Poster 881

The Conference on Retroviruses and Opportunistic Infections – Boston, March 3 to March 6, 2014

Cytomegalovirus, and Possibly Epstein-Barr Virus, Shedding in Breast-milk is Associated with HIV-1 Transmission by Breastfeeding

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Financial Support: This study was supported by funding from Wellcome Trust (VTS Grant Nr: 063009/Z/00/Z) and ANRS (Grant Nr: 2008 – 033).

Acknowledgement: We are grateful to the women and children enrolled and the dedicated field, clinic and data staff who worked on the Vertical Transmission Study.

INTRODUCTION

In the absence of antiretroviral prophylaxis, breastfeeding accounts for almost one-third to one-half of mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 (HIV-1). Breast-milk (BM) is also a vehicle for the transmission to the infant of other viruses infecting leukocytes, such as cytomegalovirus (CMV), human T-cell lymphoma/leukemia virus (HTLV-1) and possibly Epstein-Barr virus (EBV). However, since the vast majority of breastfed infants of HIV-1 infected mothers escapes infection even in the absence of maternal or infant prophylactic treatment, some unidentified factors must drive HIV-1 transmission by breastfeeding. It has been suggested that co-infection with CMV and EBV may be associated with increased risk of HIV-1 transmission through mechanisms enhancing reciprocal viral replication. BM is a main source of MTCT of CMV and is estimated to occur in 40-66% of breast-fed infants in the first year of life. In HIV-1 infected mothers, levels of CMV in BM and maternal CD4+ counts have been shown to be independently associated with early infant CMV acquisition. It is important to understand the pathogenesis of HIV-1 MTCT and to identify factors associated with postnatal MTCT of HIV-1. Here we report the findings of a case-control study on the relationship between CMV DNA levels and EBV DNA detection in BM and the risk of mother-to-child transmission of HIV-1 via breastfeeding.

MATERIALS and METHODS

Study Population and Design

This was a case-control study that was nested in the Vertical Transmission Study (VTS), a large infant feeding intervention cohort with enrollment between August 2001 and September 2004. This study aimed to examine breastfeeding and HIV-1 transmission in a community with a high HIV-1 prevalence [1]. Cases were 62 mothers with established HIV-1 infection and proven postnatal transmission of HIV-1 to their infants via breastfeeding. The age of estimated timing of infant HIV-1 acquisition was taken as the midpoint between the last negative and the first positive HIV-1 RNA test result. The BM sample selected for testing was the sample obtained immediately prior to the estimated timing of infant HIV-1 acquisition. Control subjects were 62 HIV-positive mothers with HIV-uninfected infants matched for infant age at the time of BM sample collection in a 1:1 ratio.

Cell-free and cell-associate HIV-1 RNA and DNA in BM were quantified as previously described [2,3]. CMV and EBV DNA viral load quantification was performed on pooled left and right BM cell pellets, using commercially available assays (PrimerDesign™ GeneSig qPCR Kit, Southampton, UK). Maternal plasma viral load (Nuclisens EasyQ HIV-1 assay, Biomerieux, Boxtel, Netherlands) and CD4+ count (Epics XL, Beckman Coulter, CA, USA) were obtained from samples taken at enrollment, 6 weeks or 26 weeks post-delivery. All study participants provided informed consent, and the VTS and BM analyses were approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal.

RESULTS

Maternal HIV-1 plasma viral load was significantly higher in cases (4.5 $\log_{10}$ copies/ml) [IQR 3.9-4.8] than in controls (3.8 $\log_{10}$ copies/ml) [IQR 3.1-4.4] (p=0.003) and there was a trend towards lower CD4+ counts. The median (IQR) BM HIV-1 RNA level was significantly higher in cases than controls, 1,404.50 (188-6,776.0) and 188 (188-188) copies/ml, respectively (p<0.001). In univariate analysis, MTCT of HIV-1 was significantly associated with BM HIV-1 RNA detection (Table). BM HIV-1 DNA was detected significantly more frequently in cases (24/29; 82.8%), than in controls (10/40; 25%) (p<0.001). The median (IQR) BM HIV-1 DNA level was significantly higher in cases, 5,002 (1,993-14890), than in controls, 280 (280-461.5) copies/10^6 BM cells (p<0.001) CD4+ count was marginally associated with MTCT of HIV-1 (p=0.068).

Detection and quantification of CMV DNA and EBV DNA in breast-milk

CMV DNA was detectable in the majority of BM samples of cases and controls (both 96.8%, 60 out of 62). Median (IQR) CMV DNA viral load was significantly higher in cases, 88,044 (18,586-233,904), than in controls, 11,167 (3,221-31,152) copies/10^6 BM cells (p<0.001) (Fig A).

Table: Univariate and multivariate analysis of Mother-to-Child transmission of HIV-1

Variable	Crude OR[1] (95% CI)	p	Adjusted OR[2] (95% CI)	p	Adjusted OR[3] (95% CI)	p
DNA EBV (detection) [4]	3.17 (1.27 – 7.93)	0.014				
DNA CMV (log)	2.49 (1.50 – 4.12)	<0.001	1.94 (1.08 - 3.40)	0.0263[a]	2.52[c] (1.28 – 4.99)	0.008
Breast milk HIV RNA (detection)	10.67 (3.27– 34.83)	<0.001	7.84 (2.28 – 27.00)	<0.001[b]		
Plasma HIV (Log10 RNA)	1.897 (1.20 – 3.00)	0.0057			1.58[d] (0.91 – 2.74)	0.104
CD4+ T cells (cells/µl)	0.270 (0.07 – 1.10)	0.0678				

[1] Crude Odds Ratio using univariate analysis;
[2] Adjusted Odds Ratio Model 1. Analysis of HIV MTCT according to DNA CMV levels taking into account BM HIV RNA shedding[(a)]. Analysis of HIV MTCT according to BM HIV RNA shedding taking into account the BM CMV DNA levels [(b)];
[3] Adjusted Odds Ratio Model 2.Analysis of HIV MTCT according to DNA CMV levels taking into account plasma HIV RNA level[(c)]. Analysis of HIV MTCT according to plasma HIV RNA level taking into account the BM CMV levels[(d)];
[4] This variable was removed because it caused a loss of model quality.

