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Judul Artikel	:	Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
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Decision letter:
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Correlation between Maternal Hemoglobin and Serum Transferrin Receptor with Lactoferrin Concentration of Breastfeeding Mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

I am pleased to inform you that your manuscript, entitled: Correlation between Maternal Hemoglobin and Serum Transferrin Receptor with Lactoferrin Concentration of Breastfeeding Mothers, might be accepted for publication in our journal, pending some minor changes suggested by reviewers (see below).

Please revise your paper strictly according to the attached Reviewers comments. Your manuscript won't be taken into consideration without the revisions made according to the recommendations.

Authors are requested to prepare a revised version of their manuscript and the detailed reply to Reviewers with a list of all made changes as soon as possible. All changes in the revised version should be clearly indicated (by colored background or colored fonts).

Thank you for submitting your work to us.

Kindest regards,
Katarzyna Szwamel
Associate Editor
Family Medicine & Primary Care Review

Review 1:

Assessment of the paper's content:

Achievement of the paper's aim:
Satisfying

Extent of knowledge presented in the paper:
Satisfying

Accuracy of methods used in the study:
Satisfying

Quality of findings and statistical analyses, and assessment of their interpretation:
Satisfying

Assessment of summaries and conclusions:
Satisfying

Assessment of the cited literature:
Satisfying

Is the manuscript in accordance with the Editorial Board's instructions:
Yes

Keywords compatible with MeSH (https://meshb.nlm.nih.gov):
Yes

Do authors have demonstrated knowledge of the achievements of Family Medicine &Primary Care Review?
Yes

Usefulness of the paper to family doctors:
Satisfying

- english is poor and needs to be corrected by native speaker
- in Abstract section - the way of collecting milk samples and correlations is not clear. The correlations are tested between samples from pregnant and lactating women, right?
- in Background section - there are too many imprecise terms ("longer periods", "living food")
- please show the results of measurement of sTfR and hemoglobin (mean, min, max)

Review 2:

Assessment of the paper's content:

Achievement of the paper's aim:
Satisfying

Extent of knowledge presented in the paper:
Satisfying

Accuracy of methods used in the study:

High

Quality of findings and statistical analyses, and assessment of their interpretation:
High

Assessment of summaries and conclusions:
Satisfying

Assessment of the cited literature:
Satisfying

Is the manuscript in accordance with the Editorial Board's instructions:
Yes

Keywords compatible with MeSH (https://meshb.nlm.nih.gov):
No - should be:
Milk, Human; Lactoferrin; Hemoglobins; Receptors, Transferrin
Do authors have demonstrated knowledge of the achievements of Family Medicine &Primary Care Review?
Yes

Usefulness of the paper to family doctors:
Satisfying

Interesting paper on the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in breast milk. Although the mechanism of homeostasis of lactoferrin in breast milk is not fully understood and further studies on the subject are needed, this study is important to rule out a hypothesis.
I ask that the methodology be better explained, that there were two moments of data collection for the study, what was collected in the third trimester of pregnancy and what was collected after delivery. All pregnant women included initially did the second data collection or gave up.
I also request that bibliographic references be placed to support the first paragraph of the Background "Researches throughout the year have proved that breastfeeding is important for both mother and child. Children who are breastfed for longer periods have lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence than those who are breastfed for shorter periods, or not breastfed. This inequality persists until later in life. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life."
The keywords must be changed according to the MeSH terms, as recommended.

Family Medicine & Primary Care Review

Revised Manuscript Draft ver1

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Type

Original paper

Keywords

Human milk, lactoferrin, hemoglobins, transferrin receptors

Abstract

Background
Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives
This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods
This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained during the third trimester of pregnancy, while lactoferrin concentration was measured after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results
There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions
The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Explanation letter

Dear Reviewers,

Here are some changes to the manuscript made according to the recommendations :
- All changes in the revised version of the manuscript were indicated with red font color
- English has been corrected by proofreading service "Scribendi" based in Canada (native speaker)
- Results of measurement of sTfR and hemoglobin (median, min, max) have already stated in Table 4
- The explanation about two stages of data collection was added in 'Material and methods' section
- Bibliographic reference to support the first paragraph has been included which is [1]
- Key words have been changed according to the MeSH terms, as recommended.

Thank you.

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Summary

Background. Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.
Objectives. This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.
Material and methods. This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained during the third trimester of pregnancy, while lactoferrin concentration was measured after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.
Results. There was no correlation between maternal hemoglobin and lactoferrin concentration (p = 0.636). There was also no correlation between serum transferrin receptor and lactoferrin concentration (p = 0.688). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.
Conclusions. The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Key words: human milk, lactoferrin, hemoglobins, transferrin receptors.

Background

Over the years, many studies have proved that breastfeeding is important for both mothers and children. Breastfeeding is associated with lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence in children. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life. Mothers who breastfeed their children receive many benefits, such as preventing breast cancer, improved

birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommendations, children should be exclusively breastfed for the first six months; breastfeeding should then continue for up to two years while also being provided with nutritionally adequate and safe complementary food [3].

One of the factors that has a role in the benefits of breastfeeding is the content of human milk, which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients [4]. It contains biologically active components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 different proteins in which the concentration differs according to the child's age and other characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, lactoferrin may impact gut health and gut-immune development and functioning, decreases the risk of lower respiratory tract illness, and decreases the burden of colonization by some

parasites in underdeveloped settings [9]. In terms of nutritional function, lactoferrin transports iron and detoxifies free radicals in biological fluids so that it is beneficial for people with iron deficiency [8].

Iron deficiency commonly occurs during pregnancy due to the increase in iron demand. It develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores are completely depleted, iron accessibility to the tissues declines, leading to symptomatic anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 48.9% in 2018 [12].

Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected by IDA considering that maternal nutritional status is closely associated with the quality of human milk; therefore, impairment in human milk content may occur due to maternal anemia. Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content [14].

The WHO and Centers for Disease Control and Prevention (CDC) Technical Consultation have established hemoglobin (Hb) and sTfR concentration as an indicator of iron status in the population. Hb concentration is a measure of anemia while sTfR, which is derived mostly from developing red blood cells, reflects the balance between cellular iron requirements and iron supply, and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis [15].

A study on anemic and non-anemic breastfeeding mothers found that, even though Hb concentration increases after iron supplementation, the lactoferrin concentration in both groups was similar at the end of 30 days of supplementation [16]. From our previous study we know that mothers with better nutritional status have a higher lactoferrin concentration in their milk [17]. However, there have been few studies related to maternal iron status and its effect on the quality of human milk, and it is poorly understood. Considering that lactoferrin is the main protein of human milk and has many benefits for infants' growth and development during the breastfeeding period, this study aims to analyze its correlation with iron status.

Objectives

This study aimed to analyze the correlation between Hb and sTfR concentration during pregnancy with the lactoferrin concentration of breastfeeding mothers.

Material and methods

Design and subject

This was a quantitative study with an analytical design and a cross-sectional approach. The study was conducted for three months, from September to November 2017, in the working areas of Kedungmundu, Bangetayu, and Genuk primary health centers in Semarang City, Indonesia. The subjects were 79 pregnant mothers who were selected using purposive sampling. The sample size was determined with Slovin's formula.

Measurement

The data for this study were collected in two periods. The first data collection was during the third trimester of pregnancy; this included data on subject characteristics, anthropometric measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The second data collection was after delivery in which milk samples were collected for lactoferrin concentration analysis. All subjects participated in both stages of data collection.

Data on subject characteristics, such as education level and occupation, were obtained through interviews with the subjects. Education level was categorized into two groups: (a) primary, which was six years in elementary school and three years in junior high school; and (b) secondary or higher, which was three years in senior high school and about three to four years at college.

Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive days, with food pictures to help subjects determine the food portions they consumed. Food intake was recorded in the form of household portions such as tablespoons, teaspoons, cups, etc. This was then converted into grams and analyzed using NutriSurvey software to calculate the nutrition intake. The data were then compared with the Indonesian recommended dietary allowance (RDA) which is based on the 2019 Republic of Indonesia Ministry of Health Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 cm were categorized as at risk of chronic energy deficiency (CEM) [19].

For the analysis of Hb and sTfR, about 5 mL of venous blood was taken from the subjects once in the morning between 8 am and 10 am. Hb concentration was measured using cyanmethemoglobin, while STfR was measured using a Quantikine IVD Human STfR Immunoassay (R&D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay (ELISA) Reader 680 using a quantitative sandwich technique. Subjects were categorized as anemic if Hb concentration was below 11 g/dL [19] and sTfR concentration was greater than or equal to 21.0 nmol/L [20].

