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Review

Association between breast milk fatty acids and HIV-1 transmission through breastfeeding

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ABSTRACT

A residual mother-to-child transmission of HIV through breastfeeding persists despite prophylaxis. We identified breast milk fatty acids (FA) associated with postnatal HIV transmission through breastfeeding in a case-control study. Cases ($n=23$) were HIV-infected women with an infant who acquired HIV after 6 weeks of age. Controls ($n=23$) were matched on infant's age at sample collection. Adjusting for maternal antenatal plasma CD4 T cell count, cis-vaccenic acid (18:1n-7) and eicosatrienoic acid (20:3n-3) were associated with HIV transmission in opposite dose–response manner: OR (tertile 3 versus tertile 1): 10.8 and 0.16, p for trend = 0.02 and 0.03, respectively. These fatty acids correlated with HIV RNA load, T helper-1 related cytokines, IL15, IP10, and β2 microglobulin, positively for cis-vaccenic acid, negatively for eicosatrienoic acid. These results suggested a change in FA synthesis by mammary gland cells leading to increased cis-vaccenic acid in milk of mothers who transmitted HIV to their infant during breastfeeding.

1. Introduction

The 2013 WHO guidelines recommend that HIV-infected mothers breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months, with prophylactic antiretroviral therapy delivered to the mother and a short prophylactic course to the infant [1]. However, there is a residual postnatal mother-to-child transmission (MTCT) of HIV through breastfeeding despite prophylaxis [2,3]. Identifying the role of breast milk components in HIV MTCT through breastfeeding would add to the scientific knowledge base for clinical practice and prevention of postnatal HIV transmission.

Human breast milk is a complex body fluid containing compounds with immunological, antibacterial and antiviral functions [4]. *In vivo* experiments have shown that whole human breast milk inhibits oral transmission of cell-free or cell-associated HIV-1 in humanized bone marrow/liver/thymus mice [5]. *In vitro* experiments have also shown that skim human breast milk inhibits cell-free HIV-1 infection but not cell-associated HIV-1 infection of CD4+ T cells [6]. These findings suggest that the lipid fraction of breast milk contains inhibitors of cell-associated and/or cell-free HIV that may both be associated with postnatal MTCT of HIV [7,8]. Interestingly, Villamor et al. have shown that long chain polyunsaturated fatty acids (LC-PUFA) of the n-6 family in breast milk are associated with a lower risk of postnatal transmission of HIV and a lower level of breast milk HIV RNA [9]. However, this study did not investigate the relationship of PUFA with breast milk immune components.

Beyond nutritional and developmental functions, human milk is involved in the modulation of inflammation and immune response. LC-PUFA acts as lipid mediators that regulate inflammation: prostaglandins and leukotrienes derived from either n-6 or n-3 LC-PUFA, resolvins or protectins derived only from n-3 LC-PUFA [10]. A beneficial anti-inflammatory profile is recognized for the n-3 family while the n-6 family is more likely associated

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with inflammatory effects [11]. Breast milk contains also several cytokines with pro-inflammatory effect such as IL-1β, IL-6, TNF-α, and IFN-γ and cytokines of the CXC and CC chemokine family such as IL-8, monocyte chemotactic protein-1 (MCP-1), RANTES and macrophage inflammatory protein-1a (MIP-1a). Cytokines of breast milk are transcribed and secreted by both mammary epithelial cells and breast milk leukocytes [12]. Previous data reported a relationship between breast milk cytokines and FA composition [13], in agreement with the recognized immune and inflammatory modulating functions of FA. Although the FA profile in breast milk could be one factor driving immune activation observed in breast milk, no study determined the relationship between breast milk FA composition and cytokines levels in regard to a mother-to-child transmission (MTCT) of HIV through breastfeeding.

The present study aimed as first objective to identify breast milk fatty acids (FA) associated with postnatal transmission of HIV through breastfeeding and to determine as second objective the associations of FA with inflammatory and immune factors and with HIV RNA load, in breast milk.

