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Total Protein, Secretory Immunoglobulin A and Lactoferrin Concentrations in the Breastmilk of Lactating Women and their Correlation with Nutritional Intake

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Abstract

Background and objectives: Mothers' breastmilk is the best source of nutrition for infants; hence, its nutritional adequacy is essential. The purpose of this study is to evaluate the total protein, secretory immunoglobulin A (sIgA) and lactoferrin (LF) concentrations of breastmilk from lactating mothers in Jakarta and analyse their correlation with average daily calory, macronutrient and vitamin C and E intakes.

Methods and study design: A cross-sectional study was conducted in two primary health centres in Jakarta, Indonesia. The breastmilk's total protein concentration was measured with the Warburg–Christian method, while sIgA and LF concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Macronutrient and vitamin C and E intake was assessed through 24-hour food recall and a semi-quantitative food frequency questionnaire (FFQ), respectively.

Results: Thirty-six lactating women were recruited. The mean plasma protein was 8.17 g/dl, breastmilk protein was 3.36 g/dL, sIgA was 4.37 g/L and LF was 14.36 g/L. The total energy, carbohydrate, protein, fat and vitamin C and E daily intakes were 1828.61 kcal/day, 234.76, 71.15, 72.31 gram/day, 109.7 mg/day and 5.60 mg/day, respectively. Neither plasma and breastmilk total protein nor sIgA and LF concentrations were correlated with total energy, macronutrients or vitamin C and E daily intake.

Conclusions: The plasma total protein, breastmilk total protein, sIgA and LF concentrations of lactating women in Jakarta were within the normal range. These biochemical profiles were shown to be not significantly correlated with daily calory, macronutrient or vitamin C or E intake.

Keywords: breastmilk protein; secretory immunoglobulin A; lactoferrin; macronutrients; vitamins

Introduction

The quality and quantity of proteins in breastmilk are crucial for the healthy growth and long-term development of infants. The role of breastmilk nutrients in various mechanisms of immune modulation and antimicrobial activity is the primary reason it is the best source of nutrients for new-borns and infants.[1] The immunoactive properties of breastmilk are mainly enacted by two extensively-studied substances, namely lactoferrin (LF) and secretory immunoglobulin A (sIgA).[2]

LF is a pivotal substance for babies' innate immune system, which protects them from various types of viruses and bacteria.2 The protective mechanism of LF is conveyed through the polarisation of microorganisms' cell membrane and modulation of gene transcriptions, in particular transforming growth factor-β1 (TGF-β1).[3] Maternal immunoglobulin, which is transferred through breastfeeding, is the mainstay of new-borns' and infants' passive immune system. It also determines the composition of gut microbiota. sIgA is the predominant antibody in human breastmilk and thus plays an important role in the infant's immunity. SIgA confers epithelial protection on the infant by means of pathogen adhesion inhibition, intracellular neutralisation and agglutination, as well as protecting the intestine's epithelium.[4]

An adequate daily intake of calories, macronutrients and micronutrients by lactating women is a fundamental determinant of the quality of the breastmilk given to the baby.[5,6]. The purpose of this study is to evaluate the biochemical profile of breastmilk in terms of total protein, sIgA and LF concentrations and to evaluate the correlation of such parameters with the daily dietary intake profile of lactating women, in particular macronutrients and vitamin C and E.

Materials and Methods

This study is a part of larger research tree undertaken by the Department of Nutrition and Department of Biochemistry and Molecular Biology regarding inflammation status, biomarker concentration, nutritional concentration and oxidative stress status in breastmilk, with a special assessment of the docosahexaenoic acid (DHA), beta-carotene, zinc, C-reactive protein (CRP), superoxide dismutase (SOD), malondialdehyde (MDA), protein, immunoglobulin and LF concentrations. This research has been approved by the medical research ethical committee of Faculty of Medicine Universitas Indonesia by the registration number of KET-1007/UN2.F1/ETIK/PPM.00.02/2020.

Study population

The subjects were lactating women aged 20–40 attending routine maternal and neonatal checks in Petamburan public primary health centre (PHC) (West Jakarta) and Cilincing PHC (North Jakarta). The inclusion criteria were that the mothers breastfed exclusively and the babies were 0–6 months old and healthy, without significant congenital or acquired disorders. The exclusion criteria were mothers who had followed or started specific diet programmes (for example, a vegan diet) in the last year or had any kidney disease, grade II obesity, history of preeclampsia/eclampsia, preterm birth or other autoimmune/inherited metabolic diseases, as determined by medical history and a simple physical examination by the PHC doctor.