For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a refrigerator during the visit and further stored in a freezer at −20°C. Storage time both in the refrigerator and the freezer was recorded and considered during analysis to ensure it did not affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding mothers was confirmed by the day breastfeeding began and the infants' age at the time of collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanograms/ml.

Statistical analysis

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using Pearson's product moment test, while the rank Spearman test was used to assess the correlation of variables if the data distribution was not normal.

Results

The mean age of the subjects at the time of study was 27.95 ± 5.08 years old. During the third trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had secondary or higher education levels (68.4%), were housewives (65.8%), and had normal nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature (34.2%) (Table 1).

Table 1. Characteristics distribution of subjects

Variables	Value
Age (years)	27.95 ± 5.08
MUAC at third trimester (cm)	25.0 (19.8–35.0)
Level of education:	
Primary	25 (31.6%)
Secondary or higher	54 (68.4%)
Occupation:	
Housewife	52 (65.8%)
Laborer	8 (10.1%)
Entrepreneur	7 (8.9%)
Private employee	4 (5.1%)
Civil servant	8 (10.1%)
Nutritional status:	
CEM	1 (1.3%)
Normal	78 (98.7%)
Lactation stage:	
Colostrum (g/L)	28 (35.4%)
Transition (g/L)	24 (30.4%)
Mature (g/L)	27 (34.2%)

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). Nutritional intake that had a significant relationship to Hb concentration was protein, dietary cholesterol, fat, all types of vitamin B (thiamin, riboflavin, niacin, vitamin B6, folic acid, and vitamin B12), vitamin C, iron, and zinc. The sTfR concentration was significantly related to the intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, the rank Spearman test found no correlation with lactoferrin concentration (Table 3).

Table 2. Nutritional intake of subjects during the third trimester of pregnancy

Variables	Value	Min.	Max.
Energy (kcal)	2,595.58 ± 401.88		
Carbohydrate (g)	283.98 ± 60.49		
Protein (g)	113.81 ± 23.65		
Dietary cholesterol (mg)	377.0	0.0	1,314.0
Fat (g)	77.42 ± 26.61		
Dietary fiber (g)	37.2	31.9	51.0
Vitamin A (RE)	967.0	49.0	9,462.0
Thiamin (mg)	1.8	1.1	2.9
Riboflavin (mg)	2.13 ± 1.29		
Niacin (mg)	12.01 ± 4.66		
Vitamin B6 (mg)	1.4	0.6	4.6
Folic acid (μg)	716.0	545.0	1,346.0
Vitamin B12 (μg)	6.1	0.1	65.4
Vitamin C (mg)	93.0	51.1	388.0
Vitamin E (μg)	15.0	15.0	16.2
Iron (mg)	40.5	26.3	47.3
Zinc (mg)	20.09 ± 3.15		

Table 3. Nutritional intake and its correlation to maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration

Variables	*p*-value		
	Hemoglobin (g/dL)	Serum transferrin receptor (nmol/L)	Lactoferrin (g/L)
Energy (kcal)	0.001	0.125	0.783
Carbohydrate (g)	0.073	0.692	0.405
Protein (g)	0.002	0.072	0.848
Dietary cholesterol (mg)	0.001	0.235	0.665
Fat (g)	0.009	0.516	0.342
Dietary fiber (g)	0.517	0.929	0.708
Vitamin A (RE)	0.078	0.312	0.278
Thiamin (mg)	0.002	0.036	0.952
Riboflavin (mg)	0.012	0.098	0.986
Niacin (mg)	0.022	0.046	0.099
Vitamin B6 (mg)	0.021	0.171	0.599
Folic acid (μg)	0.001	0.024	0.768
Vitamin B12 (μg)	0.015	0.192	0.750
Vitamin C (mg)	0.001	0.001	0.969
Vitamin E (μg)	0.337	0.133	0.701
Iron (mg)	0.001	0.001	0.443
Zinc (mg)	0.001	0.207	0.460

Mothers did not suffer from anemia, which was shown through Hb concentration above 11 g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5). However, Hb and sTfR concentration showed a significant inverse correlation ($p = 0.001$, $r = -0.438$).

Table 4. Hemoglobin, serum transferrin receptor, and lactoferrin concentration of breastfeeding mothers

Variables	Median	Min.	Max.
Hemoglobin (g/dL)	11.3	8.9	14.3
Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
Lactoferrin (g/L)	1.52	0.38	2.94

Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration of breastfeeding mothers

Variables	Lactoferrin concentration (g/L)	
	R	*p*-value
Hemoglobin (g/dL)	0.054	0.636
Serum transferrin receptor (nmol/L)	0.046	0.686

Discussion

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third trimester was adequate. This was reflected in the MUAC, which was equal or more than 23.5 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not have anemia which could be a result of adequate iron intake as well as other important nutrients that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, polyphenols, calcium, and proteins in milk products, and will increase the absorption of both native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role in the formation of red blood cells [22].

Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case of anemia, Hb concentration became lower while sTfR concentration rose. This result was supported by other studies in which Hb and sTfR were negatively correlated [23, 24].

In the present study, lactoferrin concentration was found to be lower compared to mean lactoferrin concentration in several countries in Asia, namely Bangladesh (5.72 g/L), India (3.71 g/L), Japan (4.17 g/L), and Thailand (2.27 g/L) [25]. However, the concentration was in a similar range as China, which varied from 0.99 g/L to 1.91 g/L across its 11 provinces [26].

In our previous study, we found that maternal nutritional status was a predictor of lactoferrin concentration in human milk [17], but evidently it was not the case for maternal iron status. Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin concentration was low and they were not significantly correlated. Another study supported this result, which found that lactoferrin concentration in human milk did not depend on maternal iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between maternal iron status and lactoferrin concentration in human milk at birth and during early lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27].

A study in Brazil found that total protein levels in human milk were higher in anemic mothers regardless of their lactation stage (colostrum, transition, mature) [28]. Since lactoferrin is the main whey protein in human milk, and all mothers in the present study were non-anemic, we assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further studies are needed to explain the mechanism.

Conclusions

Maternal iron status as indicated by Hb and sTfR concentration did not affect lactoferrin concentration in human milk. However, the mechanism of milk lactoferrin homeostasis is not completely understood. Thus, further studies are needed to help promote better health for mothers and their children.

Acknowledgments. Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion. Financial support was provided by the Directorate of Community Nutrition from the Ministry of Health, Republic of Indonesia, with grant number HK.03.01/V/365/2017.

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Conflicts of interest: The authors declare no conflicts of interest.

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September 14, 2020
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Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

I am pleased to inform you that your manuscript, entitled: Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers, might be accepted for publication in our journal, pending some minor changes suggested by reviewers (see below).

Please revise your paper strictly according to the attached Reviewers comments. Your manuscript won't be taken into consideration without the revisions made according to the recommendations.

Authors are requested to prepare a revised version of their manuscript and the detailed reply to Reviewers with a list of all made changes as soon as possible. All changes in the revised version should be clearly indicated (by colored background or colored fonts).

Thank you for submitting your work to us.

Kindest regards,
Katarzyna Szwamel
Associate Editor
Family Medicine & Primary Care Review

Review 1:

Assessment of the paper's content:

Achievement of the paper's aim:
Satisfying

Extent of knowledge presented in the paper:
Satisfying

Accuracy of methods used in the study:
Satisfying

Quality of findings and statistical analyses, and assessment of their interpretation:
Satisfying

Assessment of summaries and conclusions:
Satisfying

Assessment of the cited literature:
Satisfying

Is the manuscript in accordance with the Editorial Board's instructions:
Yes

Keywords compatible with MeSH (https://meshb.nlm.nih.gov):
Yes

Do authors have demonstrated knowledge of the achievements of Family Medicine &Primary Care Review?
Yes

Usefulness of the paper to family doctors:
Satisfying

The revision is satisfactory.
I suggest to change 2 things:
1. In Abstract: Hemoglobin and serum transferrin receptor data were obtained FROM BLOOD during the third trimester of pregnancy, while lactoferrin concentration was measured IN MILK after delivery.
2. The title of Table 4:
Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of nursing mothers (or similar)

with kind regards,

Attachment
Maternal Hemoglobin - revised - MGed.docx

Family Medicine & Primary Care Review

Revised Manuscript Draft ver 2

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Type

Original paper

Keywords

Human milk, lactoferrin, hemoglobins, transferrin receptors

Abstract

Background
Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives
This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods
This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results
There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions
The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Explanation letter

Dear Editor,

This manuscript has been revised based on the reviewer recommendation as follows :
1. In Abstract: The sentences has been changed as recommended which is "Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery."
2. The title of Table 4 has been changed to "Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers"

Thank you.

