


Polyunsaturated Fatty Acid Composition in Breast Milk Plasma of HIV-infected and Uninfected Mothers in Relation to Infant Clinical Outcomes

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ABSTRACT

BACKGROUND: The increased coverage of prevention of mother to child transmission (PMTCT) services has significantly reduced paediatric HIV infection incidence. The aim of the study was to compare breast milk omega-6 and omega-3 polyunsaturated fatty acid profiles of HIV infected and uninfected mothers and determine the association between fatty acid profiles and postnatal transmission of HIV, morbidity/mortality of HIV exposed and unexposed infants.

METHODS: A prospective cohort study of 57 HIV infected and 57 HIV uninfected lactating mothers was conducted in Gweru, Zimbabwe from July 2019 to March 2020. The women's 114 babies (term and preterm) were also enrolled and stratified by HIV exposure and infection status. The mother-infant pairs were followed up at 6 weeks, 16 weeks and 6 months postpartum to determine, HIV transmission rate, breast milk polyunsaturated fatty acid profiles as well as infant clinical outcomes.

RESULTS: The mean breast milk docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels in HIV uninfected mothers (0.82 ± 0.92 ; $0.47 \pm 0.75 \mu\text{g/ml}$ respectively) at 6 weeks postpartum were significantly higher compared to those of HIV infected mothers (0.33 ± 0.32 ; $0.08 \pm 0.14 \mu\text{g/ml}$) respectively. The same pattern was observed at 16 weeks postpartum in terms of DHA and EPA mean concentration. However, the arachidonic acid (AA) levels and AA/DHA ratio measured at 6 weeks postpartum were significantly higher in HIV infected mothers (2.31 ± 2.01 ; 17.18 ± 52.47 respectively) compared to HIV uninfected mothers (0.82 ± 0.54 ; 9.71 ± 21.80 ; $P < .001$). A higher morbidity rate was observed amongst HIV exposed infants than HIV unexposed infants (3.26 ± 0.13 ; 2.49 ± 0.09 ; $P < .001$) respectively. A significant positive correlation was observed between AA and infant morbidity ($r = .388$; $P < .001$).

CONCLUSION: Deficiencies in breast milk omega-3 fatty acids were observed in HIV infected women. Maintaining a healthy balance between omega-6 and omega-3 fatty acid diets is critical for breast feeding mothers regardless of their HIV status. The adverse clinical outcomes observed amongst HIV exposed infants emphasise their vulnerability under conditions of maternal universal antiretroviral therapy.

KEYWORDS: Polyunsaturated fatty acids, HIV, breast milk, morbidity, infant clinical outcome

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Introduction

Lipids are the most abundant macronutrient components of breast milk and these include especially long-chain polyunsaturated fatty acids (LC-PUFAs), which possess both a metabolic and nutritional functionality.¹ LC-PUFAs are a group of fatty acids which contain 18 to 20 or more carbons, and include the 2 families of the omega-3 (n-3) and omega-6 (n-6) fatty acids. LC-PUFAs are converted endogenously from the precursors; alpha-linolenic acid (ALA) and linoleic acid (LA) through a

series of desaturation and chain elongation steps present in the omega-3 and omega-6 pathways.² Arachidonic acid (AA), Eicosapentaenoic acid (EPA) and Dicosahaexanoic acid (DHA) are important for infant and foetal growth and development¹. In a Danish trial, supplementation of maternal diet with fish oil during lactation led to an increased body mass index (BMI) and waist circumference at 2.5 years of age in infants.³

Sufficient evidence exists from animal experiments and basic research on the critical role of LC-PUFAs in infant



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neurological development. The concentrations of LC-PUFAs among membrane phospholipids determine membrane fluidity, which is important for membrane functions, including receptors, membrane-bound enzymes and ion channels which can influence neuronal functions.⁴ In addition to influencing membrane properties, omega-3 LC-PUFAs determine the quantity of neurotransmitters such as serotonin and dopamine.⁵ Previous studies have established that poor accumulation of retina and brain DHA often causes poor visual acuity, abnormal retinal physiology, increased stereotyped behaviours duration of visual fixation and locomotor activity.⁶ However, performance levels can be restored through dietary supplementation of DHA in animals with reduced brain DHA concentration. Makrides⁷ reported that fortified infant formula containing 0.58% EPA, 0.36% DHA and 0.27% γ -linolenic acid exhibited enhanced visual evoked potential acuity compared to control infant formula without DHA.