Daily total calory, macronutrient, vitamin C and vitamin E intake assessment

The daily total calory, carbohydrate, protein and fat intake was assessed through the 24-hour food recall method over two non-consecutive days. Meanwhile, the daily vitamin C and vitamin E intake was assessed through a semi-quantitative food frequency questionnaire (FFQ) by asking the subjects the amounts of vitamin-source foods

consumed daily on average for a month. The intake of macronutrients and vitamins was categorised as adequate if it reached 80–100% of the 2013 Indonesian recommended daily allowance (RDA).

Measurement of plasma total protein

Plasma total protein concentration was measured by means of the Warburg–Christian method. A 1-mL plasma sample (diluted 200 times) and 1 mL BSA with seven different concentrations were prepared (0.05, 0.1, 0.15, 0.2, 0.3, 0.4 and 0.5 mg/mL). The absorbance of the sample mixtures was then measured with a spectrophotometer with a wavelength of 280 nm. The obtained absorbance values were substituted for x in the y = ax + b equation to find y, after which the obtained y values were multiplied by a dilution factor of 200 to find the total protein concentration per g/dL unit.

Measurement of breastmilk lactoferrin and secretory immunoglobulin A concentration

Breastmilk sample collection

Before the breastmilk sample was collected, the mother breastfed with the breast from which the sample was to be taken. The milk sample was collected through manual breast pumping for 15–20 minutes until no more breast milk appeared. The fore milk and hind milk obtained from the pumping were then shaken until homogenous and poured into a sterile plastic container which was stored in a cool box at a temperature of 10–15oC in the collection sites. The samples were sent to the laboratory, where they were stored in the freezer at -80oC before the analysis. The LF and sIgA concentration of all subjects was measured together after all the subjects had undergone breastmilk sample collection.

Biochemical assay

The LF and sIgA concentrations were measured using a sandwich ELISA kit (Elabscience®, Bethesda, MD). The detection range of the kit is 0.3–20 ng/mL with an intra-assay and inter-assay coefficient of variation of less than 10%. The fat contents of the breastmilk were discarded by centrifugation. The assay was conducted in accordance with the manual guide. Breast milk samples were diluted 10 ,times. 100µL of standard

LF/sIgA and sample solutions were added to the well, after which the wells were incubated for 60 min at 37°C. The solutions were then removed from the well, and Biotinylated Detection Ab working solutions were immediately added to each well. After the wells had been aspirated and washed three times, 100µL HRP conjugate working solution was added and incubated for 30 minutes, after which they were washed again. A 90µL substrate reagent was then added and incubated for 15 minutes. After the stop solution had been added, the plates were read at a wavelength of 450 nm.

Results

Thirty-six subjects were measured for the primary outcomes. The subjects' characteristics are provided in **Table 1.** Most were 27 years old and had two children, with the most recent infant aged three months. Average maternal plasma protein was 8.17 g/dL, breastmilk total protein content was 3.36 g/dL, sIgA concentration was 4.37 and LF concentration was 14.36.

The macronutrient intake profile of the mother and percentage of adequacy according to RDA are given in **Table 2.** The total energy, carbohydrate, protein and fat daily intakes obtained by the 24-hour food recall method were 1828.61 kcal/day, 234.76 gram/day, 71.15 gram/day and 72.31 gram/day, respectively, while, daily vitamin C and vitamin A intake was 109.7 and 5.60 mg/day, respectively. Daily intake of all macronutrients was within an adequate range, according to the Indonesian RDA.

As presented in **Table 3**, the correlation of plasma and breastmilk total protein concentration with maternal age, parity or infant's age was not statistically significant. The breastmilk sIgA and

LF concentrations were not significantly correlated with maternal age or maternal parity. Recent infant's age was significantly correlated with the sIgA concentration but not with the LF concentration.

Table 4 provides the Spearman correlation tests of macronutrients as well as vitamin C and vitamin E intake with the maternal plasma protein, breastmilk protein, sIgA and LF concentrations. None of the maternal macronutrient intakes from 24-hour food recall were correlated significantly with the maternal plasma protein, breastmilk protein, sIGA or breastmilk LF concentration ($p>0.05$). Daily vitamin C and E intakes were similarly not correlated with plasma and breastmilk protein, sIgA or LF, but vitamin C intake was significantly correlated with maternal plasma protein concentration ($p=0.026$).