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Summary

Background. Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives. This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods. This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results. There was no correlation between maternal hemoglobin and lactoferrin concentration (p = 0.636). There was also no correlation between serum transferrin receptor and lactoferrin concentration (p = 0.688). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions. The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Key words: human milk, lactoferrin, hemoglobins, transferrin receptors.

Background

Over the years, many studies have proved that breastfeeding is important for both mothers and children. Breastfeeding is associated with lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence in children. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life. Mothers who breastfeed their children receive many benefits, such as preventing breast cancer, improved

birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommendations, children should be exclusively breastfed for the first six months; breastfeeding should then continue for up to two years while also being provided with nutritionally adequate and safe complementary food [3].

One of the factors that has a role in the benefits of breastfeeding is the content of human milk, which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients [4]. It contains biologically active components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 different proteins in which the concentration differs according to the child's age and other characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, lactoferrin may impact gut health and gut-immune development and functioning, decreases the risk of lower respiratory tract illness, and decreases the burden of colonization by some

parasites in underdeveloped settings [9]. In terms of nutritional function, lactoferrin transports iron and detoxifies free radicals in biological fluids so that it is beneficial for people with iron deficiency [8].

Iron deficiency commonly occurs during pregnancy due to the increase in iron demand. It develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores are completely depleted, iron accessibility to the tissues declines, leading to symptomatic anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 48.9% in 2018 [12].

Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected by IDA considering that maternal nutritional status is closely associated with the quality of human milk; therefore, impairment in human milk content may occur due to maternal anemia. Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content [14].

The WHO and Centers for Disease Control and Prevention (CDC) Technical Consultation have established hemoglobin (Hb) and sTfR concentration as an indicator of iron status in the population. Hb concentration is a measure of anemia while sTfR, which is derived mostly from developing red blood cells, reflects the balance between cellular iron requirements and iron supply, and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis [15].

A study on anemic and non-anemic breastfeeding mothers found that, even though Hb concentration increases after iron supplementation, the lactoferrin concentration in both groups was similar at the end of 30 days of supplementation [16]. From our previous study we know that mothers with better nutritional status have a higher lactoferrin concentration in their milk [17]. However, there have been few studies related to maternal iron status and its effect on the quality of human milk, and it is poorly understood. Considering that lactoferrin is the main protein of human milk and has many benefits for infants' growth and development during the breastfeeding period, this study aims to analyze its correlation with iron status.

Objectives

This study aimed to analyze the correlation between Hb and sTfR concentration during pregnancy with the lactoferrin concentration of breastfeeding mothers.

Material and methods

Design and subject

This was a quantitative study with an analytical design and a cross-sectional approach. The study was conducted for three months, from September to November 2017, in the working areas of Kedungmundu, Bangetayu, and Genuk primary health centers in Semarang City, Indonesia. The subjects were 79 pregnant mothers who were selected using purposive sampling. The sample size was determined with Slovin's formula.

Measurement

The data for this study were collected in two periods. The first data collection was during the third trimester of pregnancy; this included data on subject characteristics, anthropometric measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The second data collection was after delivery in which milk samples were collected for lactoferrin concentration analysis. All subjects participated in both stages of data collection.

Data on subject characteristics, such as education level and occupation, were obtained through interviews with the subjects. Education level was categorized into two groups: (a) primary, which was six years in elementary school and three years in junior high school; and (b) secondary or higher, which was three years in senior high school and about three to four years at college.

Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive days, with food pictures to help subjects determine the food portions they consumed. Food intake was recorded in the form of household portions such as tablespoons, teaspoons, cups, etc. This was then converted into grams and analyzed using NutriSurvey software to calculate the nutrition intake. The data were then compared with the Indonesian recommended dietary allowance (RDA) which is based on the 2019 Republic of Indonesia Ministry of Health Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 cm were categorized as at risk of chronic energy deficiency (CEM) [19].

For the analysis of Hb and sTfR, about 5 mL of venous blood was taken from the subjects once in the morning between 8 am and 10 am. Hb concentration was measured using cyanmethemoglobin, while STfR was measured using a Quantikine IVD Human STfR Immunoassay (R&D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay (ELISA) Reader 680 using a quantitative sandwich technique. Subjects were categorized as anemic if Hb concentration was below 11 g/dL [19] and sTfR concentration was greater than or equal to 21.0 nmol/L [20].

For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a refrigerator during the visit and further stored in a freezer at −20°C. Storage time both in the refrigerator and the freezer was recorded and considered during analysis to ensure it did not affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding mothers was confirmed by the day breastfeeding began and the infants' age at the time of collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanograms/ml.

Statistical analysis

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using Pearson's product moment test, while the rank Spearman test was used to assess the correlation of variables if the data distribution was not normal.

Results

The mean age of the subjects at the time of study was 27.95 ± 5.08 years old. During the third trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had secondary or higher education levels (68.4%), were housewives (65.8%), and had normal nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature (34.2%) (Table 1).

Table 1. Characteristics distribution of subjects

Variables	Value
Age (years)	27.95 ± 5.08
MUAC at third trimester (cm)	25.0 (19.8–35.0)
Level of education:	
Primary	25 (31.6%)
Secondary or higher	54 (68.4%)
Occupation:	
Housewife	52 (65.8%)
Laborer	8 (10.1%)
Entrepreneur	7 (8.9%)
Private employee	4 (5.1%)
Civil servant	8 (10.1%)
Nutritional status:	
CEM	1 (1.3%)
Normal	78 (98.7%)
Lactation stage:	
Colostrum (g/L)	28 (35.4%)
Transition (g/L)	24 (30.4%)
Mature (g/L)	27 (34.2%)

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). Nutritional intake that had a significant relationship to Hb concentration was protein, dietary cholesterol, fat, all types of vitamin B (thiamin, riboflavin, niacin, vitamin B6, folic acid, and vitamin B12), vitamin C, iron, and zinc. The sTfR concentration was significantly related to the intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, the rank Spearman test found no correlation with lactoferrin concentration (Table 3).

Table 2. Nutritional intake of subjects during the third trimester of pregnancy

Variables	Value	Min.	Max.
Energy (kcal)	2,595.58 ± 401.88		
Carbohydrate (g)	283.98 ± 60.49		
Protein (g)	113.81 ± 23.65		
Dietary cholesterol (mg)	377.0	0.0	1,314.0
Fat (g)	77.42 ± 26.61		
Dietary fiber (g)	37.2	31.9	51.0
Vitamin A (RE)	967.0	49.0	9,462.0
Thiamin (mg)	1.8	1.1	2.9
Riboflavin (mg)	2.13 ± 1.29		
Niacin (mg)	12.01 ± 4.66		
Vitamin B6 (mg)	1.4	0.6	4.6
Folic acid (µg)	716.0	545.0	1,346.0
Vitamin B12 (µg)	6.1	0.1	65.4
Vitamin C (mg)	93.0	51.1	388.0
Vitamin E (µg)	15.0	15.0	16.2
Iron (mg)	40.5	26.3	47.3
Zinc (mg)	20.09 ± 3.15		

Table 3. Nutritional intake and its correlation to maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration

Variables	*p*-value		
	Hemoglobin (g/dL)	Serum transferrin receptor (nmol/L)	Lactoferrin (g/L)
Energy (kcal)	0.001	0.125	0.783
Carbohydrate (g)	0.073	0.692	0.405
Protein (g)	0.002	0.072	0.848
Dietary cholesterol (mg)	0.001	0.235	0.665
Fat (g)	0.009	0.516	0.342
Dietary fiber (g)	0.517	0.929	0.708
Vitamin A (RE)	0.078	0.312	0.278
Thiamin (mg)	0.002	0.036	0.952
Riboflavin (mg)	0.012	0.098	0.986
Niacin (mg)	0.022	0.046	0.099
Vitamin B6 (mg)	0.021	0.171	0.599
Folic acid (µg)	0.001	0.024	0.768
Vitamin B12 (µg)	0.015	0.192	0.750
Vitamin C (mg)	0.001	0.001	0.969
Vitamin E (µg)	0.337	0.133	0.701
Iron (mg)	0.001	0.001	0.443
Zinc (mg)	0.001	0.207	0.460

Mothers did not suffer from anemia, which was shown through Hb concentration above 11 g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5). However, Hb and sTfR concentration showed a significant inverse correlation (p = 0.001, r = −0.438).

Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers

Variables	Median	Min.	Max.
Hemoglobin (g/dL)	11.3	8.9	14.3
Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
Lactoferrin (g/L)	1.52	0.38	2.94

Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration of breastfeeding mothers

Variables	Lactoferrin concentration (g/L)	
	R	*p*-value
Hemoglobin (g/dL)	0.054	0.636
Serum transferrin receptor (nmol/L)	0.046	0.686

Discussion

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third trimester was adequate. This was reflected in the MUAC, which was equal or more than 23.5 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not have anemia which could be a result of adequate iron intake as well as other important nutrients that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, polyphenols, calcium, and proteins in milk products, and will increase the absorption of both native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role in the formation of red blood cells [22].

Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case of anemia, Hb concentration became lower while sTfR concentration rose. This result was supported by other studies in which Hb and sTfR were negatively correlated [23, 24].

In the present study, lactoferrin concentration was found to be lower compared to mean lactoferrin concentration in several countries in Asia, namely Bangladesh (5.72 g/L), India (3.71 g/L), Japan (4.17 g/L), and Thailand (2.27 g/L) [25]. However, the concentration was in a similar range as China, which varied from 0.99 g/L to 1.91 g/L across its 11 provinces [26].

In our previous study, we found that maternal nutritional status was a predictor of lactoferrin concentration in human milk [17], but evidently it was not the case for maternal iron status. Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin concentration was low and they were not significantly correlated. Another study supported this result, which found that lactoferrin concentration in human milk did not depend on maternal iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between maternal iron status and lactoferrin concentration in human milk at birth and during early lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27].

A study in Brazil found that total protein levels in human milk were higher in anemic mothers regardless of their lactation stage (colostrum, transition, mature) [28]. Since lactoferrin is the main whey protein in human milk, and all mothers in the present study were non-anemic, we assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further studies are needed to explain the mechanism.

Conclusions

Maternal iron status as indicated by Hb and sTfR concentration did not affect lactoferrin concentration in human milk. However, the mechanism of milk lactoferrin homeostasis is not completely understood. Thus, further studies are needed to help promote better health for mothers and their children.

Acknowledgments. Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion. Financial support was provided by the Directorate of Community Nutrition from the Ministry of Health, Republic of Indonesia, with grant number HK.03.01/V/365/2017.

Source of funding: This work was financed by the Directorate of Community Nutrition from the Ministry of Health, Republic of Indonesia.

Conflicts of interest: The authors declare no conflicts of interest.

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Manuscript body

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Authors:
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Decision letter:
September 17, 2020
FAMILY-00831-2020-03
Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

I am pleased to inform you that your manuscript, entitled: Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers, might be accepted for publication in our journal, pending some minor changes suggested below:
1. Section „material and methods" should be described strictly according to STROBE checklist. I think about providing relevant subtitles : study design, setting, participants, variables, data sources etc.
2. Section "discussion" should be also provided strictly according to STROBE (key results, interpretation, generalisability).
3. Limitations of the study should be described.
4. Please put the legend under table 1 (explain CEM, MUAC under the table)
5. Please put this sentence below into the section "material and methods" :
" Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion"

Please revise your paper strictly according to the attached Reviewers comments. Your manuscript won't be taken into consideration without the revisions made according to the recommendations.

Authors are requested to prepare a revised version of their manuscript and the detailed reply to Reviewers with a list of all made changes as soon as possible. All changes in the revised version should be clearly indicated (by colored background or colored fonts).

Thank you for submitting your work to us.

Kindest regards,
Katarzyna Szwamel
Associate Editor
Family Medicine & Primary Care Review

Revised Manuscript Draft ver 3

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Type

Original paper

Keywords

lactoferrin, Human milk, hemoglobins, transferrin receptors

Abstract

Background
Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives
This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods
This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results
There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions
The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Explanation letter

Dear Editor,

We have revised the manuscript based on recommendation as follows (indicated by red font color) :
1. Section "Material and methods" and "Discussion" has been described according to STROBE Checklist
2. Limitations of the study have been added in section "Discussion" as suggested by STROBE Checklist (subtitle "Limitations")
3. Explanation for MUAC and CEM in Table 1 has been added under the table
4. The sentence “Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion” has been moved to section "Material and methods"

Thank you.

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Summary

Background. Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.
Objectives. This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.
Material and methods. This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.
Results. There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.
Conclusions. The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Key words: human milk, lactoferrin, hemoglobins, transferrin receptors.

Background

Over the years, many studies have proved that breastfeeding is important for both mothers and children. Breastfeeding is associated with lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence in children. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life. Mothers who breastfeed their children receive many benefits, such as preventing breast cancer, improved

birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommendations, children should be exclusively breastfed for the first six months; breastfeeding should then continue for up to two years while also being provided with nutritionally adequate and safe complementary food [3].

One of the factors that has a role in the benefits of breastfeeding is the content of human milk, which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients [4]. It contains biologically active components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 different proteins in which the concentration differs according to the child's age and other characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and whole blood, lactoferrin concentration is low, varying from 0.02 μg/ml to 1.52 μg/ml. Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, lactoferrin may impact gut health and gut-immune development and functioning, decreases the risk of lower respiratory tract illness, and decreases the burden of colonization by some

parasites in underdeveloped settings [9]. In terms of nutritional function, lactoferrin transports iron and detoxifies free radicals in biological fluids so that it is beneficial for people with iron deficiency [8].

Iron deficiency commonly occurs during pregnancy due to the increase in iron demand. It develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores are completely depleted, iron accessibility to the tissues declines, leading to symptomatic anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 48.9% in 2018 [12].

Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected by IDA considering that maternal nutritional status is closely associated with the quality of human milk; therefore, impairment in human milk content may occur due to maternal anemia. Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content [14].

The WHO and Centers for Disease Control and Prevention (CDC) Technical Consultation have established hemoglobin (Hb) and sTfR concentration as an indicator of iron status in the population. Hb concentration is a measure of anemia while sTfR, which is derived mostly from developing red blood cells, reflects the balance between cellular iron requirements and iron supply, and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis [15].

A study on anemic and non-anemic breastfeeding mothers found that, even though Hb concentration increases after iron supplementation, the lactoferrin concentration in both groups was similar at the end of 30 days of supplementation [16]. From our previous study, we know that mothers with better nutritional status have a higher lactoferrin concentration in their milk [17]. However, there have been few studies related to maternal iron status and its effect on the quality of human milk, and it is poorly understood. Considering that lactoferrin is the main protein of human milk and has many benefits for infants' growth and development during the breastfeeding period, this study aims to analyze its correlation with iron status.

Objectives

This study aimed to analyze the correlation between Hb and sTfR concentration during pregnancy with the lactoferrin concentration of breastfeeding mothers.

Material and methods

Study Design

This was a quantitative study with an analytical design and a cross-sectional approach.

Setting

The study was conducted for three months, from September to November 2017, in the working areas of Kedungmundu, Bangetayu, and Genuk primary health centers in Semarang City, Indonesia. The data for this study were collected in two periods. The first data collection was during the third trimester of pregnancy; this included data on subject characteristics, anthropometric measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The second data collection was after delivery in which milk samples

were collected for lactoferrin concentration analysis. All subjects participated in both stages of data collection.

Participants

The subjects were 79 pregnant mothers who were selected using purposive sampling. The sample size was determined with Slovin's formula. The inclusion criteria were willingness to participate in the study, giving birth in September 2017, breastfeeding mothers, had singleton child, had children born at a normal weight (> 2500 g) and had children without abnormalities that made suckling difficult.

Variables

The variables assessed in this study were maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration.

Data Sources/ Measurement

Data on subject characteristics, such as education level and occupation, were obtained through interviews with the subjects. Education level was categorized into two groups: (a) primary, which was six years in elementary school and three years in junior high school; and (b) secondary or higher, which was three years in senior high school and about three to four years at college.

Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive days, with food pictures to help subjects determine the food portions they consumed. Food intake was recorded in the form of household portions such as tablespoons, teaspoons, cups, etc. This was then converted into grams and analyzed using NutriSurvey software to calculate the nutrition intake. The data were then compared with the Indonesian recommended dietary allowance (RDA) which is based on the 2019 Republic of Indonesia Ministry of Health

Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 cm were categorized as at risk of chronic energy deficiency (CEM) [19].

For the analysis of Hb and sTfR, about 5 mL of venous blood was taken from the subjects once in the morning between 8 am and 10 am. Hb concentration was measured using cyanmethemoglobin, while STfR was measured using a Quantikine IVD Human STfR Immunoassay (R&D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay (ELISA) Reader 680 using a quantitative sandwich technique. Subjects were categorized as anemic if Hb concentration was below 11 g/dL [19] and sTfR concentration was greater than or equal to 21.0 nmol/L [20].

For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a refrigerator during the visit and further stored in a freezer at −20°C. Storage time both in the refrigerator and the freezer was recorded and considered during analysis to ensure it did not affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding mothers was confirmed by the day breastfeeding began and the infants' age at the time of collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanograms/ml.

Statistical Methods

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using

Pearson's product moment test, while the rank Spearman test was used to assess the correlation of variables if the data distribution was not normal.