It is postulated that LC-PUFAs particularly EPA and DHA contribute in various functions of the immune system. LC-PUFAs regulate lymphocyte activation, neutrophil, macrophage and natural killer cell function. These roles are facilitated by modulation of lipid peroxidation and eicosanoid pathways. The fatty acid composition of immune and inflammatory cells is determined by dietary intake.⁸ Breast milk fatty acids possess direct gastrointestinal tract antimicrobial activity in infants. Epithelial cells of the intestine maintain a balance between the absorption of essential nutrients such as fatty acids and blocking the proliferation of pathogens.^{9,10} Anti-inflammatory effects of gamma-linolenic acid (GLA), DHA, ALA and EPA, were evaluated in the IL-1 β -mediated inflammation model in the Caco-2 cell line.¹¹ The LC-PUFAs decreased the release of inflammatory cytokines which include IL-8 and IL-6. In n-3 LC-PUFA activated cells, this effect was facilitated by the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPAR γ).

In addition to antiviral properties in infants during the early stages of life, LC-PUFAs have potential to reduce the risk of vertical transmission of HIV by blocking pathogens that affect mucosal barriers resulting in MTCT of HIV.¹² Studies have documented that LC-PUFAs, including DHA and AA, are essential for the development of T cell function in infants.¹³ In previous studies, infants who received formula milk containing DHA and AA displayed high lymphocyte populations, increased cytokine levels, and immune cell maturation that was similar to those of breastfed infants. These findings concluded that dietary uptake of DHA and AA, whether through infant formula or breast milk contributes to immune development.¹³ Maternal nutritional status affects breast milk fatty acid composition and subsequent risk of HIV transmission through breast feeding. Some studies have suggested that LC-PUFAs effectively inactivate enveloped viruses by inhibiting HIV-1 reverse transcriptase or by enhancing the viability of HIV-targeted CD4+ T cells.^{14,15}

Globally, over 1 million HIV exposed and uninfected (HEU) children are born to HIV positive women each year¹⁶ and evidence suggests that these children especially from middle and low income countries are at risk of adverse clinical outcomes. They have increased risk of impaired growth, neurodevelopmental abnormalities, morbidity and mortality than their HIV unexposed peers born from HIV uninfected mothers.¹⁷⁻²¹ In Zimbabwe, previous studies have recorded significant prevalence of under-nutrition, anaemia and decreased LC-PUFA status in 7 to 10 year old children born in a national prevention of mother to child transmission (PMTCT) programme.^{22,23} However, these studies were undertaken in the era of short periods of antiretroviral therapy (ART) for PMTCT. Therefore, there is need to examine polyunsaturated fatty acid status in the setting of fully suppressive maternal ART throughout pregnancy, Option B+ therapy, which is now the standard of care. The purpose of this study was to compare breast milk omega-3 and omega-6 fatty acid profiles in HIV infected and uninfected lactating women and to determine the association between breast milk polyunsaturated fatty acid profiles and infant morbidity/mortality within the first 6 months of life.

Methodology

Study participants

A prospective study of 114 HIV infected breast feeding women receiving universal antiretroviral therapy and HIV-uninfected lactating women together with their infants (114) was conducted. Participants were stratified equally by HIV infection status; HIV infected women and their infants (n=114) and HIV-uninfected women and their infants (n=114). Information regarding maternal HIV status, antiretroviral use, duration and regimen was obtained and recorded. Participants were recruited from Gweru district polyclinics in Zimbabwe at 6 weeks post-delivery after obtaining written informed consent from each participant. Eligible infants were those whose mothers had given written informed consent, at 6 weeks of age, born singleton, being breastfed exclusively for 6 months and visiting the same clinic with their mothers for post antenatal care. Mothers were considered for the study if they had no severe obstetric complications or psychiatric disorders.