Table 1: The subjects' characteristics

Parameters	Data (n=36)
Mothers' age (years)	27.58 ± 4.68
Infants' age (months)	3.0 (1 – 5)
Parity	2 (1 – 4)
Maternal plasma protein	8.17 (6.74 – 13.27)
Breast milk protein concentration (g/dL)	3.36 (1.78 – 7.92)
Secretory immunoglobulin A (g/L)	4.37 ± 1.30
Breast milk lactoferrin concentration (g/L)	14.36 (1.157 – 92.651)

Table 2: Macronutrient intake profile of the mother and percentage of adequacy according to recommended dietary allowances

Macro/micronutrients	**Data (n=36)**
Total energy (kcal/day)	1828.61 (1055.48 – 3359.05)
adequate, n(%)	32 (88.9)
deficient, n(%)	4 (11.1)
Carbohydrate (gram/day)	234.76 (117.39 – 415.69)
adequate, n(%)	36 (100%)
deficient, n(%)	0 (0)
Protein (gram/day)	71.15 (45.81 – 143.27)
adequate, n(%)	22 (61.1)
deficient, n(%)	14 (38.9)
Fat (gram/day)	72.31 (36.80 – 129.88)
adequate, n(%)	23 (63.9)
deficient, n(%)	13 (36.1)
Vitamin C (mg/day)	109.70 (37.50 – 479.20)
adequate, n(%)	15 (41.7)
deficient, n(%)	21 (58.3)
Vitamin E (mg/day)	5.60 (2.10 – 43.10)
adequate, n(%)	34 (94.4)
deficient, n(%)	2 (5.6)

Table 3: The correlation between maternal and infant demographic characteristics and the maternal plasma protein, breast milk protein concentration, sIgA and breast milk lactoferrin concentration.

Parameters	Maternal age		Maternal parity		Recent infant's age	
	Correlation coefficient	*P-value*	Correlation coefficient	*P-value*	Correlation coefficient	*P-value*
Maternal plasma protein	-0.015	0.929[k]	0.021	0.905[k]	0.224	0.189[k]
Breast milk protein concentration (g/dL)	0.107	0.536[k]	0.200	0.241[k]	-0.147	0.392[k]
Secretory immunoglobulin A	0.245	0.149[p]	0.133	0.440[k]	0.550	0.001[*k]
Breast milk lactoferrin concentration	-0.115	0.504[k]	-0.095	0.582[k]	-0.302	0.073[k]

Table 4: The correlation between macronutrient and vitamin C and E intake with the maternal plasma protein, breast milk protein concentration, sIgA and lactoferrin concentration

Nutrients	Maternal plasma protein		Breast milk protein		sIgA		Breast milk lactoferrin	
	r	*P-value*	r	*P-value*	r	*P-value*	r	*P-value*
Total energy	-0.40	0.817	-0.203	0.235	0.110	*0.523*	-0.075	*0.665*
Carbohydrate	0.211	0.218	-0.195	0.254	-0.068	0.693	-0.053	0.758
Protein	-0.014	0.935	-0.194	0.258	-0.090	0.602	-0.095	0.582
Fat	-0.086	0.618	-0.167	0.329	0.114	0.507	-0.018	0.918
Vitamin C	-0.370	0.026[*]	0.125	0.468	-0.262	0.123	-0.019	0.913
Vitamin E	-0.290	0.086	-0.200	0.243	0.147	0.392	-0.008	0.965

r = correlation coefficient

All the correlation coefficients and p-values were obtained from a Spearman correlation test.

Discussion

Breastmilk proteins are the most important macronutrients for the growth, development and immunity of the infant. The protein concentrations depend on the stage of lactation and time after delivery.[7] Breastmilk proteins can be distinguished as true and bioactive proteins, both of which decrease gradually as the infants increase in age but remain relatively constant in ratio. Bioactive proteins mainly serve in immunomodulation and antimicrobial activities, digestive functions and gut development and are carriers for other nutrients. LF and sIgA are among the most extensively studied bioactive proteins which confer immunity on the baby.[1]

A large-scale study in China by Cai et al. involving 248 lactating women showed a trend that LF concentration decreased steadily during the first 30 days after birth and remained constant from day 31 to 330. The median values (p25–p75) of LF during the first 1–7 days of a newborn's life were 3.85 (3.13–4.12) g/L, decreasing to 1.19 (0.98–1.46) g/L at the 241–330-day stage. This study revealed that dietary pattern and maternal BMI were not correlated with breastmilk LF concentration. Its concentration in mature milk had no significant correlation with

various protein sources, vegetable consumption or maternal serum iron/haemoglobin concentrations. Meanwhile, there was significant variability in LF concentration among ethnicities and ages, as this study covered eight different regions across the nation. Older lactating women (≥30 years old) tended to have lower LF than those in the younger group (20–25 years old).[8]

