ORIGINAL ARTICLE

Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian human immunodeficiency virus-infected lactating mothers

Sheu K. Rahamon, Ganiyu O. Arinola, Moses O. Akiibinu[1]

Immunology Unit, Department of Chemical Pathology, College of Medicine, University of Ibadan, [1]Department of Chemical Pathology, Olabisi Onabanjo University, Ago-Iwoye, Oyo State, Nigeria

Background: The effect of Human Immunodeficiency Virus (HIV) on the immune system is well documented however; its impact on the nutritional and immunological qualities of the breast milk is scarce. **Aim:** Levels of some essential trace metals, albumin and antioxidant status in the plasma and breast milk of Nigerian HIV-infected lactating mothers were determined. **Materials and Methods:** Essential trace metals and total antioxidant potential were measured using spectrophotometric method while albumin was measured using single radial immunodiffusion technique. **Results:** Only the mean plasma level of albumin in HIV infected lactating mothers (HIM) was significantly reduced when compared with HIV free lactating mothers (HFM) [9.39 (4.46) g/L vs 26.18 (18.43) g/L, *P*=0.000], others had no significant difference. The mean breast milk levels of total antioxidant potential (1776.82(564.26) µmolTE/L vs. 2384.67 (679.00) µmolTE/L, *P* = 0.0000), Cu [67.68 (5.04) µg/dL vs. 71.10 (5.45) µg/dL, *P* = 0.033] and Fe [66.21 (6.31) µg/dL vs. 71.20 (6.48) µg/dL, *P* = 0.011] were significantly reduced in HIM compared with HFM. No significant differences were observed in other parameters. **Conclusion:** It could be concluded from this study that hypoalbuminemia is a feature of HIV-infected lactating mothers and that breast milk of HIM has low antioxidant capacity.

Key words: Breast milk, essential trace metals, human immunodeficiency virus, lactating mothers, total antioxidant potential

INTRODUCTION

Human milk is a complex mixture of interacting compounds, which differ markedly within the lactation period. It contains a variety of bioactive and immunologic factors acting synergistically to protect the suckling infant at a time when its immune system is still immature, enhancing quick gut maturation and, attenuating inappropriate inflammatory reactions.[1] The effect of Human Immunodeficiency Virus (HIV) on the immune system is well documented but its impact on the nutritional and immunological qualities of the breast milk is scarce.

Essential trace metals and their corresponding antioxidants play important roles in growth, development and effective immune responses.[2] Abnormalities in plasma mineral and trace elements, especially of zinc, copper, iron and magnesium have been reported in HIV-infected individuals.[3] Loss of appetite, decreased absorption of nutrients, diarrhea, urinary losses of nutrients, and redistribution of trace metals from plasma to tissues as a result of response to infection[4] are some abnormalities associated with infection. Samba and Tang[5] reported that low levels of zinc and selenium have been associated with adverse clinical outcomes during HIV infection, and this was related with increased activation of NF-kB, which is a key regulator of HIV.[6]

Human milk is a complex biological fluid with a high bioavailability of trace metals.[1] For effective exclusive breastfeeding, milk must provide adequate amounts of all the essential nutrients, including the trace minerals needed for normal growth and development.[7] Borkow *et al*.[8] reported that copper has potent virucidal properties. Levels of trace elements, such as selenium, depend to a large extent on the type of food taken, which in lactating women affect the trace elements content of breast milk.[9]

The adverse effects of HIV on the immune system and certain plasma antioxidant levels are well documented,[10] but impact of HIV infection on the nutritional and immunological qualities of the breast milk is scarce. More so, there have been relatively few studies of the effect of infection on milk quality and quantity.

Since, HIV is a blood borne pathogen and the production of human milk (lactogenesis) is dependent on factors in the blood,[11] it is therefore, hypothesized that HIV

Address for correspondence: Mr. Sheu Kadiri Rahamon, Immunology Unit, Department of Chemical Pathology and Immunology, College of Medicine, University of Ibadan, Nigeria. E-mail: adekunlesheu@rocketmail.com
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infection which affectsplasma levels of some micronutrients and antioxidant indices may also have corresponding effects on the components of breast milk. The relationship that exists between HIV infection and breast milk quality is an area of research that is yet to be explored, thus necessitating this study.