On recruitment, participants were subjected to HIV antibody testing using Determine test kits (Abbott Diagnostics, Abbott Park, Illinois, U.S.A.) to confirm infection status. HIV negative mothers were retested after a further 6 weeks using the same protocol to detect possible sero-conversion. Blood plasma (5 ml) was collected from HIV infected mothers for determination of baseline plasma HIV RNA viral load. Matured breast milk samples (10 ml) were collected by manual expression into labelled sterile conical tubes from all the participants at 6 and 16 weeks postpartum for determination of LC-PUFAs (EPA, DHA and AA). Milk samples were stored at -80°C pending analysis. The mother-infant pairs were

followed up from enrolment to 6 months post-delivery to determine HIV transmission rate, infant morbidity and mortality. Anthropometric measurements including mid upper arm circumference, height and weight were recorded at enrolment. Ethical approval was granted by the Medical Research Council of Zimbabwe (MRCZ/A/2466) before commencement of study.

Determination of HIV viral load

Maternal plasma was quantified for HIV-1 RNA load using an automated Roche COBAS® TaqMan® 96 Analyser (Roche Diagnostics Ltd, Rotkreuz, Switzerland) with a lower detection limit of 20 copies/ml.

Fatty acid methyl ester (FAME) extraction and gas chromatography analysis

Whole milk samples were first homogenised for 30 seconds using a vortex mixer (Heidolph Vortex Shaker REAX 1. 220 V. 30W Germany). Fatty acid extraction was conducted using a method by Kelishadi et al.²⁴ The esterified sample extract (1 µl) was injected into the gas chromatography machine (GC-7890A, Agilent Technologies, USA) coupled with 5975C VL MSD with Triple-axis detector equipped with a splitless capillary intel system. Separation was achieved on capillary column (29.5 m × 250 µm × 0.25 µm; Agilent 19091S-433HP-MS) using helium gas as carrier gas. Oven temperature was maintained at 100°C for 5 minutes (min), then increased by 5°C/min to 200°C and held there for 20 minutes followed by increase by 5°C/minutes to 220°C and followed by another 5°C/minutes increase to 300°C. The temperature was maintained for 10 minutes. The total analysis time was 85 minutes. The injector and the detector temperatures were set at 200°C and 250°C, respectively. Fatty acids of interest (DHA 22:6n-3, AA 20:4n-6, EPA 20: 5n-3) were identified by comparing the retention times of sample FAME with a standard FAME mixture (SUPELCO 37 Component, FAME mix, Sigma Aldrich, USA). Samples were analysed in duplicate. Results were expressed as concentration of fatty acids in microgrammes per millilitre (µg/ml).

Sample size calculation

Schumann's sample size calculation formula was used to determine the minimum sample size (n) as

$$n = \frac{3CV^2 \times (Z_\alpha + Z_\beta)^2}{d^2}, \text{ where coefficient of variation}$$

(CV) = 50% is the expected inter-individual variability in fatty acid composition, $Z_\beta = 0.84$, the standard value for a normal distribution at power (β) = 80%, $Z_\alpha = 1.96$ the critical value for a standard normal distribution at level of significance (α) = 5%, $d = 20\%$, the difference in fatty acid exposure considered

clinically significant in HIV infected or un-infected mothers,

$$n := \frac{2CV^2 \times (Z_\alpha + Z_\beta)^2}{d^2} = \frac{2 \times 0.5^2 \times (1.96 + 0.84)^2}{0.20^2} = 114.$$

Statistical analysis

Statistical analysis was performed using STATA version 14.2 software (Stata Corporation, College Station, Texas, USA). Data was summarised by proportions (%), mean ± standard deviation for normally distributed data and median interquartile range (IQR) for non-normal data. The Pearson's Chi square test was used to assess differences in categorical variables. The independent t-test was used for analysis of normally distributed continuous variables. The mean fatty acids concentrations between the lactating mothers were compared using Kruskal-Wallis test and the change in fatty acid concentration over time was determined using the Wilcoxon rank sum test. Pearson's correlation coefficient was used to determine the association between breast milk fatty acids with HIV viral load, duration of ART or infant morbidity. A P -value < .05 was considered to be statistically significant.

