ZDV/NVP arm compared to the ZDV/placebo arm at week 2 ($p < 0.001$). Among women in the ZDV/NVP arm, the median HIV-1 RNA level increased significantly from week 2 to month 2 for 49 patients with samples available from both time points ($p < 0.001$). In contrast, 53 women on the ZDV/placebo arm with samples available from both time points showed a significant decrease in median HIV-1 RNA level from week 2 to month 2 ($p < 0.001$). At months 2 and 5, median HIV-1 RNA levels in patients with detectable milk viral load were comparable between ZDV/NVP and ZDV/placebo recipients (Table 1).

Thirty-one women had HIV-1 RNA measured in plasma and breast milk at the birth time point (within 3 days of delivery); 13 were randomized to placebo and 18 to sdNVP. A total of 153 women had HIV-1 RNA measured in plasma and breast milk at the 2 week time point (within 20 days of each other); 71 were randomized to placebo and 82 to sdNVP. Overall no statistically significant correlation was seen between HIV-1 RNA in breast milk and plasma at delivery ($r = 0.32$, $p = 0.07$, Spearman's correlation); however, in analysis of individual study arms there was a correlation within the placebo arm ($r = 0.60$, $p = 0.038$). There was also an interaction between breast milk viral load and study arm for the 2 week time point. At 2 weeks, overall there was a correlation between plasma viral load and breast milk viral load ($r = 0.24$, $p = 0.003$), but when analyzed independently, only the placebo arm had a significant correlation ($r = 0.45$, $p < 0.001$). Fewer plasma samples were available at later time points, precluding further comparisons.

TABLE 1. VIRAL LOAD IN HIV-1 RNA COPIES IN CELL-FREE BREAST MILK

Time point	ZDV/NVP					ZDV/placebo				
	N	< 40 copies ^a	Median VL ^b	(IQR) ^b	vs. week 2 ^c	N	< 40 copies ^a	Median VL ^b	(IQR) ^b	vs. week 2 ^c
Birth	19	14 (73.6%)	268	(116–306)		13	7 (53.8%)	1139	(131–1139)	
2 weeks	87	59 ^d (67.8%)	244 ^e	(104–686)		78	30 ^d (38.5%)	663 ^e	(211–3420)	
2 months	89	39 (43.8%)	386	(145–1040)	$n = 49$ ($p < 0.001$)	93	50 (53.8%)	719	(157–3510)	$n = 53$ ($p < 0.001$)
5 months	86	52 (60.5%)	231	(134–2800)	$n = 45$ ($p = 0.005$)	62	40 (64.5%)	225	(74–577)	$n = 27$ ($p = 0.18$)

^aNumber of samples with undetectable viral load < 40 copies/ml.

^bMedian VL and interquartile range are calculated from the data points, which have a detectable number of copies (> 40 copies/ml) in copies/ml.

^cTwo-sided p -value from signed rank test to allow inclusion of VL < 40 .

^dTwo-sided p -value < 0.001 from Fisher's exact test comparing study arms.

^eTwo-sided p -value < 0.001 from Wilcoxon rank sum test comparing study arms.

Detailed MTCT results from the parent Mashi study have been published elsewhere.^{7,9,10} Among 558 infants randomized to the breastfeeding arm of the parent study who were monitored at 1 month of age, only seven seroconverted between birth and 1 month postpartum, representing infections that occurred either intrapartum or within that first month of breastfeeding. Receipt of single-dose NVP was not a significant predictor of HIV-1 transmission in the first month postpartum ($p = 0.45$). Both higher baseline maternal plasma HIV-1 RNA ($p < 0.001$) and higher breast milk HIV-1 RNA ($p < 0.001$) were among the univariate predictors of late MTCT (transmission after 1 month postpartum).

