Short Communication

Biochemical Nutritional Parameters in Breast-milk and Plasma of HIV Infected Lactating Nigerian Mothers on Anti-Retroviral Therapy

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ABSTRACT
Undernourishment in HIV infected individuals exacerbates immunosuppression, acceleration of HIV replication and CD4 + T cell depletion. The production of human milk (lactogenesis) is dependent on factors in the blood therefore deranged blood parameters in HIV patients are expected to reflect in the components of breast milk. Study on effects of HIV infection on nutritional components of breast milk and plasma is scarce. This study assessed the impact which HIV infection might have on the nutritional quality of human breast milk and plasma by determining the levels of biochemical nutritional factors such as albumin, pre-albumin, transferrin and retinol binding in HIV infected lactating mothers (n=20) and HIV-negative lactating mothers (n=30) using immunoplates. The mean plasma level of albumin was significantly reduced in HIV infected lactating mothers (HIM) compared with HIV-negative lactating mothers (control). Breast milk transferrin was significantly increased in HIM compared with the control. It is concluded from this study that hypoalbuminaemia is a common feature in HIV-infected lactating mothers.

INTRODUCTION
Human Immunodeficiency Virus (HIV) is a retrovirus that primarily infects vital organs and cells of the human immune system thereby culminating in low cellular immunity especially when the CD4^{+} T cells are less than two hundred (<200) per microliter (μL) of blood (Kallings, 2008). Human milk contains myriad of anti-inflammatory (IL-10, PAF-acetyl hydrolase, Lactoferrin, Lysozyme, Antitrypsin), anti-microbial (sIgA, IgM, IgG, Lactoferrin, Lysozyme, Complement factors) and anti-modulatory substances (Memory T cells, IL-4, IL-10, IL-12, G-CSF, IFN-α, IFN-γ, RANTES). Others are trace elements (such as Cu, Zn, Se, Fe), α-lactalbumin, soluble TNF-α receptors, interleukin-1 (IL-1) receptor antagonist, transforming growth factor β, antioxidants (vitamins and minerals), hormones, adiponectin, and glycans (Newburg and Walker, 2007).

Human milk also contains more than 10^9 leukocytes (Macrophages, Neutrophils, T cells and B-cells) per

liter for the first several months of lactation, with the highest number in the initial days and weeks. By six months of lactation, milk cells are 80% epithelial cells (Buescher, 2001). Previous studies reported polyclonal B cell activation, oxidative stress, hyperzincaemia, reduced Vitamin E, Se, Mg, Fe, Mn, Cu, Cr and Cd in the plasma of HIV infected Nigerian patients especially in patients with 200-499 CD4/μL blood (Arinola *et al.*, 2004). There have been relatively few studies on the effect of infection on milk quantity and quality (Filteau, 2000).

Since approximately 40% of breast milk samples from HIV-infected mothers contain HIV-RNA (John-Stewart *et al.*, 2004), the question becomes "Will the breast milk expressed by HIV infected lactating mothers be beneficial to their babies?" This present study provided part of the answers by determining the plasma and breast milk levels of biochemical nutritional factors (albumin, pre-albumin, transferrin and retinol binding protein in HIV -

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infected lactating mothers and HIV-negative lactating mothers in Nigeria. To the knowledge of the authors, this is the first of such study among HIV infected lactating mothers in Nigeria.

MATERIALS AND METHODS
Study Subjects
The subjects were twenty (20) randomly selected asymptomatic HIV- infected lactating mothers (28 ± 6.29 years of age) and thirty (30) age-matched HIV- negative lactating mothers (26.1 ± 4.11 years of age). They were recruited from Sexually Transmitted Infections (STI) and Immunization Clinics of Adeoyo Maternity Teaching Hospital, Yemetu, Ibadan, Nigeria after obtaining an informed consent from each patient. Five milliliters (5ml) of venous blood and mature breast milk (15 days - 2 months post birth) were collected from each participant on the same day. Those on special medication (aside from anti-retroviral drugs), history of recent blood transfusion, premature delivery, hepatitis or mastitis infections were excluded from the study.

Nevirapine, anti-retroviral drug was used by HIV patients starting from 3rd trimester. This was to prevent mother-to-child transmission of HIV. The blood samples were collected into heparinised bottles to obtain plasma while the milk samples were collected into plain sterile plastic tubes. The breast milk samples were spun at 8000 x *g* for 5 minutes and the fat layer was carefully removed to obtain fat-free milk plasma. A rapid assay using agglutination procedure (Capillus HIV-1 and 2) (Trinity Biotech, USA) was used for the screening of suspected subjects and controls. Those tested positive to HIV-antibodies were confirmed with purified multiple recombinant antigens (Hexagon HIV-1 and 2) (KoRa Healthcare, Ireland).

Determination of Pre-albumin, Albumin, Retinol binding protein and Transferrin
These parameters were determined based on the principle of single radial immunodiffusion in immunoplates (Charles-Davies *et al.*, 2006). Wells of the prepared immunoplates were filled with standard proteins (25%, 50%, 100% and 200%) or plasma samples. The plates were incubated for 4 hours at room temperature and the diameters of precipitin rings were measured using an illuminated Hyland viewer with a micrometer eyepiece.