Results

Baseline maternal characteristics

Altogether, 114 participants were enrolled into the study. These comprised 57 HIV infected and 57 HIV uninfected mothers. The mean age of HIV infected mothers was significantly higher than that of HIV-uninfected mothers ($P < .001$). However, there were no significant differences in terms of weight, height, parity, gravidity and mid upper arm circumference (MUAC) between groups by HIV status. The majority of HIV infected mothers were married ($n = 25$, 43.9%), whilst the majority of HIV negative mothers were single ($n = 23$, 40.3%). No significant differences in education level or employment status were observed between HIV infected and uninfected mothers (both $P > .05$). Of the HIV-infected mothers, 50 (87.8%) were on Tenolam E ART regimen whilst only 7 (12.3%) were on Tenolam N. On the other hand, 10.5% of the participants had HIV viral load levels >1000 copies/ml whilst 89.5% had viral loads <1000 copies/ml. The median duration on ART was 48 months, IQR: 8 to 60 months (Table 1).

Baseline infant characteristics

A total of 114 mother-infant pairs were included in the study. Among 57 infants born from HIV infected mothers, 5 were HIV infected by 6 weeks of life, whilst 52 were HIV exposed but uninfected. On the other hand, 57 HUU were also included in the study. There were significant differences in mean birth weights between HIV exposed and HUU infants (2.9 ± 0.3 ; 3.2 ± 0.5 respectively, $P < .001$). The mean head circumference at birth was significantly lower for HIV exposed infants compared to their unexposed counterparts (33.7 ± 1.7 ; 35.1 ± 2.5)

Table 1. Demographic and clinical characteristics of breast feeding mothers.

CHARACTERISTICS		HIV-INFECTED N=57	HIV-UNINFECTED N=57	P1
Age (years)	Mean (SD)	30.6 (5.4)	26.5 (5.9)	<0.001
Marital status (n %)	Married	25 (43.90)	20 (35.1)	0.530
	Divorced	10 (17.5)	10 (17.5)	
	Single	16 (28.1)	23 (40.4)	
	Widowed	6 (10.5)	4 (7)	
Education (n %)	Primary	7 (12.3)	1 (1.8)	0.083
	Secondary	34 (59.6)	40 (70.2)	
	Tertiary	16 (28.1)	16 (28.1)	
Employment (n %)	Employed	15 (26.3)	14 (24.6)	0.835
	S-employed	25 (43.9)	23 (40.4)	
	Unemployed	17 (29.8)	20 (35)	
ART Regimen n %				
TDF + 3TC + EFV600	Tenolam E	50 (87.7)	N/A	
TDF + 3TC + NVP	Tenolam N	7 (12.3)	N/A	
Viral load copies/ml	Median(IQR)	56 (0-215)	N/A	
Viral load > 1000	n %	6 (10.5) N/A	N/A	
Duration of ART (m)	Median(IQR)	48 (8-60)	N/A	
Monthly income US\$	Median(IQR)	81 (69-98)	69 (58-87)	0.009
Monthly income <\$50	n %	3 (5.3)	9 (15.8)	0.067
Gravidity	Mean (SD)	2.9 (1.2)	2.6 (1.4)	0.240
Parity	Mean (SD)	2.4 (1)	2.5 (1.2)	0.932
MUAC (cm)	Mean (SD)	26.4 (3.4)	26.2 (3.6)	0.847
Height (cm)	Mean (SD)	163.3 (5.8)	164.3 (95.9)	0.366
Weight (kg)	Mean (SD)	64.2 (12.6)	63.4 (12)	0.728

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; EFV600, Efavirenz 600mg; IQR, interquartile range; m, months; MUAC, mid upper arm circumference; N/A, non-applicable; NVP: nevirapine; SD, standard deviation; S-employed, self-employed; TDF, tenofovir disoproxil fumarate; US\$, United States dollar. P1: Comparison of characteristics of HIV-infected and HIV-uninfected mothers.

respectively, $P < .001$). A significantly higher proportion of HEU infants were born preterm before 37 weeks (82.7%) compared to the unexposed infants (31.6%), $P < .001$. There were significant differences in mean birth weight, birth length, head circumference and gestational age between HEU and HUU infants (Table 2).

