HIV Transmission Through Breastfeeding: Is There a Critical "Cut Off" of Plasma Viral Load?

N.Z. Nyazema, E. Gomo*, H. Friis, P. Ndhlovu*** and the Edith Opperman Maternity Clinic HIV/AIDS Study Group******

Department of Clinical Pharmacology/ICHE, Univ. of Zimbabwe, Harare, Zimbabwe
**Blair Research Laboratory, HIV/AIDS Section, Harare, Zimbabwe*
***Department of Human Nutrition, Danish Royal Vet University, Copenhagen, Denmark*
****Dept.of Med.Lab.Sc. Univ.of Zimbabwe, Harare, Zimbabwe*
*****City of Harare, Health Department, Harare, Zimbabwe*

Summary

HIV-1 infection found in children in Zimbabwe is a reflection of high prevalence of HIV-1 among pregnant mothers who are invariably encouraged to breastfeed their babies after birth. At present policy makers are still struggling to reconcile the contrasting roles for breast-feeding. There is little information on the biologic features of breast milk, including factors that increase the risk for transmission of the virus. To investigate whether there is a critical "cut off" of plasma viral load which breaks the breast-blood-barrier, used the NASBA-based amplification of isolated HIV-1 RNA from paired plasma and blood samples collected from 30 HIV positive women. Virus loads 810 and 350 copies/mL were detected in the breast milk of two women whose plasma viral load were 74 000 and 43 000 copies/mL and none from the women whose loads were even higher. HIV RNA quantity ranged from undetectable to 810 copies/mL in breast milk and 65 to 110 000 copies/mL in plasma. There appeared to no positive correlation even with the CD4 counts. RT-PCR done showed that there were no natural mutations associated with resistance to AZT, 3TC and nevirapine, drugs to be used in the planned MTCT programmes. The results, which still need to be substantiated with a much bigger

sample, seem to suggest that shedding of the virus in breast milk may depend on many yet unknown factors.

Introduction

For anyone working with mothers and infants in Zimbabwe, it is distressing to learn that HIV can be transmitted through breast milk, making maternal HIV-1 infection the primary source of all child infection. HIV-1 infection found in children is a reflection of the high prevalence of HIV-1 infection among pregnant women. Zimbabwe has an estimated 33% rate of HIV infection among pregnant women presenting at antenatal clinics where they are still generally encouraged to breastfeed after birth. Current estimates of the risk of breast milk transmission suggest that breast-feeding may account for up 50% of mother-to-infant transmission in a developing country such as Zimbabwe. At present public health policy makers are struggling to reconcile the contrasting roles for breast-feeding. There is still not information, particularly on the biologic features of breast milk.

It is still not known what time during lactation is highest risk for HIV-1 transmission. There has been data presented to make the case for highest risk during early lactation (colostrums and transitional milk) as well as later during lactation. It is exceedingly difficult to determine what the net effect of these competing or diverging factors is on the final outcome. However, among these factors is the quantity of virus in the breast milk. HIV-1 viral particles have been detected as both free virus and cell associated virus (Dunn et al 1992)

A study in South Africa where HIV-1 subtype C is found among heterosexual population showed that breast milk viral load at different post-delivery ages was positively correlated with plasma viral load. The viral load, which ranged from undetectable to 227, 586 copies/mL, and was not influenced by vitamin A supplementation (Pillay et al 2000). A similar study in India established a critical "cut off" of plasma HIV-1, i.e. 40,000 copies/mL, which was seen to break the selective "blood-breast-barrier" (Sapalya et al 2000). This cut off was recommended as diagnostic to reduce horizontal transmission where breast-feeding was culturally desirable like in Zimbabwe.

We decided to investigate whether there was a critical "cut off" of plasma viral load that broke "the blood-breast-barrier" and what this meant for the national infant feeding policy, and the proposed use of ARVs in MTCT prevention.

Methods

The Medical Research Council of Zimbabwe approved the study. Written informed consent was obtained from all the women who participated in the study. Breast milk and plasma were collected into sterile plastic containers

from 30 HIV-1 seropositive women who were enrolled in a prospective trial evaluating the role of multi-micronutrient supplementation in reducing mother-to-child transmission of the virus. This cohort of women has been described previously (Friis et al 2001). Maternal data obtained during this study was used in the analysis of the breast milk data. Briefly, HIV-1-seropositive women had been recruited at between 28 and 32 weeks' gestation and randomised to receive multi-micronutrient supplementation or placebo through the prenatal period.

Full blood, CD4+ and CD8+ counts, in-house HIV-PCR and other relevant biochemical determinations that were routinely carried out at any antenatal clinic were done. The blood samples used were those that had been collected at the same time as the breast milk and at enrolment into the study. The NASBA-based amplification of isolated HIV-1 RNA was later used on the plasma and breast milk samples. The lower limit of reliable detection of virus by this technique is 250 copies/mL. An RT-PCR was done to determine naturally occurring mutations associated with AZT, 3TC and nevirapine.

Pearson's correlation coefficients were used to investigate associations between continuous variables

Results

Plasma viral load was found to be between 65 and 110 000 copies/mL (Average 21 206 copies/mL) and in breast milk, between below detectable limit i.e. <250 in 90% of the samples and 810 copies/mL (Table 1). There was no correlation between plasma and breast milk viral loads. Generally speaking, the plasma viral load was much higher than breast milk viral load. $CD4^+$ counts ranged from 108 – 1222 cells/mL with an average of 459cells/mL and had no correlation with maternal plasma viral load. All biochemical parameters were in their normal ranges. There were no natural mutations associated with resistance to AZT, 3TC and nevirapine found in the plasma HIV-1 RNA. It made no difference whether the women had received multi-micronutrient supplementation or not.

