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Submission date: 06-May-2021 09:30AM (UTC+0700)
Submission ID: 1579203822
File name: A29_-_Lactoferrin_association_with.pdf (141.51K)
Word count: 4055
Character count: 21499

ISSN: 2347-467X, Vol. 08, No. (1) 2020, Pg. 174-181

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

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Abstract

Background: Previous research has not been consistently found that Lactoferrin (LF) is influenced by maternal factors, during pregnancy and postpartum. In Indonesia, the effect of mother's nutritional status to their milk quality has rarely been studied.

Objective: This study aimed to determine how the mother's nutritional status during pregnancy and the lactation period is associated with LF.

Methods: This cross sectional study was performed from September to November 2017 at three primary health care centres working area in Semarang, Indonesia. Seventy-nine lactating mothers were recruited. LF was analysed from about 5 ml of human milk. Data on the mother's general characteristics and anthropometry (weight, height, and mid-upper-arm-circumference (MUAC)) were collected.

Results: Mother's average age was 28±5 years old, mostly multipara and non-working. Average haemoglobin concentration at the third trimester pregnancy was 11.3±1.09 mg/dL MUAC at the third trimester pregnancy and postpartum was 25 cm and 26.4 cm, respectively. Body mass index at postpartum was 23.74 kg/m^2. Median human milk LF was 1.52 g/L. Milk was collected from mothers with ten-day-old infants (median), at 10.00 a.m. and stored 73 days before analysed. Median LF in colostrum (1.60 g/L) did not differ significantly from transition (1.99 g/L), but did with mature milk (1.07 g/L).

Conclusion: Better nutritional statuses of mothers during pregnancy (as indicated by MUAC) and early stages of lactation resulted in significantly higher LF concentration in human milk.

Article History

Received: 28 October 2019
Accepted: 20 December 2019

Keywords

Human Milk;
Lactoferrin;
Lactation Stages;
Maternal Nutritional Status.

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Doi: http://dx.doi.org/10.12944/CRNFSJ.8.1.16

Introduction

Lactoferrin (LF) is a protein that has various functions in the body's metabolism in the form of iron-binding glycoproteins, which are often referred to as metalloproteins.[1] Based on its structure, LF has a similarity concentration to transferrin serum (sTFr) of 60%. Initially, LF was found in cow's milk, and was later studied in breast milk, saliva, tears and pancreatic fluid.[1] LF is synthesised from different cell groups, including neutrophils (poly-morphonuclear lymphocytes), macrophages and glandular epithelial cells, which are mostly secreted as an inflammatory response.[2]

Studied maternal factors that have a relationship with variations in LF concentrations are race/ethnicity, maternal age, parity, socioeconomic status, nutritional status, smoking, mastitis, chorioamnionitis, postpartum infection and other infections.[3] Many studies conducted between the 1980s to 2017 inconsistently proved that these mentioned factors are associated with LF concentration in human milk.[3–7] Other studies reveal the connection between the infant's health status and the LF concentration in the mother's milk to consider the role and benefit of LF in inflammation and infection. The relation of LF concentrations in breast milk to gastrointestinal and respiratory symptoms in infants was studied in in mother–baby dyads in Argentina. Researchers found that LF concentrations were higher if babies had an infection in the past month or got sick the following month; however, there were no differences between breast milk LF concentrations at the time of infection.[8] The factor that consistently showed decreased LF concentration as the infant grow older was the stage of lactation; the highest LF concentration was found in colostrum, while the lowest was in mature milk.[3,4]

A study among anaemic lactating mothers found that iron supplementation improved the mothers' haemoglobin concentration as well as iron in their milk, but did not affect the LF concentration.[9] However, the relationship between iron status during pregnancy and LF in postpartum human milk has not been sufficiently explained in previous studies.[10] Additionally, the effect of the mother's nutritional status on her milk LF concentration has barely been studied, especially in Indonesia. Given the inconsistent results of previous work and the need to consider maternal factors from the start of pregnancy, this study attempts to analyse the relationship that maternal nutritional status factors during pregnancy and postpartum have with LF concentrations in breast milk.