Maternal breast milk fatty acid profile during lactation

The mean (SD) breast milk DHA and EPA levels in HIV uninfected mothers (0.82 ± 0.92 ; $0.47 \pm 0.75 \mu\text{g/ml}$) at 6 weeks

postpartum were significantly higher compared to those of HIV infected mothers (0.33 ± 0.32 ; $0.08 \pm 0.14 \mu\text{g/ml}$) respectively. The same pattern was observed at 16 weeks postpartum in terms of DHA and EPA mean concentrations in HIV infected (0.18 ± 0.23 ; 0.25 ± 0.55) and uninfected mothers (8.31 ± 9.25 ; 1.76 ± 4.93) respectively. In contrast, the AA levels and AA/DHA ratio measured at 6 weeks postpartum were significantly elevated ($P < .001$) in HIV infected mothers (2.31 ± 2.01 ; 17.18 ± 52.47) than in HIV uninfected mothers (0.82 ± 0.54 ; 9.71 ± 21.80). The same pattern was observed at 16 weeks postpartum in terms of breast milk plasma levels of AA and AA/DHA ratio in HIV infected (11.92 ± 10.96 ;

Table 2. Baseline infant characteristics by HIV status.

INFANT CHARACTERISTICS		CHE INFANTS N=57	HEU INFANTS N=52	HUU INFANTS N=57	P1	P2	P3
Gender n %	Female	30 (52.6)	27 (51.9)	29 (50.9)	0.730	0.851	0.767
Birth weight (kg)	Mean (SD)	2.9 (0.3)	3 (0.4)	3.2 (0.5)	0.531	<0.001	<0.001
Birth weight (<2.5 kg)	n %	6 (10.5)	6 (11.5)	3 (5.3)	0.422	0.297	0.234
Birth length (cm)	Mean (SD)	49.7 (1.8)	49.6 (1.9)	50.6 (1.7)	0.675	0.004	0.004
Head circumference, cm	Mean (SD)	33.7 (1.7)	33.8 (1.7)	35.1 (2.5)	0.345	<0.001	0.001
Gestational age (weeks)	Mean (SD)	35.5 (1.3)	35.4 (1.4)	37.1 (1.2)	0.373	<0.001	<0.001
Preterm birth <37 weeks	n %	47 (82.5)	43 (82.7)	18 (31.6)	0.880	<0.001	<0.001

Abbreviations: CHE, combined HIV –exposed; HEU, HIV –exposed and uninfected; HUU, HIV-unexposed and uninfected; SD, standard deviation.

P1: Comparison of characteristics of CHE and HEU infants.

P2: Comparison of characteristics of CHE and HUU infants.

P3: Comparison of characteristics of HEU and HUU infants.

Independent t-test and Pearson's Chi square test were used to compare demographic characteristics.

Table 3. Breast milk fatty acid profiles of HIV infected and uninfected lactating mothers.

FATTY ACID	GROUP	N	6TH WEEK POSTPARTUM	N	16TH WEEK POSTPARTUM	P ^A
			MEAN ± SD		MEAN ± SD	
DHA (µg/ml)	HU	57	0.82 ± 0.92***	57	8.31 ± 9.25 ***	<0.001
	HI	57	0.33 ± 0.32	57	0.18 ± 0.23	<0.001
EPA (µg/ml)	HU	57	0.47 ± 0.75***	57	1.76 ± 4.93 ***	0.063
	HI	57	0.08 ± 0.14	57	0.25 ± 0.55	0.889
AA (µg/ml)	HU	57	0.82 ± 0.54	57	0.46 ± 0.59	<0.001
	HI	57	2.31 ± 2.01***	57	11.92 ± 10.96 ***	<0.001
AA/DHA	HU	57	9.71 ± 21.80	57	5.43 ± 7.54	0.791
	HI	57	17.18 ± 52.47***	57	7.85 ± 31.80***	0.033

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HI, HIV infected; HU, HIV uninfected; SD, differences in fatty acids between groups at the same time point were compared using Kruskal-Wallis test.

P^a: Changes in fatty acid profiles over time.