MATERIALS AND METHODS

Subjects

The subjects were 20 asymptomatic HIV-infected lactating mothers (HIM) (28 ± 6.29 years of age) and 30 age-matched HIV-free lactating mothers (HFM) (26.1 ± 4.11 years of age). They were recruited from the Sexually Transmitted Infections (STI) and Immunization Clinics of Adeoyo Maternity Teaching Hospital, Yemetu, Ibadan, Nigeria after obtaining an informed consent from each patient. Ethical approval was also obtained from the Adeoyo Hospital Management (AMH/OG/1208).

Five milliliters (5 ml) 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, history of recent blood transfusion, hepatitis or mastitis infections, those infected post-partum and those with pre-term delivery were excluded from the study. The blood samples were collected into heparinized bottles to obtain plasma while the milk samples were collected into trace metal-free plastic tubes. The breast milk samples were spun at 8000Xg for 5 min and the fat layer was carefully removed to obtain fat-free milk plasma.

Determination of trace metals

All the materials (glass and plastic) used were thoroughly cleaned with hot solution of nitric acid (20%, v/v) for 48 h and rinsed five times with deionized water. Trace metals (Cu, Zn, Se, Fe, Mn and Mg) levels were determined using Beck 200 Atomic Absorption Spectrophotometer as described by Arinola *et al*.[12] The method is based on the principle that atoms of the elements vapourize when aspirated into the AAS and absorb light of the same wavelength as that emitted by the element when in the excited state.

Determination of total antioxidant potential (TAP)

Ferric reducing-antioxidant power (FRAP) assay was used to determine the total antioxidant potential as described by Benzie and strain.[13] The method is based on the reduction of the Fe^{3}+-TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferrous form at low pH. Samples were mixed with FRAP reagent (1:10) and incubated at room temperature for 5 min to allow reduction of Fe^{3}+-TPTZ to ferrous form by the antioxidants in the sample. The reduction was determined by measuring the absorbance of each sample at 593 nm expressed as µmol of Trolox-equivalents/L. The concentration of TAP in each sample was read from the standard curve plotted using absorbance values of serial standard samples.

Determination of albumin

Single radial immunodiffusion technique was used for the determination of albumin.[14] The diameter of precipitin ring formed after antigen-antibody reaction in a buffered agar gel is proportional to the concentration of albumin present in either the plasma or breast milk plasma. A volume of diluted monospecific antiserum was properly mixed with noble agar and poured on glass plate. Wells of equal diameter were made in the antibody/agar gel and filled with standard plasma or test. The plates were incubated for 4 h at room temperature and the diameters of precipitin rings were measured using an illuminated Hyland viewer with a micrometer eyepiece.

Statistical analysis

Student's *t*-test was used to compare the differences between the mean. Pearson's correlation coefficient was used to correlate values of blood plasma with milk plasma using SPSS version 15.0 (*http://www.spss.com*). $P < 0.05$ value was considered significant.

RESULTS

As shown in Table 1, the mean blood plasma levels of Cu, Se, Zn, Fe, Mn, Mg and TAP were not significantly different when HIM was compared with HFM. However, significant reduction in the mean blood plasma level of albumin was observed in HIM compared with HFM.

In Table 2, significant reduction was observed in the levels of Cu and Fe in the breast milk plasma of HIM compared with HFM. Also, TAP was significantly reduced in the breast milk plasma of HIM compared with HFM.

Only Mn in milk plasma of HIM showed significant positive correlation with Mn in blood plasma ($r = 0.469$, $P = 0.043$) as shown in Table 3.

DISCUSSION

Human immunodeficiency virus (HIV) causes gradual and progressive failure in immune response. Nutritional factors play an important role in maintaining normal immunity[12] hence, a compromised nutritional status, such as malnutrition, which is a common observation in HIV patients, may complement reduced $CD4^{+}$ T cells in aggravating the disease condition. The unique significance of milk to the health and growth of newborn mammals has been known for ages.[15-18] However, interaction between infections, especially HIV infection, and breast milk quality has been a neglected area of study.