Ethical consideration

Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion.

Results

The mean age of the subjects at the time of study was 27.95 ± 5.08 years old. During the third trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had secondary or higher education levels (68.4%), were housewives (65.8%), and had normal nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature (34.2%) (Table 1).

Table 1. Characteristics distribution of subjects

Variables	Value
Age (years)	27.95 ± 5.08
MUAC[a] at third trimester (cm)	25.0 (19.8–35.0)
Level of education:	
Primary	25 (31.6%)
Secondary or higher	54 (68.4%)
Occupation:	
Housewife	52 (65.8%)
Laborer	8 (10.1%)
Entrepreneur	7 (8.9%)
Private employee	4 (5.1%)
Civil servant	8 (10.1%)
Nutritional status:	
CEM[b]	1 (1.3%)
Normal	78 (98.7%)
Lactation stage:	
Colostrum (g/L)	28 (35.4%)
Transition (g/L)	24 (30.4%)
Mature (g/L)	27 (34.2%)

[a] Mid-upper arm circumference; [b] Chronic energy deficiency

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). Nutritional intake that had a significant relationship to Hb concentration was protein, dietary cholesterol, fat, all types of vitamin B (thiamin, riboflavin, niacin, vitamin B6, folic acid, and vitamin B12), vitamin C, iron, and zinc. The sTfR concentration was significantly related to the intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, the rank Spearman test found no correlation with lactoferrin concentration (Table 3).

Table 2. Nutritional intake of subjects during the third trimester of pregnancy

Variables	Value	Min.	Max.
Energy (kcal)	2,595.58 ± 401.88		
Carbohydrate (g)	283.98 ± 60.49		
Protein (g)	113.81 ± 23.65		
Dietary cholesterol (mg)	377.0	0.0	1,314.0
Fat (g)	77.42 ± 26.61		
Dietary fiber (g)	37.2	31.9	51.0
Vitamin A (RE)	967.0	49.0	9,462.0
Thiamin (mg)	1.8	1.1	2.9
Riboflavin (mg)	2.13 ± 1.29		
Niacin (mg)	12.01 ± 4.66		
Vitamin B6 (mg)	1.4	0.6	4.6
Folic acid (µg)	716.0	545.0	1,346.0
Vitamin B12 (µg)	6.1	0.1	65.4
Vitamin C (mg)	93.0	51.1	388.0
Vitamin E (µg)	15.0	15.0	16.2
Iron (mg)	40.5	26.3	47.3
Zinc (mg)	20.09 ± 3.15		

Table 3. Nutritional intake and its correlation to maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration

Variables	*p*-value		
	Hemoglobin (g/dL)	Serum transferrin receptor (nmol/L)	Lactoferrin (g/L)
Energy (kcal)	0.001	0.125	0.783
Carbohydrate (g)	0.073	0.692	0.405
Protein (g)	0.002	0.072	0.848
Dietary cholesterol (mg)	0.001	0.235	0.665
Fat (g)	0.009	0.516	0.342
Dietary fiber (g)	0.517	0.929	0.708
Vitamin A (RE)	0.078	0.312	0.278
Thiamin (mg)	0.002	0.036	0.952
Riboflavin (mg)	0.012	0.098	0.986
Niacin (mg)	0.022	0.046	0.099
Vitamin B6 (mg)	0.021	0.171	0.599
Folic acid (µg)	0.001	0.024	0.768
Vitamin B12 (µg)	0.015	0.192	0.750
Vitamin C (mg)	0.001	0.001	0.969
Vitamin E (µg)	0.337	0.133	0.701
Iron (mg)	0.001	0.001	0.443
Zinc (mg)	0.001	0.207	0.460

Mothers did not suffer from anemia, which was shown through Hb concentration above 11 g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in

human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5). However, Hb and sTfR concentration showed a significant inverse correlation ($p = 0.001$, $r = -0.438$).

Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers

Variables	Median	Min.	Max.
Hemoglobin (g/dL)	11.3	8.9	14.3
Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
Lactoferrin (g/L)	1.52	0.38	2.94

Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration of breastfeeding mothers

Variables	Lactoferrin concentration (g/L)	
	R	*p*-value
Hemoglobin (g/dL)	0.054	0.636
Serum transferrin receptor (nmol/L)	0.046	0.686

Discussion

Key Results

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third trimester was adequate. This was reflected in the MUAC, which was equal or more than 23.5 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not have anemia which could be a result of adequate iron intake as well as other important nutrients that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, polyphenols, calcium, and proteins in milk products, and will increase the absorption of both native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role in the formation of red blood cells [22].

Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case of anemia, Hb concentration became lower while sTfR concentration rose. This result was supported by other studies in which Hb and sTfR were negatively correlated [23, 24].

In the present study, lactoferrin concentration was found to be lower compared to mean lactoferrin concentration in several countries in Asia, namely Bangladesh (5.72 g/L), India (3.71 g/L), Japan (4.17 g/L), and Thailand (2.27 g/L) [25]. However, the concentration was in a similar range as China, which varied from 0.99 g/L to 1.91 g/L across its 11 provinces [26].

Limitations

Data on nutritional intake were obtained with 24-hours recall method that might not represent the long-term dietary habits of the subjects. Moreover, there was a possibility that the subjects could not remember all the food they consumed for the day which would lead to an inaccurate data record.

Interpretation

In our previous study, we found that maternal nutritional status was a predictor of lactoferrin concentration in human milk [17], but evidently it was not the case for maternal iron status. Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin concentration was low and they were not significantly correlated. Another study supported this result, which found that lactoferrin concentration in human milk did not depend on maternal iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between maternal iron status and lactoferrin concentration in human milk at birth and during early lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27].

A study in Brazil found that total protein levels in human milk were higher in anemic mothers regardless of their lactation stage (colostrum, transition, mature) [28]. Since lactoferrin is the main whey protein in human milk, and all mothers in the present study were non-anemic, we assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further studies are needed to explain the mechanism.

Generalisability

The present study had small sample size hence it was not possible to generalize the result. More studies in different cities in Indonesia are needed to increase generalizability.

Conclusions

Maternal iron status as indicated by Hb and sTfR concentration did not affect lactoferrin concentration in human milk. However, the mechanism of milk lactoferrin homeostasis is not completely understood. Thus, further studies are needed to help promote better health for mothers and their children.

Acknowledgments. This study was approved by Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, (No. 252/EC/FKM/2016). Financial support was provided by the Directorate of Community Nutrition from the Ministry of Health, Republic of Indonesia, with grant number HK.03.01/V/365/2017.

Source of funding: This work was financed by the Directorate of Community Nutrition from the Ministry of Health, Republic of Indonesia.

Conflicts of interest: The authors declare no conflicts of interest.

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Manuscript body

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Editorial Comments Revised 4

September 29, 2020
FAMILY-00831-2020-04
Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

We have carefully evaluated your manuscript, entitled: Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers, and feel that as it stands we cannot accept it. We might, however, be able to accept it if you could respond adequately to the points that have been raised during the review process (see below).

Please revise your manuscript strictly according to the attached Reviewers' comments. Your manuscript won't be taken into consideration without the revisions made according to the recommendations.
The discussion requires a detailed editing, without division into subsections, with an extension of the references.

Authors are requested to prepare a revised version of their manuscript and the detailed reply to Reviewers with a list of all made changes as soon as possible. All changes in the revised version should be clearly indicated (by colored background or colored fonts).

Thank you for submitting your work to our journal.

Yours sincerely,
Editorial Office of FM&PCR

Revised Manuscript Draft ver 4

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Type

Original paper

Keywords

lactoferrin, Human milk, hemoglobins, transferrin receptors

Abstract

Background
Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives
This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods
This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results
There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions
The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Explanation letter

Dear Editor,

We have revised the manuscript based on recommendation. In section "Discussion", we have added more detailed explanation without division into subsections. Since we added more explanation, there is an extension of the references.
All changes are indicated with red font color.

Thank you.

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Summary

Background. Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.
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Background

Over the years, many studies have proved that breastfeeding is important for both mothers and children. Breastfeeding is associated with lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence in children. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life. Mothers who breastfeed their children receive many benefits, such as preventing breast cancer, improved

birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommendations, children should be exclusively breastfed for the first six months; breastfeeding should then continue for up to two years while also being provided with nutritionally adequate and safe complementary food [3].

One of the factors that has a role in the benefits of breastfeeding is the content of human milk, which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients [4]. It contains biologically active components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 different proteins in which the concentration differs according to the child's age and other characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, lactoferrin may impact gut health and gut-immune development and functioning, decreases the risk of lower respiratory tract illness, and decreases the burden of colonization by some

parasites in underdeveloped settings [9]. In terms of nutritional function, lactoferrin transports iron and detoxifies free radicals in biological fluids so that it is beneficial for people with iron deficiency [8].