Changes in fatty acid profile over time were determined using Wilcoxon Rank Sum test.

***indicate P < .001

7.85 ± 31.80) versus HIV uninfected mothers (0.46 ± 0.59; 5.43 ± 7.54) respectively (Table 3).

Over the course of lactation (6-16 weeks), there was a significant decrease in DHA concentrations in HIV infected (0.33 ± 0.32-0.18 ± 0.23 µg/ml; P < .001) whilst a significant increase in DHA concentration was observed in uninfected mothers (0.82 ± 0.92-8.31 ± 9.25 µg/ml; P < .001). In addition, a significant increase in AA concentrations over time was observed in HIV infected mothers (2.31 ± 2.01-11.92 ± 10.96; P < .001). However, a significant reduction in AA concentration was observed in the HIV uninfected group (0.82 ± 0.54-0.46 ± 0.59 µg/ml; P < .001). A significant

change in AA/DHA ratio was observed over the course of lactation in the HIV infected group (17.18 ± 52.47-7.85 ± 31.80 µg/ml; P = 0.033) (Table 3).

Clinical outcome of infants by HIV status over the first 6 months of life

Infant morbidity was defined by the number of documented infant sick clinic visits during the study period. There were significant differences in mean number of infant sick clinic visits between the CHE and HUU infants respectively (3.26 ± 0.13; 2.49 ± 0.09; P < .001). A mortality rate of 1.75% was recorded amongst combined HIV exposed infants whilst no deaths were

Table 4. Morbidity and mortality of HIV exposed and HIV unexposed infants.

CHARACTERISTIC	CHE INFANTS N=57	HUU INFANTS N=57	P VALUE
Morbidity mean (SD)	3.26 (0.13)	2.49 (0.09)	<.001
Mortality n %	1 (1.75)	0	.315

Abbreviations: CHE, combined HIV –exposed. HUU, HIV unexposed and uninfected. Morbidity is represented by the number of infant sick clinic visits. Bold indicate the significance of P value < .001.

witnessed amongst HIV unexposed and uninfected infants over the first 6 months postpartum (Table 4)

Association between early fatty acid profile (6 weeks) with infant morbidity, HIV viral load and duration of ART

There was a significant moderate positive correlation between AA and infant morbidity ($r=.388$; $P<.001$). However, no association was observed between breast milk EPA and infant morbidity ($r=.09$; $P=.338$). In addition, a weak positive insignificant correlation, was observed between breast milk DHA and infant morbidity ($r=.149$; $P=.004$). Significant positive correlations were observed between breast milk DHA, AA and the plasma HIV viral load ($r=.476$; $r=.505$; $P<.001$) respectively. Furthermore, no association was also observed between EPA and the plasma viral load ($r=.077$; $P=.568$). Breast milk DHA and EPA and AA levels were not associated with duration of ART ($r=-.064$; $P=.637$; $r=.051$; $P=.709$; $r=.192$; $P=.152$) respectively (Table 5).

Discussion

The present study sheds some important revelations with regards to breast milk LC-PUFA profiles in HIV infected women taking option B⁺ therapy for HIV treatment as well as HIV uninfected women. Higher concentrations of breast milk omega-3 fatty acids; EPA and DHA were observed in HIV uninfected mothers compared to HIV infected mothers whose concentrations were far below the recommended limits. However, higher breast milk omega-6 fatty acids; AA were observed in the HIV infected group. This is consistent with findings from previous studies which demonstrated significantly lower levels of polyunsaturated fatty acids (EPA, DHA and DPA) at baseline in HIV infected participants compared to their HIV uninfected counterparts.²⁵ HIV infection and treatment with ART have been reported to alter serum lipid profiles.^{26,27} There are several reports on the association of ART and the lipodystrophy syndrome; a disturbance of lipid metabolism often characterised by dyslipidaemia, insulin resistance, and fat maldistribution, which presents as cervical fat pad accumulation and visceral abdominal obesity.^{28,29}

A study conducted in Tanzania, reported significantly elevated breast milk omega-6 LC-PUFAs including AA and dihomo- gamma linolenic acid in mothers who did not vertically transmit HIV to their foetuses compared to those who did.¹⁴ However, there were no significant differences in terms of omega-3 fatty acid concentration between the 2 groups. Furthermore, it was concluded that higher omega-6 fatty acid concentrations reduced MTCT of HIV by about 79% possibly by dissolving the HIV viral envelopes.^{15,30} Therefore, the significantly elevated AA levels observed in the current study in patients on universal antiretroviral therapy could have contributed to the low MTCT of HIV rates (8.8%) witnessed in the study.

