Immunoglobulin Classes and Nutritional Factors in Plasma and Breast Milk of Lactating Mothers in Nigeria

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ABSTRACT

Background: Breast milk is important for the overall well-being of infants. Although lactation is relatively robust in the face of poor nutrition, the implication of poor nutrition on non-nutritive factors in breast milk is inconclusive. **Objective:** This study was designed to find associations between nutritional and immune factors in maternal blood and breast milk with the aim to improve the needed public and individual strategies for a healthy infant. **Method:** A cross sectional study was conducted on 61 lactating Nigerian women aged 23-40years within the first 3 months postpartum. Anthropometric measurements were obtained while nutritional factors (total protein, albumin) and immunoglobulin classes (IgG, A and M) were estimated by Biuret, Bromocresol green and single radial immunodiffusion methods respectively in maternal plasma and breast milk. **Results:** Most (73.5%) of the lactating mothers had normal mean body mass index (i.e. not under weight nor obese) and the mean levels of plasma total protein, albumin, IgG, IgA and IgM were within normal reference ranges in these mothers. Nutritional and immunological indices increase in the plasma with length of lactation but decrease in breast milk with lactation. There were no correlation between BMI, plasma indices and milk indices in these lactating mothers. **Conclusion:** This study supports the superiority of colostrum over transitional or matured milk for the protection and nourishment of infants.

Keywords: Breast milk, Immune status, Nutritional status, Anthropometric measurements, Infant health

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INTRODUCTION

Breast milk is a complex fluid, rich in nutrients with anti-infective properties for the healthy, physical and mental development of babies in the first four to six months of life (1, 2, 3). The incident and severity of certain bacterial and viral infections of the intestinal tract and respiratory system are significantly lower in breast-fed compared to non-breast-fed infants (4). Apart from contraceptive effect of breast-feeding, when the breast-fed infant ingests pathogenic bacteria during nursing, often no enteric symptoms develop (4, 5).

Secretory antibodies against bacteria and viruses in human colostrum and milk are known to be protective factors for the breast-fed infant. Colostrum has been shown to contain IgA and IgM antibodies to a number of autoantigens (native DNA, actin, myosin, myoglobin, laminin, transferrin and thyroglobulin). Natural autoantibodies may contribute to the selection process of physiological repertoire during postnatal period in breast-fed infants resulting in lower frequency of inflammatory and auto-immune diseases and lymphomas seen in later life (4). Hypogammaglobulinemia and deficient antibody responses to injected antigens do occur in very young infants that are not properly breast fed (1). The previous observations above lead to the prediction that the protection afforded by human milk was due to antimicrobial agents in human milk and these agents may vary in quality and quantity with stage and length of breast-feeding.

A study showed that poorly nourished mothers had normal lactational experience (2), however, the implication of poor nutrition on non-nutritive factors in breast milk is inconclusive (5, 6). Moreover, Rasmussen and Kjolhede (7) related overweight and obesity to early lactation failure (7). These reports (5,6,7) conducted on breast milk were largely qualitative and without laboratory based studies (8,9).

Studies on immunological factors in breast milk are scarce in Nigeria. Kassim et al (10) demonstrated significant in-vitro growth inhibition of *P. falciparum* by 144 breast milk samples from Nigerians, Oyedele et al (11) showed that anti-measles IgA dropped below protective cut-off within the 1st 2 weeks of birth in 55 colostrum and 347 breast milk samples from Nigerians, Adu and Adeniji (12) suggested that very little level of measles antibody is passed through the breast milk.

This study was designed to find the levels of nutritional and immune factors in maternal blood and breast milk, and also to find association between these factors in maternal blood and breast milk.

MATERIALS AND METHODS

Subjects. A total of 61 lactating apparently healthy Nigerian women aged 23-40 years were recruited from Adeoyo State Hospital, Ibadan, Nigeria. They were within the first 3 months (1-96days) postpartum. The mean (+S.E) age of mothers was 25.4 (±0.7) yrs while their parity was 2.94 (±0.2). To avoid bias, mothers with complaints of ill health and preterm babies were excluded.

Anthropometric characteristics such as body weight and height of mothers were taken using sensitive weighting balance and meter rule respectively. Body mass index (BMI) was calculated as weight (kg)/height(m)^2.

Sample Collection. Informed consent was obtained from mothers before the collection of whole blood and breast milk. Blood was obtained by venous puncture into lithium heparin tubes and spun at 3000rpm for 5 minutes to obtain plasma and stored at -20^{0}C until analysis. Milk samples were collected by manual expression and stored at -20^{0}C. Before used for analysis, frozen milk was thawed and defatted as described (9,13).