Table 1: Levels of the essential trace metals, total antioxidant potential and albumin in blood plasma of human immunodeficiency virus infected lactating mothers and human immunodeficiency virus free lactating mothers

Biochemical parameters	HIM (*n*=20)	HFM (*n*=30)	*t* values	*P* values
Zn (µg/dL)	147.11±12.17	154.27±25.78	1.130	0.264
Cu (µg/dL)	71.05±2.72	71.23±3.94	0.175	0.862
Fe (µg/dL)	77.74±3.56	76.67±4.40	0.890	0.378
Se (µg/L)	74.95±3.31	74.27±4.48	0.570	0.571
Mn (µg/dL)	67.74±3.14	67.47±3.33	0.283	0.779
Mg (mg/L)	13.04±1.02	13.93±2.06	1.761	0.085
TAP (µmolTE/L)	850.11±230	884.75±545.74	0.262	0.795
ALB (g/L)	9.39±4.46	26.18±18.43	0.389	*0.000

Zn=Zinc; Cu=Copper; Fe=Iron; Se=Selenium; Mn=Manganese; Mg=Magnesium; TAP=Total antioxidant potential; ALB=Albumin; Values are presented as mean±standard deviation; **P* is significant at *P*<0.05 value

Table 2: Levels of the essential trace metals, total antioxidant potential and albumin in breast milk of human Immunodeficiency Virus infected lactating mothers and human Immunodeficiency Virus free lactating mothers

Biochemical parameters	HIM (*n*=20)	HFM (*n*=30)	*t* values	*P* values
Zn (µg/dL)	145.68±19.70	147.97±15.54	0.451	0.654
Cu (µg/dL)	67.68±5.04	71.10±5.45	2.198	*0.033
Fe (µg/dL)	66.21±6.31	71.20±6.48	2.652	*0.011
Se (µg/L)	64.11±6.26	65.23±3.17	0.835	0.408
Mn (µg/dL)	61.32±7.11	64.07±4.67	1.638	0.108
Mg (mg/L)	12.66±1.03	13.09±0.91	1.509	0.138
TAP (µmolTE/L)	1776.82±564.26	2384.67±679.00	1.949	*0.000
ALB (g/L)	4.62±0.00	4.62±0.00	4.025	0.999

Zn=Zinc, Cu=Copper; Fe=Iron; Se=Selenium; Mn=Manganese; Mg=Magnesium; TAP=Total antioxidant potential; ALB=Albumin; Values are presented as mean±standard deviation; **P* is significant at *P*<0.05 value

Table 3: Correlation between milk plasma parameters with blood plasma parameters in human Immunodeficiency virus infected lactating mothers and human immunodeficiency virus free lactating mothers

Biochemical parameters	HIM group (*n*=20)		HFM group (*n*=30)	
	r values	*P* values	r values	*P* values
Cu (µg/dL)	−0.355	0.135	−0.093	0.626
Zn (µg/dL)	0.042	0.866	−0.249	0.184
Se (µg/L)	−0.112	0.647	0.299	0.108
Fe (µg/dL)	−0.022	0.928	0.233	0.215
Mn (µg/dL)	0.496	*0.043	−0.157	0.406
Mg (mg/L)	0.282	0.242	−0.187	0.321
TAP (µmolTE/L)	−0.111	0.651	0.000	0.999

Zn=Zinc; Cu=Copper; Fe=iron; Se=Selenium; Mn=Manganese; Mg=Magnesium; TAP=Total antioxidant potential; **P* is significant at *P*<0.05 value