Discussion

The results obtained in the present study did not agree with what had been previously reported i.e. positive correlation of plasma and breast milk viral load (Pillay et 2000; Sapalya 2000). This could, may be, partly be explained by the different method of quantification used to determine the viral load. Both the Indian and the South African studies used Amplicor HIV quantification test, which may have less interference from inhibitors in breast milk. Until different assays are compared, the optimal method of determining the viral load in breast milk will remain undetermined. Be that as it may, the study in South Africa showed that plasma HIV RNA

Table 1. Shows the women's age, plasma viral load, CD4, baby weight and breast milk viral load.

	Age (yrs)	V L plasma (copies/mL	CD4 (cells/mL)	Baby wt (Kg)	V Load breast (copies/mL
1	28	7200	ND	3.5	<250
2	19.9	ND	1045	2.84	"
3	28	400	580	3.3	"
4	31.6	3400	588	3.66	"
5	21.1	34000	624	2.9	"
6	0	ND	280	2.5	"
7	16.4	43000	ND	2.9	350
8	25.7	ND	1226	3.1	<250
9	19.1	ND	398	ND	"
10	29.9	15000	ND	3	"
11	20	93000	627	2.4	"
12	21.4	9300	ND	3.02	"
13	31	2300	ND	3.7	"
14	23.4	3600	267	3.5	"
15	29.6	74000	129	2.9	810
16	26.7	22000	ND	3.8	<250
17	21.5	65	526	3.3	"
18	22.5	9200	425	2.72	"
19	25.5	16000	336	ND	"
20	23.7	2800	169	2.8	"
21	34.2	110000	ND	2.9	"
22	27.6	7200	181	ND	"
23	27.4	6400	ND	3.2	"
24	29.4	27000	108	ND	"
25	23.1	7500	ND	3.3	"
26	29	4800	377	4.2	"
27	25.6	1400	ND	3.7	"
28	22.8	2400	432	3.2	"
29	20.2	ND	ND	ND	ND
30	ND	ND	418	0	ND

ND = not determined

levels were significantly higher in 33% of the women with undetectable HIV RNA in breast milk (mean $\log_{10}$ 4.38) than in women with detectable HIV RNA in breast milk. If indeed there were correlation of cell-free HIV-1 in plasma, colostrums and breast milk as suggested by the Indian study, this would have been the case in all the women enrolled in the study carried out in South Africa. Instead the correlation was seen in 66% of the women.

The load of the virus in breast milk in the present study was undetectable in 90% of the women. This agrees with a study carried out in Kenya, which showed low viral loads with most samples near the limit of detection the assay they used (Lewis et al 1998). In the present study the maximum breast milk viral load found was 810 copies/mL in a woman who had 74 000 copies/mL in the plasma. As shown in Table 1, a woman who had

110 000 copies/mL in her blood had <250 copies/mL in the breast milk. A delay of 30 days after delivery has been shown to make no difference in milk viral load (Pillay et al 2000). This suggests a role for local maternal factors in determining the presence and concentration of the virus in breast milk, and that there cannot be a general critical "cut-off" of plasma HIV-1 viral load which can be used as a surrogate marker of potential transmission through breast milk; hence may be used as a diagnostic to reduce MTCT in Zimbabwe where breast-feeding is culturally desirable. It would be impossible to implement a selective intervention strategy for women with high plasma viral load during pregnancy to discourage breast-feeding or reduce/sustain low plasma viral load using ARVs. Luckily at the moment as shown by the study there are natural mutations associated with the ARVs are meant to be used in the proposed MTCT programmes in Zimbabwe.

Conclusions

The results obtained seemed to suggest that there was no critical viral load that could describe as responsible for breaking the blood-breast-barrier in the women studied. There is, however, a need to carry out the study with a bigger sample size before any proposals to change current recommendations on infant feeding are made. Shedding of the virus in breast milk may depend on many yet unknown factors.

References

DUNN DT, NEWELL ML, ADES AE and PECKHAM CS. Risk of human immunodeficiency virus type 1 through breast-feeding. Lancet 340:585-588,1992.

FRIIS H, GOMO E, NYAZEMA NZ and NDHLOVU P. Presentation of a randomised control trial in Zimbabwe. The effect of maternal multi micronutrient supplementation on mother-to-child HIV transmission. Int J App Sc Basic Nutr 13(3): 275, 1997.

LEWIS P, NDUATI R, KREISS JK et al. Cell free human immunodeficiency virus type 1 in breast milk. J Infect Dis 177:34-39, 1998.

PILLAY K, COUTSOUDIS A, DENNIS Y, LOUISE K and COOVADIA H. Cell free virus in breast milk of HIV-1-seropositive women. JAIDS 24:330-336, 2000.

SAPALYA R, JAYKAR A, DONGAONKAR D, ANGADI S, HIRA S NARKHEDE N and FERNANDES C. Correlation of cell-free HIV-1 in plasma, colostrums and breast milk. The XIII International AIDS 2000, TuPeB3249, 2000.