Biochemical Analysis. Total protein, albumin and immunoglobulin classes were estimated by Biuret method, Bromocresol Green method (14) and single radial immunodiffusion technique (15) respectively.

Nutritional Status. Total protein and albumin in plasma and maternal BMI were used as maternal nutritional indices while total protein and albumin in milk were used as milk nutritional indices. BMI was used to categories mothers as shown below:

BMI (kg/m^2)	Categories	
<16.5	Grade 3	underweight
16.5-17.4	Grade 2	underweight
17.5-18.4	Grade 1	underweight
18.5-24.9	Normal	
25.0-29.9	Overweight/ Grade 1-obesity	
30-39.9	Grade 2-obesity	
>40	Grade 3 –obesity	

Stages of Lactation. This was based on a previous description (1)
Days 1-5 of lactation = 1st stage of lactation and milk obtained was colostrum.
Days 6-15 of lactation = 2nd stage of lactation and milk obtained was transitional milk.
Days16 – 96 of lactation = 3rd stage of lactation and milk obtained was mature milk.

Statistics. Statistical software SPSS version 10 was used for statistical analysis. Pearson's correlation coefficient was used to measure the closeness of association between maternal and milk nutritional and immunological indices. ANOVA and Student's t-test were used to compare mean ± SE of variables and to show their statistical significance. $p \leq 0.05$ was considered significant.

RESULTS

Anthropometric Measurements. The mean (S.E) of weight, height and BMI of all mothers recruited for this study was 54.7(1.1)kg, 1.57(0.02)m, 22.4 (0.4)kg/m^2 respectively. Based on the BMI, 7(11.4%) of the mothers were found to be underweight, 45(73.8%) women had normal BMI, and 9 (14.7%) were obese (Table 1).

Table 1. Maternal nutritional status as assessed by body mass index

BMI (kg/m^2)	Nutritional Status	No. of subjects	%
<16.5	Grade 3 underweight	0	0
16.5-17.4	Grade 2 underweight	1	1.6
17.5-18.4	Grade 1 underweight	6	9.8
18.5-24.9	Normal	45	73.8
25.0-29.9	Obesity grade 1	6	9.8
30-39.9	Obesity grade 2(obese)	3	4.9
>40	Obesity grade 3(obese)	0	0

Nutritional and Immunological Indices in Plasma of Mothers. The mean (S.E) of total protein, albumin, IgG, IgA and IgM in plasma of mothers were 6.8(0.1)g/100ml, 3.6(0.1)g/100ml, 1920.7(141)mg/100ml, 241.1(13.7)mg/100ml, and 332.7(31.2)mg/100ml respectively. These values are within the local reference ranges. The mean (S.E) of total protein, albumin, IgG, IgA and IgM in plasma of mothers at different stages of lactation are shown in Table 2. It showed that the least levels of total protein, albumin, IgG, IgA and IgM were observed in mothers at their 1st stage of lactation. However, only total protein, albumin and IgG showed statistically significant differences when the 3 groups were compared ($p<0.05$).

Table 2. Comparison of nutritional and immunological indices in plasma of mothers at various stages of lactation

Index	Stage 1 n=10	Stage 2 n=10	Stage 3 n=30	F	P
Total protein (g/dl)	5.9(0.4)	6.6(0.2)	7.1(0.1)	10.8	0.00
Albumin (g/dl)	2.9(0.2)	3.4(0.2)	3.8(0.2)	5.9	0.01
1gG (mg/dl)	1066.7(144.1)	1571.3(242.4)	2321.5(212.0)	6.8	0.00
IgA (mg/dl)	221.3(29.4)	248.5(32.5)	241.3(18.4)	0.2	0.80
IgM (mg/dl)	363.3(78.3)	381.3(100.0)	318.5(43.3)	0.3	0.80

Nutritional and Immunological Indices in Colostrum, Transitional, and Mature Milk. The mean (S.E) of biochemical indices in colostrum, transitional and mature milk are shown on Table 3. Comparison of indices showed decreases from colostrum to transitional to mature milk. However, only in total protein and albumin showed statistically significant different ($p<0.05$). IgG was higher in mature than transitional milk although the difference was statistically insignificant ($p>0.05$).

Table 3. Comparison of nutritional and immunological indices of breast milk at various stages of lactation (colostrum, transitional, and matured milk)

Index	Colostrum n=4	Transitional milk n=11	Mature milk n=27	F	P
Milk protein (g/dl)	5.0(1.0)	2.9(0.3)	2.8(0.2)	5.6	0.01
Milk albumin (g/dl)	0.7(0.1)	0.4(0.07)	0.5(0.08)	3.4	0.04
Milk IgG (mg/dl)	21.8(10.6)	12.9(1.5)	17.2(2.0)	1.2	0.30
Milk IgA (mg/dl)	67.0(22.3)	47.5(5.6)	37.8(4.1)	3.0	0.06
Milk IgM (mg/dl)	53.5(38.1)	30.9(6.9)	17.6(4.0)	2.9	0.07

Correlations between Nutritional and Immunological Indices in Maternal Plasma and Breast Milk. As shown in Table 4 and Table 5, no correlation existed between nutritional and immunological indices in maternal plasma and breast milk.