Methods

Study Design

This cross sectional study study took place from September to November 2017 in the working area of Kedungmundu, Bangetayu and Genuk Primary Health Centre, Semarang City, Indonesia. Based on minimum sample size calculation for estimating a population mean with power of the test 0.95, population mean of 0.019 g/L, population standard deviation of 0.0815 g/L,[11] estimation of 10% drop out, and several inclusion criteria, 79 mothers in lactation period were recruited as subjects. Inclusion criteria used were women willing to participate, women giving birth in September 2017, women who were still breastfeeding, women with singleton babies, women with babies born at a normal weight (>2500 g) and women with babies without abnormalities that made suckling difficult.

Data Collection

Data on general characteristics and anthropometry (body weight, height and mid-upper-arm-circumference (MUAC)) were collected. The subject's anthropometric profiles were collected from body weight using a digital weighing scale, height using microtoise and MUAC with MUAC tape. Subject's nutritional statuses were expressed in Body Mass Index (BMI) (kg/m^2) and MUAC (cm). A cut off 18.5-22.9 kg/m^2 of BMI was used for the normal nutritional status among woman in the Asian population. Above 23 kg/m^2 was categorised as overweight.[12] MUAC parameters were used to determine protein-energy malnutrition (PEM) among women; parameters less than 22 cm were categorised as PEM.[13] Data on subject's haemoglobin at the third trimester were collected at the preliminary study with HemoCue. Blood haemoglobin concentration of less than 11 g/dL was used as cut off for anaemia among pregnant woman.[14]

Human Milk Samples and LF Analysis

About 5 ml of subjects' milk was collected door to door with a sterilised human milk-pump and placed inside a sterile glass bottle for LF analysis. The LF analysis on human milk used Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanogram/mL. Prior to analysis, samples were gathered in refrigerator after field collection, and further stored at freezer at -20°C until all samples fulfilled 79 samples. Time in refrigerator and freezer storage was recorded and calculated into data analysis to guarantee that storage time does not affect LF concentrations.[15] To prevent the effects of diurnal variations during milk collection, data on collection times were also recorded and analysed. LF data according to lactation stage was confirmed by the day breastfeeding began and the babies' ages at the time of collection.

Statistical Analysis

Frequency was analysed with univariate analysis, while differences of LF concentration between lactation stages were calculated with the Kruskal–Wallis and Mann–Whitney tests. Spearman's rank test was performed to find correlation between LF concentration and other variables. A multivariate linear regression analysis was performed to predict which factors influence the exclusive breastfeeding practice. Prior to analysis, data which did not normally distributed were transformed with square root.[16] Factors which were significantly correlated to LF by bivariate analysis ($p<0.05$) and other factors which also considered as potential predictors ($p<0.250$) where then tested further as predictive variable in multivariate analysis.[17] Due to colinearity test ($r>0.5$), age of infant and total storage time were excluded from the candidate predictors in further analysis. All predictors included in the linear regression analysis are shown in Table 4. The analysis was run using backward method. A significant level (p) of 0.05 was used as threshold for significance.

This study has obtained ethical approval from the Health Research Ethics Committee of the Faculty of Public Health, Diponegoro University (No. 197/EC/FKM/2017). All participants obtained written information about this study and were free to ask questions before signing the consent form. Participation was voluntarily and their detailed identities were kept confidential.

Results

Characteristics of Subjects

Subjects in this study were of productive age and mostly housewife mothers (Table 1). According to haemoglobin concentration at the third trimester of pregnancy, almost half of all subjects were anaemic (49.4%, n=39). Most subject's had normal MUAC, 8.9% (n=7) of subjects were categorised as PEM during pregnancy. After delivery, only 1 subject was PEM. The median BMI revealed that most subjects were overweight, yet this condition was a backup during the lactation period.