Iron deficiency commonly occurs during pregnancy due to the increase in iron demand. It develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores are completely depleted, iron accessibility to the tissues declines, leading to symptomatic anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 48.9% in 2018 [12].

Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected by IDA considering that maternal nutritional status is closely associated with the quality of human milk; therefore, impairment in human milk content may occur due to maternal anemia. Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content [14].

The WHO and Centers for Disease Control and Prevention (CDC) Technical Consultation have established hemoglobin (Hb) and sTfR concentration as an indicator of iron status in the population. Hb concentration is a measure of anemia while sTfR, which is derived mostly from developing red blood cells, reflects the balance between cellular iron requirements and iron supply, and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis [15].

A study on anemic and non-anemic breastfeeding mothers found that, even though Hb concentration increases after iron supplementation, the lactoferrin concentration in both groups was similar at the end of 30 days of supplementation [16]. From our previous study, we know that mothers with better nutritional status have a higher lactoferrin concentration in their milk [17]. However, there have been few studies related to maternal iron status and its effect on the quality of human milk, and it is poorly understood. Considering that lactoferrin is the main protein of human milk and has many benefits for infants' growth and development during the breastfeeding period, this study aims to analyze its correlation with iron status.

Objectives

This study aimed to analyze the correlation between Hb and sTfR concentration during pregnancy with the lactoferrin concentration of breastfeeding mothers.

Material and methods

Study Design

This was a quantitative study with an analytical design and a cross-sectional approach.

Setting

The study was conducted for three months, from September to November 2017, in the working areas of Kedungmundu, Bangetayu, and Genuk primary health centers in Semarang City, Indonesia. The data for this study were collected in two periods. The first data collection was during the third trimester of pregnancy; this included data on subject characteristics, anthropometric measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The second data collection was after delivery in which milk samples

were collected for lactoferrin concentration analysis. All subjects participated in both stages of data collection.

Participants

The subjects were 79 pregnant mothers who were selected using purposive sampling. The sample size was determined with Slovin's formula. The inclusion criteria were willingness to participate in the study, giving birth in September 2017, breastfeeding mothers, had singleton child, had children born at a normal weight (> 2500 g) and had children without abnormalities that made suckling difficult.

Variables

The variables assessed in this study were maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration.

Data Sources/ Measurement

Data on subject characteristics, such as education level and occupation, were obtained through interviews with the subjects. Education level was categorized into two groups: (a) primary, which was six years in elementary school and three years in junior high school; and (b) secondary or higher, which was three years in senior high school and about three to four years at college.

Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive days, with food pictures to help subjects determine the food portions they consumed. Food intake was recorded in the form of household portions such as tablespoons, teaspoons, cups, etc. This was then converted into grams and analyzed using NutriSurvey software to calculate the nutrition intake. The data were then compared with the Indonesian recommended dietary allowance (RDA) which is based on the 2019 Republic of Indonesia Ministry of Health

Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 cm were categorized as at risk of chronic energy deficiency (CEM) [19].

For the analysis of Hb and sTfR, about 5 mL of venous blood was taken from the subjects once in the morning between 8 am and 10 am. Hb concentration was measured using cyanmethemoglobin, while STfR was measured using a Quantikine IVD Human STfR Immunoassay (R&D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay (ELISA) Reader 680 using a quantitative sandwich technique. Subjects were categorized as anemic if Hb concentration was below 11 g/dL [19] and sTfR concentration was greater than or equal to 21.0 nmol/L [20].

For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a refrigerator during the visit and further stored in a freezer at −20°C. Storage time both in the refrigerator and the freezer was recorded and considered during analysis to ensure it did not affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding mothers was confirmed by the day breastfeeding began and the infants' age at the time of collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanograms/ml.

Statistical Methods

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using

Pearson's product moment test, while the rank Spearman test was used to assess the correlation of variables if the data distribution was not normal.

Ethical consideration

Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion.

Results

The mean age of the subjects at the time of study was 27.95 ± 5.08 years old. During the third trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had secondary or higher education levels (68.4%), were housewives (65.8%), and had normal nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature (34.2%) (Table 1).

Table 1. Characteristics distribution of subjects

Variables	Value
Age (years)	27.95 ± 5.08
MUAC[a] at third trimester (cm)	25.0 (19.8–35.0)
Level of education:	
Primary	25 (31.6%)
Secondary or higher	54 (68.4%)
Occupation:	
Housewife	52 (65.8%)
Laborer	8 (10.1%)
Entrepreneur	7 (8.9%)
Private employee	4 (5.1%)
Civil servant	8 (10.1%)
Nutritional status:	
CEM[b]	1 (1.3%)
Normal	78 (98.7%)
Lactation stage:	
Colostrum (g/L)	28 (35.4%)
Transition (g/L)	24 (30.4%)
Mature (g/L)	27 (34.2%)

[a] Mid-upper arm circumference; [b] Chronic energy deficiency

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). Nutritional intake that had a significant relationship to Hb concentration was protein, dietary cholesterol, fat, all types of vitamin B (thiamin, riboflavin, niacin, vitamin B6, folic acid, and vitamin B12), vitamin C, iron, and zinc. The sTfR concentration was significantly related to the intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, the rank Spearman test found no correlation with lactoferrin concentration (Table 3).

Table 2. Nutritional intake of subjects during the third trimester of pregnancy

Variables	Value	Min.	Max.
Energy (kcal)	2,595.58 ± 401.88		
Carbohydrate (g)	283.98 ± 60.49		
Protein (g)	113.81 ± 23.65		
Dietary cholesterol (mg)	377.0	0.0	1,314.0
Fat (g)	77.42 ± 26.61		
Dietary fiber (g)	37.2	31.9	51.0
Vitamin A (RE)	967.0	49.0	9,462.0
Thiamin (mg)	1.8	1.1	2.9
Riboflavin (mg)	2.13 ± 1.29		
Niacin (mg)	12.01 ± 4.66		
Vitamin B6 (mg)	1.4	0.6	4.6
Folic acid (μg)	716.0	545.0	1,346.0
Vitamin B12 (μg)	6.1	0.1	65.4
Vitamin C (mg)	93.0	51.1	388.0
Vitamin E (μg)	15.0	15.0	16.2
Iron (mg)	40.5	26.3	47.3
Zinc (mg)	20.09 ± 3.15		

Table 3. Nutritional intake and its correlation to maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration

Variables	*p*-value		
	Hemoglobin (g/dL)	Serum transferrin receptor (nmol/L)	Lactoferrin (g/L)
Energy (kcal)	0.001	0.125	0.783
Carbohydrate (g)	0.073	0.692	0.405
Protein (g)	0.002	0.072	0.848
Dietary cholesterol (mg)	0.001	0.235	0.665
Fat (g)	0.009	0.516	0.342
Dietary fiber (g)	0.517	0.929	0.708
Vitamin A (RE)	0.078	0.312	0.278
Thiamin (mg)	0.002	0.036	0.952
Riboflavin (mg)	0.012	0.098	0.986
Niacin (mg)	0.022	0.046	0.099
Vitamin B6 (mg)	0.021	0.171	0.599
Folic acid (μg)	0.001	0.024	0.768
Vitamin B12 (μg)	0.015	0.192	0.750
Vitamin C (mg)	0.001	0.001	0.969
Vitamin E (μg)	0.337	0.133	0.701
Iron (mg)	0.001	0.001	0.443
Zinc (mg)	0.001	0.207	0.460

Mothers did not suffer from anemia, which was shown through Hb concentration above 11 g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in

human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5). However, Hb and sTfR concentration showed a significant inverse correlation (p = 0.001, r = −0.438).

Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers

Variables	Median	Min.	Max.
Hemoglobin (g/dL)	11.3	8.9	14.3
Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
Lactoferrin (g/L)	1.52	0.38	2.94

Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration of breastfeeding mothers

Variables	Lactoferrin concentration (g/L)	
	R	*p*-value
Hemoglobin (g/dL)	0.054	0.636
Serum transferrin receptor (nmol/L)	0.046	0.686

Discussion

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third trimester was adequate. This was reflected in the MUAC, which was equal to or more than 23.5 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not have anemia which could be a result of adequate iron intake as well as other important nutrients that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, polyphenols, calcium, and proteins in milk products, and will increase the absorption of both native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role in the formation of red blood cells [22].

Maternal iron intake was significantly correlated to both Hb and sTfR concentration. A study of breastfeeding mothers in Mexico showed that iron supplementation improved maternal Hb and sTfR concentration [23]. Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case of anemia, Hb concentration became lower while sTfR concentration rose. This result was supported by other studies in which Hb and sTfR were negatively correlated [24, 25].