Data Analysis
Data were presented as Mean ± Standard Deviation. Student t-test (using pooled variance) was used to test the significance of difference between the mean values. Pearson's correlation (r) was used to test for correlation between the values in the plasma and those obtained in the breast milk samples. The probability (*p*) values less than 0.05 were considered significant. The statistical analyses were done using SPSS version 15.0.

RESULTS
The mean plasma level of albumin was significantly reduced in HIM compared with the control. There were no significant differences in the mean plasma levels of transferrin, pre-albumin and retinol binding protein in HIM compared with control (Table 1). The mean breast milk level of transferrin was significantly increased in HIM compared with the control. There were no significant differences in the mean breast milk levels of albumin, prealbumin and retinol binding protein in HIM compared with control (Table 2). There were no correlations between the levels of nutritional factors in the plasma and breast milk samples of HIM or control (Table 3).

Table 1: Plasma Levels of Biochemical Nutritional Factors in HIM and Control

Biochemical Nutritional Factors	HIM (n = 20)	Control (n = 30)	*t*-values	*p*-values
Transferrin (g/L)	2.68 ± 0.96	2.66 ± 1.04	0.064	0.949
Prealbumin (g/L)	0.18 ± 0.14	0.22 ± 0.13	1.014	0.316
Retinol binding Protein (g/L)	0.08 ±0.05	0.07 ± 0.69	0.557	0.580
Albumin (g/L)	9.39 ± 4.46	26.18 ± 18.43	3.886	*0.000

*HIM = HIV-infected Lactating Mothers, *p is significant at <0.05 values (2-tailed)*

Table 2: Breast Milk Levels of Biochemical Nutritional Factors in HIM and Control

Biochemical Nutritional Factors	HIM(n = 20)	Control (n = 30)	*t*-values	*p*-values
Transferrin (g/L)	0.21 ± 0.20	0.11 ± 0.19	3.246	*0.010
Prealbumin (g/L)	0.65 ± 5.24	0.59 ± 0.12	0.750	0.788
Retinol binding protein (g/L)	0.060 ± 0.00	0.059 ± 2.64	0.793	0.832
Albumin (g/L)	4.625 ± 2.06	4.567 ± 0.38	0.619	0.690

HIM = HIV-infected Lactating Mothers, *p is significant at ‹0.05 values (2-tailed)

Table 3: Correlation between the Levels of Biochemical Nutritional Factors in the Plasma and Breast Milk Samples of HIM and Control

Biochemical Nutritional Factors	HIM group (n=20)		Control (n=30)	
	r-values	*p*-values	*r*-values	*p*-values
Transferrin (g/L)	-0.015	0.997	-0.023	0.611
Prealbumin(g/L)	0.032	0.850	-0.199	0.291
Retinol binding protein (g/L)	-0.152	0.535	0.055	0.771
Albumin (g/L)	-0.173	0.479	0.145	0.68

HIM = HIV-infected Lactating Mothers

DISCUSSION

Human immunodeficiency virus (HIV) causes gradual and progressive failure in immune system. The hallmark of this infection is a profound decrease in the number and functions of circulating CD4^{+} T cells (Alimonti *et al*., 2003). Nutritional factors play an important role in maintaining normal immunity (Maggini *et al*., 2007). Hence, a compromised nutritional status which is a common observation in HIV patients (Arinola *et al*., 2005) may complement reduced CD4^{+} T cells and aggravate the disease condition. Since most previous studies on HIV patients were not gender- and condition- specific, therefore studies on HIV-infected lactating mothers may enhance evidence-based HIV management as pregnancy is known to be an immune-modulatory period. Moreover, Shedding of HIV in breast milk continues to pose a risk for late transmission of HIV to suckling babies (Richardson *et al*., 2003; John-Stewart *et al*., 2004).

In this present study, reduced plasma albumin was observed among the HIV positive cohort. This might be due to excessive urination, diarrhoea, loss of appetite or decreased absorption induced by anti-retroviral therapy and redistribution from plasma albumin to tissues as a result of response to residual HIV infection. Serum albumin is a negative acute-phase protein which is down-regulated in inflammatory states. Also, albumin transports many drugs and is synthesized in the liver using dietary protein (Kragh-Hansen, 1990). Thus, low level of albumin in the HIV patients examined might indicate on-going inflammation and liver damage in them. The level of transferrin was significantly raised in breast milk of HIM compared with the control group. Transferrin is an antioxidant and iron transporter. Binding of transferrin with iron makes iron unavailable for replication by certain micro-organisms (Charles-Davies *et al*., 2006). Several epidemiological data have shown the benefits of breastfeeding in preventing infections (Brandtzaeg, 2003; Hanson *et al*., 2003). Therefore, raised level of transferrin in breast milk of HIM might be to provide mucosal innate immune factor to the babies.

Although, we have been able to provide a preliminary data on the biochemical nutritional quality of the breast milk of HIV-infected lactating Nigerian mothers, a major limitation of this study was in the small number of subjects investigated

which may not be a thorough representative of HIV-infected lactating mothers in Nigeria. Additionally, the study failed to measure some viral, immunological and nutritional markers of the patients such as viral load, CD4 count, presence of Fe, Cu and other trace elements, BMI and mid-upper arm circumference analyses and co-infections. Because these factors may further compromise the immune status of the subjects with important implication on the general outcomes, they are planned to be included in future studies. Nevertheless, despite high level of transferrin in breast milk reported in this study, we cannot recommend breastfeeding for HIV infected mothers pending further research.

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