Table 1: General characteristics and anthropometric profiles of mothers (N=79)

Variables	Value	Min.	Max.
Average age (years old)	28±5		
Non-working mothers (%)	67.1		
Basic education (%)	68.4		
Median income of family (IDR)	2,000,000	500,000	6,000,000
Median parity (children)	2	1	3
Average haemoglobin at third trimester (mg/dL)	11.3±1.09		
Anthropometry profiles of mothers:			
Median MUAC at third trimester (cm)	25	19.8	35
Average height (cm)	152.9±5.7		
Median MUAC at lactation period (cm)	26.4	21	42
Median body weight at lactation period (kg)	55.7	37.9	88.9
Median BMI at lactation period (kg/m^2)	23.74	15.65	42.65

Table 2: LF content in milk and collection details (N=79)

Variables	Median	Min.	Max.
LF (g/L)	1.52	0.38	2.94
LF according to stage of lactation:[a]			
Colostrum (g/L)	1.60[b]	0.81	2.94
Transition (g/L)	1.99[c]	0.59	2.81
Mature (g/L)	1.07[b,c]	0.38	2.75
Day breastfeeding started after delivery (days)	0	0	15
Aged of infant at time of milk collection (days)	10	1	76
Time of milk collection (a.m./p.m.)	10 a.m.	8 a.m.	6 p.m.
Total storage time (days)	73	18	119
Storage time in refrigerator (days)	2	0	33
Storage time in freezer (days)	69	18	116

Notes: [a]=Significant association between groups, p=0.006, Kruskal – Wallis test; [b]= Significant association of colostrum – mature milk, p=0.007, Mann – Whitney test; [c]= Significant association of transition – mature milk, p=0.005, Mann – Whitney test.

LF in Human Milk and Stages of Lactation

LF data revealed that the range of all milk samples was varied depending on the stage of lactation (Table 2). Of the milk samples collected, 35.4% (n=28) was from the colostrum period (0–7 days after delivery), 30.4% (n=24) was from the transition period (8–14 days after delivery) and 34.2% (n=27) was from the mature period (more than 14 days after delivery). LF concentration was significantly different within each stage of lactation. Between colostrum and mature milk, there was a significant difference. There was also significant difference between transition and mature milk, but not between colostrum and transition milk (Table 2).

Table 3: Factors related to LF concentration in human milk (N=79)

Variables	r	p value
Age	0.041	0.717
Parity	-0.140	0.220
MUAC at third trimester	0.246	0.029[a]
MUAC at lactation period	-0.120	0.293
Body weight at lactation period	0.095	0.406
BMI at lactation period	0.019	0.866
Haemoglobin concentration at 3rd trimester	0.056	0.636
Day breastfeeding started after delivery	-0.025	0.829
Aged of infant at time of milk collection	-0.272	0.015[a]
Lactation stages	-0.294	0.009[a]
Total storage time	0.137	0.229
Time of milk collection	-0.141	0.216

Note: [a] = Significant correlation, Spearman's rank test

Factors Associated with LF in Human Milk

Results found that the factors significantly associated with LF concentration were MUAC during the third trimester and the infant's age at the time of sample collection (Table 3). The positive correlation on MUAC during pregnancy shows that the better the MUAC of the mother, the higher the LF concentration in milk. A consistent correlation occurs between LF concentrations and the age of the infant at the time of milk sampling. The younger the infant's age results higher the LF concentration. Concurrently, the LF concentration shows significant correlation with lactation stages. The initial period of lactation after birth, namely the colostrum period, was the period with highest content of LF. As the infant grows older, the LF concentrations decline.

According to multivariate linear regression analysis, the results found that better MUAC during pregnancy and early lactation period were significant predictors to LF concentration (Table 4). Although parity persisted up to model 2, it did not become a significant predictor in the last model. However, the best model equation shows that MUAC during pregnancy and lactation stages can only explain the LF concentration of 13%.

Table 4: Regression models of factors associated with LF concentration in human milk

Variables	Model 1				Model 2			
	b	SE	β	p value	b	SE	β	p value
Parity	-6.810	3.810	-0.193	0.078	-6.717	3.808	-0.190	0.082
MUAC at 3rd trimester pregnancy	6.705	3.605	0.240	0.032	7.013	3.048	0.251	0.024
Lactation period	-7.652	3.147	-0.261	0.017	-7.578	3.145	-0.259	0.018
Time of milk collection	-1.537	1.564	-0.104	0.329	-	-	-	-
Constant	27.171				22.339			
Adjusted R^2	0.130				0.130			