A study by Lis-Kuberka et al. comparing the LF and SIgA concentration of mothers with and without gestational diabetes showed that there was no significant difference between the two groups. From the first to the fifteenth day of lactation, the concentrations of LF and SIgA were negatively correlated with milk maturation of hyperglycaemic (r = -0.37 and r = -0.48, respectively) and normoglycemic mothers (r = -0.31 and r = -0.48, respectively), and the values of coefficients were at almost the same level. The mean concentration of LF in the early colostrum (days 1–3) was 9.67 ± 6.56 g/L and 9.30 ± 4.81 g/dl in groups of mothers with and without diabetes mellitus (DM), respectively. In colostrum produced during the next period, days 4–7, the LF concentration slightly decreased to 8.05 ± 1.38 and 8.09 ± 1.37 g/L, respectively. The LF contents further decreased in the transitional milk (days 8–15), to 6.45 ± 2.12 and 6.67 ± 2.01, in both groups. Meanwhile, as regards the concentration of SIgA, the early colostrum mean concentration was 14.59 ± 14.06 and 17.44 ± 17.63 in DM and non-DM patients, respectively. It significantly decreased, to 11.37 ± 8.79 and 3.98 ± 1.75 g/L, in colostrum in days 4–7 and to 3.92 ± 4.19 and 3.56 ± 1.24 g/L in the transitional milk. The mean LF found in this study, 14.36 (1.15 – 92.65) g/L, was higher than that found in the two previous studies, although it was measured in lactating women whose babies were mostly two months old. Furthermore, the mean SIgA concentration in this study, 4.37 ± 1.30 g/L, was similar to the values of transitional milk SIgA contents in the latter study.[9]

Another study from an urban region in Central Java, Indonesia found a median LF concentration of 1.52 (0.38–2.94) g/L. Further identification based on the breastmilk lactation phase showed that the median values in this population were 1.60 (0.81–2.94) in colostrum, 1.99 (0.59–2.81) in transition milk and 1.07 (0.38–2.75) in mature milk. In this study, there were three maternal factors which were significantly correlated with the LF concentrations: mid–upper arm circumference (MUAC) at third trimester (r=0.246, p=0.029), recent infant's age (r=-0.272, p=0.015) and lactation stage (-0.294, p=0.009). Age, parity, BMI during lactation and MUAC during lactation were not significantly correlated with the LF.[10] The study by Fujita et al. involving Kenyan women found that milk sIgA was not correlated with maternal iron deficiency anaemia (IDA) incidence, but it was proportionally correlated with infants' age and maternal MUAC. The mean milk total protein and milk sIgA among 202 Kenyan women were 0.98 ± 0.16 (range 0.65–1.52) g/dl and 1491.88 ± 554.86 (range 438–3073) µg/ml.[11]

Studies are limited and inconclusive about the direct correlation between daily nutrient intake or supplements and the concentrations of a specific substance in breastmilk. A meta-analysis by Keikha et al. concluded that the dietary intake of lactating women, especially fatty acids and some micronutrients, including vitamin C, affects their respective contents within the breastmilk. Studies of the correlation between dietary protein intake from diets and protein concentration in breastmilk are still inconclusive, although most have shown a proportional correlation. Fatty acid compositions in the maternal diet are positively correlated with the fat contents of breastmilk, as demonstrated in various observational and interventional studies. Positive dietary intake-breast milk contents were demonstrated in various fat-soluble vitamins, but studies regarding vitamin C and vitamin E are still limited[5]. Another meta-analysis showed inconclusive trends regarding the effects of maternal dietary intake of macronutrients and micronutrients on their respective concentrations

within the breastmilk. This meta-analysis showed that the total energy and total protein were generally not significantly correlated with daily intake. As regards the fat contents of breastmilk, most studies have shown a positive correlation with dietary fat intake. For vitamin C, similarly, most studies tend to show a proportional correlation between dietary intake and breastmilk concentration[6]. This study did not find a correlation between maternal macronutrient intake and maternal serum and breastmilk protein concentration.