EBV DNA was detectable in 23/62 (37.1%) and 10/62 (16.1%), respectively (p=0.009). In addition, levels of EBV DNA detected in BM were higher in cases than controls (p=0.023). CMV DNA viral load in BM was significantly associated with HIV-1 MTCT in univariate analysis, with a 2.5-fold increased risk of HIV-1 MTCT (Table). Furthermore, increased CMV DNA levels in BM were associated with increased HIV-1 RNA shedding in BM. MTCT of HIV-1 remained independently associated with CMV DNA level after adjustment for BM HIV-1 RNA detection and plasma HIV-1 RNA levels. In univariate analysis, EBV DNA detection in BM was also associated with risk of MTCT of HIV-1. However, in multivariate analysis adjusting for BM HIV-1 RNA detection, EBV DNA detection was no longer found to be significantly associated with postnatal transmission of HIV-1.

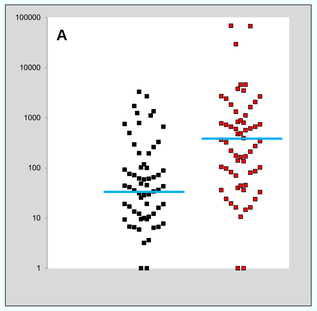
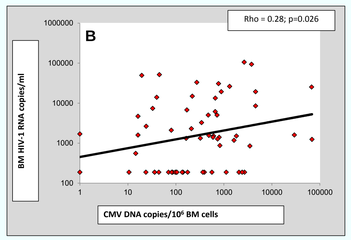


Fig A Comparison between cases and controls of breast milk CMV DNA [blue line=median value]

Association between CMV and EBV DNA in BM with HIV-1 replication and CD4+ depletion

CMV DNA levels in BM correlated positively with BM HIV-1 RNA in cases (Rho = 0.28; p=0.026) as well as in controls (Rho = 0.27; p=0.0376) (Fig B and C). A strong correlation was observed between CMV and HIV-1 DNA levels in BM for cases (Rho =0.60; p<0.001), but not for controls (Rho = 0.02; p=0.87) (Fig D and E). Levels of CMV DNA in BM correlated positively with plasma HIV-1 RNA levels in cases (Rho = 0.42; p=0.002) as well as in controls (Rho = 0.25; p=0.06) (Fig 3 A and B). A significant inverse correlation was observed between BM CMV DNA and maternal CD4+ count for cases (Rho = -0.35; p=0.005), but not for controls (Rho = -0.17; p=0.19).



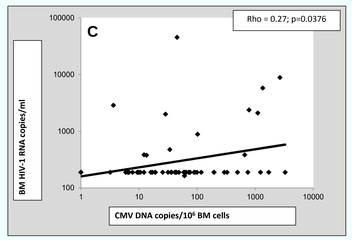
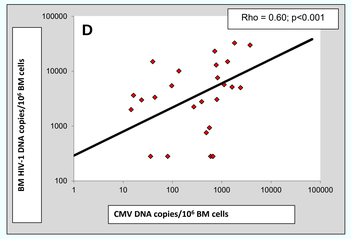


Fig B and C Correlation between BM CMV DNA and BM HIV RNA in cases (B) and controls (C).



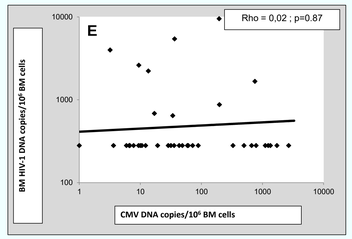


Fig D and E Correlation between BM CMV DNA and BM HIV DNA in cases (D) and controls (E).

CONCLUSIONS

CMV DNA viral load in BM is significantly associated with MTCT of HIV-1 via breastfeeding. This risk is independent of the shedding of HIV-1 RNA in BM. EBV DNA detection in BM may also facilitate MTCT of HIV-1, but only marginally so. This is the first study to demonstrate an independent association between CMV DNA in BM and postnatal MTCT of HIV-1.

Similar to previous reports, CMV DNA was detectable in the majority of BM samples studied from this population of untreated HIV-1 infected mothers, with measured CMV DNA levels being significantly higher in BM than levels previously reported in blood. In contrast to HIV-1, which we previously found to be 1-2 log lower in BM compared to blood, CMV DNA level appears to be significantly higher in BM versus blood, suggesting efficient compartmentalized CMV replication in the mammary gland.

By comparison, EBV is less frequently detected in BM. Memory B-cells are the reservoir of EBV. The proportion of B-cells in BM is lower compared to blood. B-cell count is also much lower than T-cell count in BM. This could explain in part why levels of EBV DNA were found to be lower than CMV DNA levels in BM.

In line with previous studies HIV-1 DNA and RNA levels in BM appeared strongly predictive of postnatal transmission of HIV-1 through breastfeeding. We observed a >2-fold risk of MTCT of HIV-1 with every 1 log increase in BM CMV DNA, independently of BM HIV-1 RNA level. Targeting CMV and perhaps EBV replication in the mammary gland may be of interest not only as an adjuvant to ART prophylaxis of HIV-1 MTCT, but may have the additional effect of reducing CMV burden overall.

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