In the present study, lactoferrin concentration was found to be lower compared to mean lactoferrin concentration in several countries in Asia, namely Bangladesh (5.72 g/L), India (3.71 g/L), Japan (4.17 g/L), and Thailand (2.27 g/L) [26]. However, the concentration was in a similar range as China, which varied from 0.99 g/L to 1.91 g/L across its 11 provinces [27].

Regardless of the adequate nutritional intake of mothers, it was not associated with lactoferrin concentration. Consistent with this result, Cai et al. found no significant correlation between lactoferrin concentration in mature human milk with various food intake of mothers [28]. Several studies also specifically reported that maternal protein intake did not affect lactoferrin content in human milk [27–29]. Therefore, consumption of food sources of protein such as meat, soy, or milk was not necessarily associated with lactoferrin concentration [27]. This was likely due to the synthesis from maternal stores or body tissues when nutrients in milk were insufficient to maintain the balance of milk content [30].

In our previous study, we found that maternal nutritional status was a predictor of lactoferrin concentration in human milk [17], but evidently it was not the case for maternal iron status. Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin concentration was low and they were not significantly correlated. Another study supported this result, which found that lactoferrin concentration in human milk did not depend on maternal iron status or iron supplementation [26]. Zavaleta et al. reported no correlation between maternal iron status and lactoferrin concentration in human milk at birth and during early lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [31].

A study in Brazil found that total protein levels in human milk were higher in anemic mothers regardless of their lactation stage (colostrum, transition, mature) [32]. Since lactoferrin is the main whey protein in human milk, and all mothers in the present study were non-anemic, we assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further studies are needed to explain the mechanism.

Conclusions

Maternal iron status as indicated by Hb and sTfR concentration did not affect lactoferrin concentration in human milk. However, the mechanism of milk lactoferrin homeostasis is not completely understood. Thus, further studies are needed to help promote better health for mothers and their children. A limitation of this study was that data on nutritional intake with 24-hours recall method might not represent the long-term dietary habits of the subjects. Moreover, there was a possibility that the subjects could not remember all the food they consumed for the day which would lead to an inaccurate data record.

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Conflicts of interest: The authors declare no conflicts of interest.

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Family Medicine & Primary Care Review

Authors:
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Decision letter:
November 04, 2020
FAMILY-00831-2020-05
Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

I am pleased to inform you that your manuscript, entitled: Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers, might be accepted for publication in our journal, pending some minor changes suggested by reviewers (see below).

The reviewer's recommendation was to evaluate the English language by a native speaker. Has such a verification been carried out - no information available. Further editorial proceedings will be suspended until the native speaker decides.

Please revise your paper strictly according to the attached Reviewers comments. Your manuscript won't be taken into consideration without the revisions made according to the recommendations.

Authors are requested to prepare a revised version of their manuscript and the detailed reply to Reviewers with a list of all made changes as soon as possible. All changes in the revised version should be clearly indicated (by colored background or colored fonts).

Thank you for submitting your work to us.

Yours sincerely,
Editorial Office of FM&PCR

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Type

Original paper

Keywords

lactoferrin, Human milk, hemoglobins, transferrin receptors

Abstract

Background
Human milk is rich in both nutrient and non-nutrient content which leads to many benefits for the growth and development of children's and mothers' bodies. Lactoferrin is one of the main proteins contained in human milk, and the factors that affect its concentration are important to comprehend.

Objectives
This study aimed to analyze the correlation between hemoglobin and serum transferrin receptor with lactoferrin concentration in human milk.

Material and methods
This cross-sectional study was conducted from September to November 2017. The subjects were 79 pregnant mothers in three working areas of primary health centers in Semarang City, Indonesia. Hemoglobin and serum transferrin receptor data were obtained from blood during the third trimester of pregnancy, while lactoferrin concentration was measured in milk after delivery. Hemoglobin concentration was measured using cyanmethemoglobin, serum transferrin receptor concentration using enzyme-linked immunosorbent assay (ELISA), and lactoferrin concentration using a human lactoferrin ELISA. Data analysis was performed with the rank Spearman statistical test using SPSS version 23.

Results
There was no correlation between maternal hemoglobin and lactoferrin concentration ($p = 0.636$). There was also no correlation between serum transferrin receptor and lactoferrin concentration ($p = 0.688$). Hemoglobin and serum transferrin receptors did not affect the concentration of lactoferrin in breastfeeding mothers.

Conclusions
The mechanism of lactoferrin homeostasis in human milk is still not completely understood. Further studies on this are important in order to promote a better quality of health for mothers and their children.

Explanation letter

Dear Editor,

Regarding the language evaluation for the manuscript draft, we have done that with Scribendi, a proofreading service based in Canada. Actually we have informed it since the first revision and no further adjustments need for it according to the reviewer.
Hope this clear the confusion.

Thank you.

Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

Summary

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Key words: human milk, lactoferrin, hemoglobins, transferrin receptors.

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Over the years, many studies have proved that breastfeeding is important for both mothers and children. Breastfeeding is associated with lower infectious morbidity and mortality, fewer dental malocclusions, and higher intelligence in children. Growing evidence also suggests that breastfeeding might protect against overweight and diabetes later in life. Mothers who breastfeed their children receive many benefits, such as preventing breast cancer, improved

birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommendations, children should be exclusively breastfed for the first six months; breastfeeding should then continue for up to two years while also being provided with nutritionally adequate and safe complementary food [3].

One of the factors that has a role in the benefits of breastfeeding is the content of human milk, which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, and immune-boosting properties in addition to nutrients [4]. It contains biologically active components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 different proteins in which the concentration differs according to the child's age and other characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, lactoferrin may impact gut health and gut-immune development and functioning, decreases the risk of lower respiratory tract illness, and decreases the burden of colonization by some

parasites in underdeveloped settings [9]. In terms of nutritional function, lactoferrin transports iron and detoxifies free radicals in biological fluids so that it is beneficial for people with iron deficiency [8].

Iron deficiency commonly occurs during pregnancy due to the increase in iron demand. It develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores are completely depleted, iron accessibility to the tissues declines, leading to symptomatic anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 48.9% in 2018 [12].

Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected by IDA considering that maternal nutritional status is closely associated with the quality of human milk; therefore, impairment in human milk content may occur due to maternal anemia. Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content [14].

The WHO and Centers for Disease Control and Prevention (CDC) Technical Consultation have established hemoglobin (Hb) and sTfR concentration as an indicator of iron status in the population. Hb concentration is a measure of anemia while sTfR, which is derived mostly from developing red blood cells, reflects the balance between cellular iron requirements and iron supply, and it is a marker of the severity of iron insufficiency only when iron stores have been exhausted, provided that there are no other causes of abnormal erythropoiesis [15].

A study on anemic and non-anemic breastfeeding mothers found that, even though Hb concentration increases after iron supplementation, the lactoferrin concentration in both groups was similar at the end of 30 days of supplementation [16]. From our previous study, we know that mothers with better nutritional status have a higher lactoferrin concentration in their milk [17]. However, there have been few studies related to maternal iron status and its effect on the quality of human milk, and it is poorly understood. Considering that lactoferrin is the main protein of human milk and has many benefits for infants’ growth and development during the breastfeeding period, this study aims to analyze its correlation with iron status.

Objectives

This study aimed to analyze the correlation between Hb and sTfR concentration during pregnancy with the lactoferrin concentration of breastfeeding mothers.

Material and methods

Study Design

This was a quantitative study with an analytical design and a cross-sectional approach.

Setting

The study was conducted for three months, from September to November 2017, in the working areas of Kedungmundu, Bangetayu, and Genuk primary health centers in Semarang City, Indonesia. The data for this study were collected in two periods. The first data collection was during the third trimester of pregnancy; this included data on subject characteristics, anthropometric measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The second data collection was after delivery in which milk samples

were collected for lactoferrin concentration analysis. All subjects participated in both stages of data collection.

Participants

The subjects were 79 pregnant mothers who were selected using purposive sampling. The sample size was determined with Slovin's formula. The inclusion criteria were willingness to participate in the study, giving birth in September 2017, breastfeeding mothers, had singleton child, had children born at a normal weight (> 2500 g) and had children without abnormalities that made suckling difficult.

Variables

The variables assessed in this study were maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration.

Data Sources/ Measurement

Data on subject characteristics, such as education level and occupation, were obtained through interviews with the subjects. Education level was categorized into two groups: (a) primary, which was six years in elementary school and three years in junior high school; and (b) secondary or higher, which was three years in senior high school and about three to four years at college.

Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive days, with food pictures to help subjects determine the food portions they consumed. Food intake was recorded in the form of household portions such as tablespoons, teaspoons, cups, etc. This was then converted into grams and analyzed using NutriSurvey software to calculate the nutrition intake. The data were then compared with the Indonesian recommended dietary allowance (RDA) which is based on the 2019 Republic of Indonesia Ministry of Health

Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 cm were categorized as at risk of chronic energy deficiency (CEM) [19].

For the analysis of Hb and sTfR, about 5 mL of venous blood was taken from the subjects once in the morning between 8 am and 10 am. Hb concentration was measured using cyanmethemoglobin, while STfR was measured using a Quantikine IVD Human STfR Immunoassay (R&D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay (ELISA) Reader 680 using a quantitative sandwich technique. Subjects were categorized as anemic if Hb concentration was below 11 g/dL [19] and sTfR concentration was greater than or equal to 21.0 nmol/L [20].

For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a refrigerator during the visit and further stored in a freezer at −20°C. Storage time both in the refrigerator and the freezer was recorded and considered during analysis to ensure it did not affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding mothers was confirmed by the day breastfeeding began and the infants' age at the time of collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit of 1.1 nanograms/ml.

Statistical Methods

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using

Pearson's product moment test, while the rank Spearman test was used to assess the correlation of variables if the data distribution was not normal.

Ethical consideration

Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent before inclusion.

Results

The mean age of the subjects at the time of study was 27.95 ± 5.08 years old. During the third trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had secondary or higher education levels (68.4%), were housewives (65.8%), and had normal nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature (34.2%) (Table 1).

Table 1. Characteristics distribution of subjects

Variables	Value
Age (years)	27.95 ± 5.08
MUAC[a] at third trimester (cm)	25.0 (19.8–35.0)
Level of education:	
Primary	25 (31.6%)
Secondary or higher	54 (68.4%)
Occupation:	
Housewife	52 (65.8%)
Laborer	8 (10.1%)
Entrepreneur	7 (8.9%)
Private employee	4 (5.1%)
Civil servant	8 (10.1%)
Nutritional status:	
CEM[b]	1 (1.3%)
Normal	78 (98.7%)
Lactation stage:	
Colostrum (g/L)	28 (35.4%)
Transition (g/L)	24 (30.4%)
Mature (g/L)	27 (34.2%)

[a] Mid-upper arm circumference; [b] Chronic energy deficiency

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). Nutritional intake that had a significant relationship to Hb concentration was protein, dietary cholesterol, fat, all types of vitamin B (thiamin, riboflavin, niacin, vitamin B6, folic acid, and vitamin B12), vitamin C, iron, and zinc. The sTfR concentration was significantly related to the intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, the rank Spearman test found no correlation with lactoferrin concentration (Table 3).

Table 2. Nutritional intake of subjects during the third trimester of pregnancy

Variables	Value	Min.	Max.
Energy (kcal)	2,595.58 ± 401.88		
Carbohydrate (g)	283.98 ± 60.49		
Protein (g)	113.81 ± 23.65		
Dietary cholesterol (mg)	377.0	0.0	1,314.0
Fat (g)	77.42 ± 26.61		
Dietary fiber (g)	37.2	31.9	51.0
Vitamin A (RE)	967.0	49.0	9,462.0
Thiamin (mg)	1.8	1.1	2.9
Riboflavin (mg)	2.13 ± 1.29		
Niacin (mg)	12.01 ± 4.66		
Vitamin B6 (mg)	1.4	0.6	4.6
Folic acid (µg)	716.0	545.0	1,346.0
Vitamin B12 (µg)	6.1	0.1	65.4
Vitamin C (mg)	93.0	51.1	388.0
Vitamin E (µg)	15.0	15.0	16.2
Iron (mg)	40.5	26.3	47.3
Zinc (mg)	20.09 ± 3.15		

Table 3. Nutritional intake and its correlation to maternal hemoglobin, serum transferrin receptor, and lactoferrin concentration

Variables	*p*-value		
	Hemoglobin (g/dL)	Serum transferrin receptor (nmol/L)	Lactoferrin (g/L)
Energy (kcal)	0.001	0.125	0.783
Carbohydrate (g)	0.073	0.692	0.405
Protein (g)	0.002	0.072	0.848
Dietary cholesterol (mg)	0.001	0.235	0.665
Fat (g)	0.009	0.516	0.342
Dietary fiber (g)	0.517	0.929	0.708
Vitamin A (RE)	0.078	0.312	0.278
Thiamin (mg)	0.002	0.036	0.952
Riboflavin (mg)	0.012	0.098	0.986
Niacin (mg)	0.022	0.046	0.099
Vitamin B6 (mg)	0.021	0.171	0.599
Folic acid (µg)	0.001	0.024	0.768
Vitamin B12 (µg)	0.015	0.192	0.750
Vitamin C (mg)	0.001	0.001	0.969
Vitamin E (µg)	0.337	0.133	0.701
Iron (mg)	0.001	0.001	0.443
Zinc (mg)	0.001	0.207	0.460

Mothers did not suffer from anemia, which was shown through Hb concentration above 11 g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in

human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5). However, Hb and sTfR concentration showed a significant inverse correlation (p = 0.001, r = −0.438).

Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers

Variables	Median	Min.	Max.
Hemoglobin (g/dL)	11.3	8.9	14.3
Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
Lactoferrin (g/L)	1.52	0.38	2.94

Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration of breastfeeding mothers

Variables	Lactoferrin concentration (g/L)	
	R	*p*-value
Hemoglobin (g/dL)	0.054	0.636
Serum transferrin receptor (nmol/L)	0.046	0.686

Discussion

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third trimester was adequate. This was reflected in the MUAC, which was equal to or more than 23.5 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not have anemia which could be a result of adequate iron intake as well as other important nutrients that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, polyphenols, calcium, and proteins in milk products, and will increase the absorption of both native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role in the formation of red blood cells [22].

Maternal iron intake was significantly correlated to both Hb and sTfR concentration. A study of breastfeeding mothers in Mexico showed that iron supplementation improved maternal Hb and sTfR concentration [23]. Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case of anemia, Hb concentration became lower while sTfR concentration rose. This result was supported by other studies in which Hb and sTfR were negatively correlated [24, 25].

In the present study, lactoferrin concentration was found to be lower compared to mean lactoferrin concentration in several countries in Asia, namely Bangladesh (5.72 g/L), India (3.71 g/L), Japan (4.17 g/L), and Thailand (2.27 g/L) [26]. However, the concentration was in a similar range as China, which varied from 0.99 g/L to 1.91 g/L across its 11 provinces [27].

Regardless of the adequate nutritional intake of mothers, it was not associated with lactoferrin concentration. Consistent with this result, Cai et al. found no significant correlation between lactoferrin concentration in mature human milk with various food intake of mothers [28]. Several studies also specifically reported that maternal protein intake did not affect lactoferrin content in human milk [27–29]. Therefore, consumption of food sources of protein such as meat, soy, or milk was not necessarily associated with lactoferrin concentration [27]. This was likely due to the synthesis from maternal stores or body tissues when nutrients in milk were insufficient to maintain the balance of milk content [30].

In our previous study, we found that maternal nutritional status was a predictor of lactoferrin concentration in human milk [17], but evidently it was not the case for maternal iron status. Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin concentration was low and they were not significantly correlated. Another study supported this result, which found that lactoferrin concentration in human milk did not depend on maternal iron status or iron supplementation [26]. Zavaleta et al. reported no correlation between maternal iron status and lactoferrin concentration in human milk at birth and during early lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [31].

A study in Brazil found that total protein levels in human milk were higher in anemic mothers regardless of their lactation stage (colostrum, transition, mature) [32]. Since lactoferrin is the main whey protein in human milk, and all mothers in the present study were non-anemic, we assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further studies are needed to explain the mechanism.

Conclusions

Maternal iron status as indicated by Hb and sTfR concentration did not affect lactoferrin concentration in human milk. However, the mechanism of milk lactoferrin homeostasis is not completely understood. Thus, further studies are needed to help promote better health for mothers and their children. A limitation of this study was that data on nutritional intake with 24-hours recall method might not represent the long-term dietary habits of the subjects. Moreover, there was a possibility that the subjects could not remember all the food they consumed for the day which would lead to an inaccurate data record.

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Conflicts of interest: The authors declare no conflicts of interest.

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Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers
Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

Dear Mohammad Rahfiludin,

I am pleased to inform you that your manuscript, entitled: Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers, has been finally accepted for publication in our journal.

Thank you for submitting your work to us.

Yours sincerely,
Editorial Office of FM&PCR

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Correlation between maternal hemoglobin and serum transferrin receptor with lactoferrin concentration in breastfeeding mothers

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