Table 4. Correlations between maternal plasma and milk nutritional / immunological indices

	n	r	p
Plasma tot. protein correlated with breast milk tot. protein	38	+0.17	0.32
Plasma albumin correlated with breast milk albumin	38	+0.14	0.41
Plasma IgG correlated with breast milk IgG	38	+0.08	0.63
Plasma IgA correlated with breast milk IgA	39	-0.11	0.52
Plasma IgM correlated with breast milk IgM	36	+0.12	0.48

Table 5. Correlations between maternal plasma total protein, albumin and BMI with milk nutritional / immunological indices

	Milk IgG	Milk IgA	Milk IgM	Milk Tot. prot	Milk albumin
	r p	r p	r p	r p	r p
Plasma tot. prot	-0.06,0.72	-0.02,0.92	-0.2, 0.24	+0.1,0.32	+0.11,0.52
Plasma albumin	+0.34,0.04	+0.32,0.05	+0.14,0.42	+0.04,0.83	+0.14,0.40
BMI	+0.09,0.70	+0.09,0.70	+0.03,0.20	+0.14,0.42	+0.10,0.32

DISCUSSION

Substances in colostrum and breast milk confer significant disease resistance to the breast-fed infant and these protective qualities of colostrum and milk may be influenced by maternal nutritional status (13). Malnutrition affects humoral immune system in diverse fashions (1). In this study, we observed that most of the mothers had normal BMI. Moreover plasma levels of total proteins and albumin were within normal reference range. This may be an indication of improved antenatal care in these mothers, which may explain the basis of normal levels of immunoglobulin classes as measured in these subjects.

Total protein, albumin and IgG in maternal plasma significantly increased from 1st to 3rd stage of lactation. Insignificant increase in IgA and decrease in IgM were observed from first to third stage of lactation. This may be due to continuation of Th 2 immune responses characteristic of antibody production, which is predominant in the late stage of pregnancy. Maintenance of standard postnatal care may also be conjectured. It may also be hypothesized that a shift in the synthesis of immunoglobulin classes, albumin and total proteins was concentrated to the plasma rather than breast milk. This in a way may boost maternal immunity and reduce passive immunity to the growing baby.

Breast milk contains immunostimulants, anti-inflammatory agents and promoters of protective microflora (15). Of the 3 types of milk, colostrum contained highest concentration of total protein, albumin, IgG, IgA and IgM. This is in support of a previous finding in which leucocytes are highest in the earlier phase of lactation and gradually decline during the next 2-3 months of milk production (16). Total protein, albumin, IgG, IgA and IgM levels decreased from colostrum to mature milk thus indicated the highly variable nature of breast milk and also showed the nutritional / immunological importance of colostrum The implication of increased nutritional and immunological factors in colostrum is the provision of these factors to newborn babies who are immunologically immature and are more prone to infections than at later in life.

The differences in the concentrations of immunologic factors in colostrums and matured milk may indicate adaptability in the recipient infant. The levels of immunoglobulin in the newborn are demonstrable between 1-3 months post birth. At birth, neonatal serum contains almost no IgA, small amount of IgM and adult level of IgG, almost of which is passively transferred maternal IgG. The levels of transferred immunoglobulin classes decrease with age of babies who have commenced gradual synthesis of these immunoglobulin classes (17). Reduced levels of nutritional / immunological indices in matured milk compared with colostrums may be related to physiological phenomena that enhance increase in flow of milk post birth, which may result in dilution of the breast milk content. Miranda et al (13) observed that

significant reduction in colostrum levels of IgG, IgA and C4 disappears in mature milk, concomitant with the improvement in the nutritional status of malnourished mothers.
Differences in the qualities of colostrums, transitional and matured milk may be related to compartmentalization in the source of breast milk (mammary glands) with lactation period, thus supporting the complex physical structure of human milk as earlier proposed (14, 15).
The present study did not observe relationships between plasma biochemical indices, BMI and breast milk parameters, thus it may be concluded that maternal nutrition is related to breast milk quantity but not quality. The deduction is that maternal nutrition though important might not be the only factor influencing child health. The precise ways in which defense systems in human milk operate in the recipient to fend off infections, minimize phlogistic reactions, or speed the maturation of the infant's defense systems should be the subjects of intense study.

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