The difference between the mean blood plasma levels of the trace metals (Cu, Se, Fe, Zn, Mn and Mg) in HIM and HFM were not statistically significant. The non-significant level of Cu agrees with the report of Arinola *et al*.[12] However, the non-significant levels of Se, Fe, Zn and Mg were in contrast with the reports of Fuchs *et al*.[19] Arevalo-velasco *et al*.,[20] Semba and Neville[21] and Arinola *et al*.[12] who reported low levels of these trace metals in sera of HIV-infected individuals. They reported that insufficient intake, malabsorption, diarrhea and impaired storage are some of the reasons for their observations. It is likely that supplements taken during pregnancy and post-partum might have increased the levels of the essential trace metals. It is also likely that the pathophysiology of HIV is different between sexes or physiological status (pregnancy, lactation etc). This deserves further study. Total antioxidant potential reflects the concentration and activity of many components which prevents oxidative degradation of of fats and proteins.[22] No significant change was observed in the mean blood plasma level of TAP in HIM compared with HFM. This might be due to adequate blood plasma levels of antioxidant trace metals observed in HIM and HFM. No previous studies compared TAP in HIM with HFM. Most of the previous antioxidant studies in HIV patients concentrated on plasma antioxidant trace metals in this group of people.[12,20]

Levels of copper (67.684 ± 5.04 µg/dL) and iron (66.21 ± 6.31 µg/dL) were significantly low in the breast milk plasma of HIM compared with HFMc (71.10 ± 5.45 µg/dL; 71.20 ± 6.48 µg/dL, respectively). The reduced level of Cu in HIM may be due to non-suckling of the breast milk since non-suckling was reported to culminate in low prolactin level thereby causing low level of Cu in breast milk. Suckling increases milk Cu secretion due to its direct relationship with circulating prolactin level.[23] Similarly, the level of TAP in the breast milk plasma of HIM (1776.82 ± 564.26 µmolTrolox equiv./L) was significantly low compared with HFM (2384.67 ± 679.00 µmolTrolox equiv./L). This low TAP level in the breast milk plasma of HIM could be due to low levels of Cu and Fe observed in the breast milk plasma of this group of people since TAP is an index of various classes of antioxidants.

Copper (Cu) is normally tightly bound to caeruloplasmin (CLP), a minor fraction is loosely associated to albumin and low molecular weight chelators.[24] The concentration of Cu in human milk is about 20-25% of that in the serum. During early lactation, plasma Cu concentration is high and is primarily bound to serum albumin and amino acids. In contrast, during late lactation, plasma Cu is low and it is primarily bound to CLP.[24] This shows that infants on prolong breast feeding are in negative Cu balance.[25] A report that breast milk Cu concentration decreases during lactation due to reduced Cu supply to the mammary gland as a result of decrease in serum Cu concentration supports our observation.[25]

The concentration of iron in breast milk is often considered as low, both in relation to serum iron and to estimated iron requirements of infants. Its concentration in breast milk is about 20-30% of serum iron. The relatively low

concentration of iron in human milk might be to prevent iron toxicity in babies, since term-babies are born with iron stores that can be mobilized for utilization during the first six months of life.[26] Iron concentration which declines during lactation occurs with decrease in transferrin receptor and ferroportin expression.[27] This suggests that iron uptake by the mammary gland and its secretion into milk is functionally decreased and not due to tissue iron depletion. Domelleöf *et al*.[28] reported that no correlation exists between human milk iron and iron-status variables in the serum. This is also supported by our finding.

Hypoalbuminemia found in our HIV subjects corroborate the reports of Treitinger *et al*.[29] and Arinola *et al*.[12] This was reported to be due to malabsorption and malnutrition common in HIV-infected individuals as a result of anorexia, intestinal insufficiency and chronic intestinal colonization by pathogenic microorganisms.[4] It might also be conjectured to becaused by a switch in synthesis of protein from transport protein (like albumin) to protective protein. No significant difference was however, observed in the breast milk plasma level of albumin of HIM compared with HFM.

It could be concluded from this study that hypoalbuminemia is a feature of HIV-infected lactating mothers and that breast milk of HIM has low antioxidant capacity. Although, the low total antioxidant potential in the breast milk of HIM further supports the campaign against breastfeeding by People Living with HIV and AIDS, it could be suggested that HIV-infected mothers who insist on breastfeeding their babies should supplement breastfeeding with antioxidant rich supplements.

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