On the other hand, the current study reported a significant decrease in DHA concentration over the course of lactation in HIV infected participants. A similar pattern was observed in a study conducted in Sweden involving analysis of breast milk composition of mothers who delivered preterm infants.³¹ A reduction in LC-PUFAs was observed from postnatal day 7 to 40 weeks postpartum. It has previously been reported that the duration of pregnancy impacts on milk fatty acid composition.³¹⁻³³ After preterm delivery, the immature mammary gland has a significantly reduced capacity for conversion and uptake of long chain fatty acids from circulation to milk lipids. This in turn may stimulate de novo fatty acid synthesis as a compensatory mechanism.³⁴ In this study, 82.5% of HIV infected mothers had preterm delivery whilst only 31.2% of HIV negative mothers had given birth prematurely. Therefore, the increase in breast milk DHA and EPA levels observed in the HIV negative participants from 6 to 16 weeks postpartum in the present study can be attributed to maturity of the mammary gland for uptake of LC-PUFAs since the majority of mothers had delivered at full term.

In this study, a persistently elevated AA/DHA ratio was observed in HIV infected mothers over the course of lactation (17:1; 8:1) compared to their HIV negative counterparts (10:1; 5:1) from 6 to 16 weeks respectively. The ratio of *omega-6/omega-3* is of nutritional importance because it is the key index for balanced synthesis of eicosanoids in the body.³⁵ The recommended optimum ratio range should be 4:1 to 5:1 and must not exceed 10 for optimal infant nutrition.^{36,37} Excessive amounts of omega-6 LCPUFAs and a very high omega-6/omega-3 ratio, which is typical of today's Western diets, promote the pathogenesis of cancer, cardiovascular disease, inflammatory and autoimmune diseases, whilst high levels of omega-3 fatty acids (a reduced omega-6/omega-3 ratio) are protective.³⁸

It has been postulated that individuals on ART continue to have persistently elevated levels of T cell activation and inflammation and this has been linked to mortality and end organ disease in this vulnerable group.^{39,40} Therefore, the overwhelmingly elevated AA/DHA ratio observed in this study in the presence of HIV infection indicates the possible role of

Table 5. Correlation between breast milk fatty acids with plasma viral load, duration of ART and infant morbidity.

FATTY ACID	MORBIDITY		PLASMA VIRAL LOAD		DURATION OF ART	
	R-VALUE	P-VALUE	R-VALUE	P-VALUE	R-VALUE	P-VALUE
DHA (µg/ml)	.149	.114	.476	<.001	-.064	.637
EPA (µg/ml)	.09	.338	.077	.568	.051	.709
AA (µg/ml)	.388	<.001	.505	<.001	.192	.152

Abbreviations: AA, arachidonic acid; ART: antiretroviral therapy; DHA, docosahexanoic acid; EPA, eicosapentaenoic acid.

Pearson's correlation coefficient was used to determine the association between fatty acid profiles and infant morbidity, plasma viral load and duration of ART. Bold indicate the significance of *P* value < .001.

diet as a cause of the persistent inflammation associated with antiretroviral therapy. Although a decline in the omega-6/omega-3 ratio over the course of lactation was evident, the ratio still remained above the recommended optimum levels leading to possible adverse effects in both mothers and infants. It is critical to note that although the omega-6/omega-3 fatty acid ratio was lower in HIV uninfected mothers, the values recorded were above the recommended limits. Therefore, close monitoring of maternal nutrition is prudent during the lactation period in all mothers regardless of their HIV status to maintain optimum omega-6 versus omega-3 fatty acid balance.