The Mashi trial was designed to assess the effect of the addition of sdNVP to short-course AZT on MTCT.^{9,10} In this report, we describe the effect of the two treatment arms on cell-free breast milk HIV viral load over a 5-month postpartum period. We found that in the setting of antenatal AZT monotherapy, the addition of peripartum sdNVP reduced both the percentage of women whose breast milk had detectable HIV virus as well as the median breast milk HIV viral load at 2 weeks postpartum as compared to the placebo arm ($p < 0.001$ for both). Uniquely at this time point, we also noted that in the arm that received peripartum sdNVP, breast milk viral load did not correlate with the corresponding plasma viral load, suggesting a possible preferential viral suppressive effect in breast milk. However, no statistically significant difference between the two treatment arms was observed by 2 months postpartum. Indeed, there appears to be a trend towards higher median breast milk viral loads, and a higher percentage of breast milk samples with detectable HIV virus, at the 2 month time point relative to the 2 week postpartum period in women who had received peripartum sdNVP.

Our findings agree with prior work that demonstrated that viral rebound occurs following cessation of short-course prenatal AZT monotherapy,¹¹ and that peripartum sdNVP reduces breast milk viral shedding at 3 weeks postpartum.⁸ However, in contrast to those studies, by including AZT in both treatment arms we are able to isolate the postpartum effect of sdNVP from the post-AZT rebound effect. Additionally, by extending the duration of breast milk surveillance beyond that of prior studies, we demonstrate that the effect of sdNVP is transient, and that when it wanes it may be associated with the same viral rebound described following the discontinuation of AZT as described by Manigart *et al.*¹¹

Nevirapine maintains a concentration in plasma of greater than $10\times$ the IC_{50} for 7 days following a single dose.¹² Studies such as HIVNET 012 have suggested that the prolonged effect of single-dose nevirapine in reducing HIV viral load could account for an apparently low rate of early postnatal transmission of HIV-1.^{8,13} However, our findings suggest caution in hypothesizing sustained virologic suppression in breast milk following sdNVP, and suggest that viral rebound (and potential risk) may be deferred to a later time during breastfeeding. These findings are consistent with two recently reported trials of extended infant NVP prophylaxis, both of which showed an increase in breastfeeding MTCT following the discontinuation of infant prophylaxis in the setting of ongoing breastfeeding, despite maternal sdNVP receipt in labor.^{2,14} However, the findings contrast somewhat with the observed long-term protection possibly afforded by maternal NVP in the HIVNET 012 trial. Breast milk viral load and/or proviral load is probably the most important risk factor as-

sociated with late MTCT,^{3,5,15} and these findings suggest that if maternal sdNVP provides protection against breast milk HIV transmission it is most likely to do so in the first 2 months. Viral loads may change over time in breast milk even in the absence of nevirapine chemoprophylaxis.⁵ Additionally, proviral DNA load may be even better correlated with breast milk transmission than is viral load during the first 9 months of breastfeeding.¹⁵

A primary limitation of our study was an inability to draw meaningful comparisons at the birth time point due to the fact that breast milk was not readily produced and expressed by many of the mothers shortly after delivery. Direct correlation of HIV viral load in plasma and breast milk at each time point was not possible due to the design of the parent study. For technical reasons and sample availability, breast milk supernatant (rather than whole milk) was used in this study, although breast milk viral load analysis has been shown to be more sensitive using whole milk.¹⁶ This should be taken into account when comparing these results to studies involving whole breast milk.

In conclusion, we report that women on prophylactic short-course AZT PMTCT therapy who received sdNVP in labor were less likely to have detectable virus in breast milk at 2 weeks postpartum compared with those who received placebo, but this effect faded at later time points. These findings suggest sdNVP may defer rather than blunt the viral load rebound in breast milk after discontinuation of AZT. The delayed rebound of HIV viral load in breast milk following sdNVP may have implications for the design of breastfeeding interventions or infant prophylaxis for PMTCT, suggesting that continued chemoprophylaxis throughout the breastfeeding period would be beneficial.

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Disclosure Statement

No competing financial interests exist.

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