A study involving Greek lactating women showed that nutritional intake and maternal socioeconomic factors are correlated with the macronutrient contents of breastmilk. In that study, the mean protein concentration in breastmilk was 1.90 ± 0.43 g/dL. Education level, occupation and consumption of several foodstuffs were associated with the protein contents of breastmilk.[12] A study by Zarban et al. assessing the effects of vitamin C and E supplementation on lactating women showed that it significantly increased the total antioxidant content of the breastmilk. A daily supplementation of 500 mg vitamin C in effervescent tablets and 100 IU vitamin E in chewable tablets for 30 days increased the total antioxidant content from 610 to 716 µmol/L ($p=0.017$) and remained significant compared to the control non-supplemented group ($p=0.02$).[13] A longitudinal study in Brazil showed that the dietary intake of vitamin A and vitamin E did not significantly influence their concentrations in breastmilk. The concentration of both vitamins was relatively constant over the course of the breastmilk sample collection. However, dietary vitamin A did correlate with maternal serum retinol ($r=0.403$, $p=0.007$).[14] This study did not find any correlation between daily vitamin C and vitamin E intake and LF and sIgA concentrations. This study did not assess the vitamin concentrations within the breastmilk or the total antioxidant activities.

There are several limitations in this study. First, the number of samples for sIgA and LF was small and may therefore be not completely representative of the study population; moreover, it may not adequately reflect the correlation between infants' age and LF-sIgA concentration changes. Second, there was no consideration of timing of breastmilk sample collection with regard to infant's age. Third, there was no assessment of either the nutritional adequacy or wellbeing of the infants in this study.

Conclusion

The plasma total protein and breastmilk total protein, sIgA and LF concentrations of lactating women from two PHC in Jakarta were within normal range. Those biochemical profiles were shown to not significantly correlate with the daily calory, macronutrient, vitamin C or vitamin E intake.

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References

1. Haschke F, Haiden N, Thakkar SK. Nutritive and bioactive proteins in breastmilk. Annals of Nutrition and Metabolism 2016;69(Suppl. 2):16–26.
2. Siqueiros-Cendón, T, Arévalo-Gallegos, S, Iglesias-Figueroa, B et al. Immunomodulatory effects of lactoferrin. Acta Pharmacol Sin. 2014;35: 557–566.
3. Telang S. Lactoferrin: A Critical Player in Neonatal Host Defense. Nutrients. 2018;10(9): 1228.
4. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin N Am. 2013;60:49–74
5. Keikha M, Bahreynian M, Saleki M, Kelishadi R. Macro- and micronutrients of human milk composition: Are they related to maternal diet? A comprehensive systematic review. Breastfeeding Medicine 2017;12(9):517–27.

6. Bravi F, Wiens F, Decarli A, Dal Pont A, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: A systematic review. The American Journal of Clinical Nutrition 2016;104(3):646–62.
7. Lönnerdal B, Erdmann P, Thakkar SK, Sauser J, Destaillats F: Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective. J Nutr Biochem 2016;41:1–11
8. Cai X, Duan Y, Li Y, Wang J, Mao Y, Yang Z et al. Lactoferrin level in breast milk: a study of 248 samples from eight regions in China. Food & Function 2018;9(8):4216–4222.
9. Lis-Kuberka J, Berghausen-Mazur M, Orczyk-Pawiłowicz M. Lactoferrin and immunoglobulin concentrations in milk of gestational diabetic mothers. Nutrients 2021;13(3):818.
10. Rahfiludin M, Pangestuti D. Lactoferrin association with maternal nutritional status and lactation stages. Current Research in Nutrition and Food Science Journal. 2020;8(1):174–181.
11. Fujita M, Wander K, Paredes Ruvalcaba N, Brindle E. Human milk sIgA antibody in relation to maternal nutrition and infant vulnerability in northern Kenya. Evolution, Medicine, and Public Health 2019;2019(1):201–211.
12. Koutsiaft P, SOultani G, Kechagias S, Grivea I, Malissiova E. Macronutrient composition of breast milk for full term and premature infants: correlation to nutritional and socioeconomic factors. International Journal of Caring Sciences 2021;14(1)608–616
13. Zarban A, Toroghi MM, Asli M, Jafari M, Vejdan M, Sharifzadeh G. Effect of vitamin C and E supplementation on total antioxidant content of human breastmilk and infant urine. Breastfeeding Medicine 2015;10(4):214–7.
14. Silva AG, Sousa Rebouças A, Mendonça BM, Silva DC, Dimenstein R, Ribeiro KD. Relationship between the dietary intake, serum, and breast milk concentrations of vitamin A and vitamin E in a cohort of women over the course of lactation. Maternal & Child Nutrition 2019;15(3):e12772