Discussions

The unique structural characteristics of LF provide a variety of nutritional and medicinal values.[1,18] It transports iron and detoxifies free radicals in biological fluids, making it increasingly considered a safe and effective ingredient to deliver iron in deficient people. For babies, LF helps the body regulate iron to prevent bacterial infection, inflammation and immune-modulatory diseases,[18] while human milk is their only source of nutritional intake. Nowadays, LF has become a potential supplement among pregnant mothers and infants suffering from anaemia to reduce environmental enteric dysfunction as predictor of stunting.[2,19,20]

LF concentration in this study is only half of that found in Asian countries (3.9 g/L).[21] Factors affecting LF concentration from various studies are still inconsistent. In addition, LF concentration in human milk does not appear to depend on maternal iron status and is not affected by iron supplementation.[3,9,10,22,23] Some studies have shown that maternal malnutrition negatively affects LF concentrations, while other studies have not.[3] Studies also correlate LF concentration with infection in infants.[3,7,8] However, those studies did not correlate with the nutritional status of mothers during pregnancy.

This study's LF concentration at colostrum was low compared to colostrum lactating mothers in China (3.16 g/L),[11] Thailand (2.6 g/L) and Japan (2.7 g/L).[7] However, LF analysis methods used in previous studies differ from this study. Studies in China used UPLC/MS (ultra-high performance liquid chromatography tandem/ mass spectrophotometry), while studies in Thailand and Japan used SDS-PAGE analysis. This study cannot assess the accuracy of the different analytical methods. A review of LF content from various countries found that the immunoassay method (ELISA) used in this study is a reliable method, considering the values and ranges obtained from various studies showed comparable.[21]

Similar to the study in China, this study's LF concentration at transition milk in this study was insignificant difference with colostrum.[11] A consistent decline was found to occur after the infant was one month old and remained relatively stable as mature milk.[3,21] This change is consistent during lactation in any region of the world. In general, the LF concentration in our study supports the results of previous studies that milk LF concentrations change vigorously during lactation and that milk LF concentration are highest in colostrum.

Nutritional status during lactation is a reserve when the mother is pregnant. If the mother fails to gain adequate weight during the last half of pregnancy, it will be more difficult to maintain weight while lactating.[24] A positive correlation between MUAC during the third trimester of pregnancy and LF concentration in this study may imply that nutritional status during pregnancy affect the LF, but further study needed to describe the bio molecular explanation of this. A decrease in MUAC indicates a decrease in muscle mass, subcutaneous fat or both.[13] Lipid metabolism will stimulate accumulation of fat reserves in early to mid-pregnancy, resulting in adipose tissue mobilisation at the end of pregnancy.[25] Mothers who have inadequate reserves or suffer from PEM during pregnancy will have limited protein and fat storage. Given that LF is a globule protein, the LF content in human milk will also be small.

With respect to iron homeostasis during pregnancy, since iron is main mineral of LF, unmet needs during pregnancy will have an adverse effect during lactation period.[14,25] However, this study could not reveal a significant correlation between haemoglobin concentration at third trimester of pregnancy and LF concentration. As mentioned earlier, the relationship between iron status in mothers and LF concentration from previous studies shows inconsistent results. Thus, further investigation is needed regarding other factors that can affect LF in human milk, such as fulfilment of dietary intake requirement during pregnancy. The results obtained in this study need to be confirmed by further research to see the consistency of the results.

Conclusion

Better MUAC in the third trimester of pregnancy was found to be significantly correlated with LF concentration in human milk. As consistent with other studies, LF concentration was decreased further along with lactation stages. The findings of this study can be used as insight and input for the first 1000 days' life-saving program that was adopted by Indonesia since December 2011, that prevention of malnutrition from pregnancy to lactation period will improve the quality of human milk, and positively affect infant health.

Acknowledgements

Appreciation goes to Directorate of Community Nutrition from Ministry of Health, Republic of Indonesia, for the financial support. The researchers were grateful for the collaboration of the Head and staff of Kedungmundu, Bangetayu, and the Genuk Primary Health Centre; all enumerators; trained enumerators who collected milk samples in the field; Prodia laboratory staff and all the subjects of this study.

Funding

This work was supported by the Directorate of Community Nutrition from Ministry of Health, Republic of Indonesia with a grant number HK.03.01/V/365/2017.

Conflict of Interest

All authors do not have conflict of interest in regard to this research or its funding.

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