A significant moderate positive correlation was observed between DHA, AA and the plasma viral load in HIV infected participants in the current study. However, in another study, breast milk concentrations of total omega-3 fatty acids and AA, were inversely correlated with cell free virus shedding into breast milk.¹⁴ The discordance in these findings could be attributed to the fact that the present study measured plasma viral load instead of breast milk viral load. It has been documented that there is no association between breast milk viral load and the plasma HIV viral load.⁴¹ It is also critical to note that there was no association between breast milk fatty acid concentration at 6 weeks and the duration of ART.

Poorer health outcomes in HIV exposed infants compared to their unexposed counterparts were evident during the first 6 months of life. A higher proportion of sick clinic visits was observed in this vulnerable group. Adverse consequences of infant HIV exposure have been documented particularly in low and middle income countries.⁴² Increased risk of infectious morbidity has been observed amongst HEU infants compared to HUU infants with more childhood infections and hospitalisations occurring during neonatal period due to predominantly acute respiratory infections, and bacterial infections.^{43,44} Additionally, mortality, morbidity, and long-term neurodevelopmental disability has been reported amongst preterm neonates.⁴⁵ In the current study, the majority of HIV exposed infants had a gestational age below 37 weeks. Recent studies have estimated a twofold higher child mortality amongst HEU compared to HUU children during the first to second year of life, and a persistent pattern between 2 and 5 years of age.^{46,47} However, in the current study, the mortality rate was not

significantly different between the 2 groups probably due to the shorter follow up period of only up to 6 months post-partum.

An important observation from the current study is the significant positive correlation between breast milk AA and infant morbidity. An imbalance of omega-3 and omega-6 LC-PUFAs during critical periods of development may result in adverse effects on the health of the new-borns.⁴⁸ In this study, a persistently high breast milk AA/DHA ratio was witnessed particularly in HIV infected mothers throughout lactation. In a recent study, breast milk with an increased ratio of omega-6 to omega-3 LC-PUFAs was reported as having higher soluble pro-inflammatory cytokines.⁴⁹ Therefore, adequate nutrition and a good balance of essential fatty acids is critical for breast feeding mothers due to the importance of polyunsaturated fatty acids in prevention and management of diseases and maintenance of optimum health for the developing infant.

A strength of the current study is that breast milk samples were collected at 2 time points during lactation which gave a reliable reflection of LC-PUFAs supply to the infants over the course of early lactation which is critical for infant immunity. It is therefore unlikely that fatty acids from sources other than breast milk could have confounded the associations between breast milk fatty acids with immunological parameters, infant growth or other infant clinical outcomes because all the mothers enrolled into the study committed to exclusive breastfeeding for at least 6 months. Another important strength of the study is its prospective nature and the clinical procedures involving specimen collection which were conducted by trained clinical staff as well as anthropometric measurements that were obtained using calibrated equipment.

In terms of limitations, genetic variations in breast feeding mothers cannot be totally excluded as possibly influencing variations in fatty acid proportions in breast milk. However, such gene-diet interactions in polyunsaturated fatty acid metabolism warrant further inquiry particularly genetic polymorphisms of the elongase and desaturase enzymes involved in LC-PUFA synthesis. In addition, all PMTCT mothers enrolled into the study were already on ART and the non-availability of ART naive breastfeeding maternal controls precluded discernment of possible pure effects of HIV infection from those attributable to ART on breast milk fatty acid profiles

Conclusion

The findings of grossly decreased breast milk omega-3 fatty acid concentration; DHA, EPA in HIV infected women highlights the need to support supplementation with omega-3 fatty acids during pregnancy and lactation to cater for the needs of the infants during early postnatal life. In this study, a persistently elevated breast milk AA/DHA ratio was observed over the course of lactation in all mothers. An increased omega-6/ omega-3 ratio has detrimental health effects due to its association with inflammation and development of metabolic syndrome.³⁸ Monitoring and raising awareness of the need to maintain a healthy balance in consumption of omega-6 versus omega-3 fatty acid diets is critical for breast feeding mothers regardless of their HIV status. In addition, higher infectious morbidity was observed in HIV exposed infants compared to their unexposed counterparts during the first 6 months of life. Morbidity rate was significantly correlated with breast milk concentration of omega-6 fatty acids particularly AA.

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