2. Subjects and methods

In 2008, we nested a case-control study in a large infant feeding intervention cohort (Vertical Transmission Study) of women attending 9 clinics (8 rural and 1 urban) in KwaZulu-Natal, South Africa. The Vertical Transmission Study aimed to examine HIV transmission by breastfeeding in a community with a high prevalence of HIV infection [14,15]. Single-dose nevirapine was provided to all HIV-1 infected women and their infant peripartum as prevention of MTCT prophylaxis. Breast milk samples from mothers and dried blood spot samples from infants were collected at 6 weeks after delivery and monthly thereafter; an additional dried blood spot sample was taken from infants within 72 h of delivery when possible [14,15]. Postnatal transmission was defined as infant's HIV infection acquired after the age 6 weeks. The estimated age at HIV-1 infection in infant was taken as the midpoint between the last negative RNA polymerase chain reaction (PCR) result and the first positive RNA PCR result [15]. Cases were HIV-infected women with a postnatally HIV-infected infant ("PP" group, $n=23$ women). Controls were HIV-infected women with a HIV-uninfected infant ("PN" group, $n=23$ women). A second control group was constituted with HIV-uninfected mothers ("NN" group, $n=23$ women) for comparison of the impact of HIV status on milk FA composition. Controls were matched for infant age at the time of obtainment of breast milk samples that was closest to a postnatally HIV-infected infants' age at last negative PCR result (in a 1:1:1 ratio). The minimum infant's age at HIV infection was 49.5 days. For this study, we selected women with fatty acids, immune and inflammatory soluble factors, and HIV RNA load quantified in breast milk as well with antenatal plasma HIV RNA load and CD4 cell count documented. Breast milk including the lipid fraction was collected from stored (-80 °C) whole breast milk samples. RNA was isolated from 500 μL of lactoserum with the magnetic particle-based ASPS method (Abbott), and HIV load was quantified using the HIV Charge Virale assay (Biocentric) on the MJ Mini-Opticon quantitative PCR detection platform (Biorad), with a lower detection limit of 375 copies per mL of lactoserum [16]. This method enabled accurate assessment of cell-free viral load that is preferentially entrapped by lipids [17].

2.1. Biochemical analyses

Total cholesterol and triglycerides levels were quantified (April 2009 to December 2009) in whole milk with adaptation of enzymatic routine assays on the chemistry analyzer Architect C8000 (Abbott, Rungis, France). Within and between run imprecision for total cholesterol and triglycerides measurement was $<5\%$. Composition of milk FA was determined using gas liquid chromatography with flame-ionization detector (Focus GC, Thermo Electron Corporation). Briefly, lipids were extracted with methanol/chloroform and then trans-esterified with acidified methanol. The methyl esters of FA were extracted into hexane, evaporated under nitrogen and dissolved into isooctane. FA were separated using a VARIAN Cpsil88 capillary column [50m × 0.25 mm id × 0.25 μm df] with the following temperature program: initial at 80 °C with a 1 min hold; ramp: 15 °C/min to 140 °C, 1 °C/min to 170 °C, and 15 °C/min to 220 °C with a 10 min hold. The injector and detector were set at 220 °C. FA were identified according to their retention times determined through standards from SUPELCO 37 Comp FAME Mix (SUPELCO Analytical; Sigma Aldrich, Lyon, France). Chromatograms were collected, integrated and quantified using relative area peak with AZUR software (Thermo Electron Corporation, Thermo Fisher Scientific). Results are expressed as percentage of total fatty acid. Within-run and between run imprecision was $<12\%$.

Sodium and potassium concentrations were measured in lactoserum with ion selective electrode on the AU 640 chemistry analyzer (Beckman, Villepinte, France). β2-microglobulin and high sensitive C-reactive protein (CRP) concentrations in lactoserum were measured using routine immuno-turbidimetric assays on the same analyzer. Within-run and between-run imprecision for Na and K was $<3\%$, for CRP $<5\%$ and for β-2 microglobulin $<6\%$.

2.2. Chemokine/cytokine and immune factors assays

Cytokines and chemokines were quantified on lactoserum (diluted 1:2 in phosphate buffer saline) (December 2008–December 2009) using a multiplex microbeads assay (Invitrogen Human Cytokine 25-Plex Panel, MLX-Booster program, Marne-la-Vallée, France) and a Luminex 100 apparatus (Luminex, Oosterhout, The Netherlands) according to the manufacturer's instructions. This immunoassay was dedicated to simultaneous quantification of T-helper (Th)-1 related cytokines (IFN-α, IFN-γ, IL-2, IL-2r, IL-12(p40/p70), IL-15, IP-10 CXCL-9,MIG), Th-2 related cytokines (IL-4, IL-5, IL-10, IL-13), Th17 related (IL-17), inflammation related (IL-1α, IL-1RA, IL-6, IL-8, RANTES, TNF-α), antibacterial, (CCL-2, MCP-1, MIP-1α, MIP-1β), and IL-7, GM-CSF. Standard curves were established to determine cytokines concentrations. Between run imprecision ranged from 3.6 to 9.8% depending of cytokines. Three lots of kits from the same manufacturer were used. For each factor, the highest value of the low limit of quantification among the three different lots and the lowest value of the high limit of quantification were retained. Only, cytokines and chemokines detected in more than 50% of the breast milk samples of HIV infected mothers were statistically analyzed.

2.3. Ethics

The Vertical Transmission Study and breast milk analyses were conducted according to the guidelines laid down in the declaration of Helsinki and were approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal. All women provided signed informed consent for enrollment in the study, including the sample collections and the realization of biological analyses.

2.4. Statistical methods

Twenty three pairs enabled to detect for arachidonic acid a difference in percentage of total FA of 0.10 with 80% of power, 5%