# KORESPONDENSI JURNAL

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

Penulis : Mohammad Zen Rahfiludin, Dina Rahayuning Pangestuti, Suyatno, Suroto

- Jurnal : Family Medicine & Primary Care Review
- Penerbit : Wydawnictwo Continuo

No.	Kegiatan	Tanggal	Keterangan
1.	Submission and Editorial Comments	08-08-2020	Journal System Dashboard
	Revised 1		
2.	Revised Manuscript Draft ver 1		Response to revision request
			(Journal System Dashboard)
3.	Editorial Comments Revised 2	14-09-2020	Journal System Dashboard
4.	Revised Manuscript Draft ver 2		Response to revision request
			(Journal System Dashboard)
5.	Editorial Comments Revised 3	17-09-2020	Journal System Dashboard
	Revised Manuscript Draft ver 3		Response to revision request
			(Journal System Dashboard)
	Editorial Comments Revised 4	29-09-2020	Journal System Dashboard
	Revised Manuscript Draft ver 4		Response to revision request
			(Journal System Dashboard)
	Editorial Comments Revised 5	04-11-2020	Journal System Dashboard
	Revised Manuscript Draft ver 5		Response to revision request
			(Journal System Dashboard)
6.	Accepted	04-02-2021	Journal System Dashboard
7.	Published Online	30-12-2021	Web Journal

Submission and Editorial Comments Revised 1



### Authors:

Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

### **Decision letter:**

August 08, 2020 FAMILY-00831-2020-01 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 (<u>https://meshb.nlm.nih.gov</u>): 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 (<u>https://meshb.nlm.nih.gov</u>): 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.



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

#### Туре

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

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**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.

# <sup>28</sup> 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



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<sup>35</sup> birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia <sup>36</sup> even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based <sup>37</sup> on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) <sup>38</sup> recommendations, children should be exclusively breastfed for the first six months; <sup>39</sup> breastfeeding should then continue for up to two years while also being provided with <sup>40</sup> 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, 41 which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, 42 and immune-boosting properties in addition to nutrients [4]. It contains biologically active 43 components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 44 different proteins in which the concentration differs according to the child's age and other 45 characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a 46 protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main 47 whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide 48 chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. 49 Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor 50 (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many 51 other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and 52 whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. 53 Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, 54 excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin 55 concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic 56 activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, 57 58 lactoferrin may impact gut health and gut-immune development and functioning, decreases the 59 risk of lower respiratory tract illness, and decreases the burden of colonization by some



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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 64 develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores 65 66 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 67 an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women 68 suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence 69 of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 70 48.9% in 2018 [12]. 71

<sup>72</sup> Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with <sup>73</sup> perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, <sup>74</sup> birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected <sup>75</sup> by IDA considering that maternal nutritional status is closely associated with the quality of <sup>76</sup> human milk; therefore, impairment in human milk content may occur due to maternal anemia. <sup>77</sup> Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content <sup>78</sup> [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].



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A study on anemic and non-anemic breastfeeding mothers found that, even though Hb 86 concentration increases after iron supplementation, the lactoferrin concentration in both groups 87 was similar at the end of 30 days of supplementation [16]. From our previous study we know 88 that mothers with better nutritional status have a higher lactoferrin concentration in their milk 89 [17]. However, there have been few studies related to maternal iron status and its effect on the 90 quality of human milk, and it is poorly understood. Considering that lactoferrin is the main 91 92 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. 93

#### 94 **Objectives**

<sup>95</sup> This study aimed to analyze the correlation between Hb and sTfR concentration during <sup>96</sup> pregnancy with the lactoferrin concentration of breastfeeding mothers.

97 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.

104 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



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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.

116 Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive 117 days, with food pictures to help subjects determine the food portions they consumed. Food 118 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 119 120 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 121 Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm 122 circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 123 124 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 immunosorb ent 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].



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For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with 133 a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a 134 refrigerator during the visit and further stored in a freezer at  $-20^{\circ}$ C. Storage time both in the 135 refrigerator and the freezer was recorded and considered during analysis to ensure it did not 136 affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed 137 to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding 138 mothers was confirmed by the day breastfeeding began and the infants' age at the time of 139 140 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 141 142 of 1.1 nanograms/ml.

#### <sup>143</sup> 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.

#### 148 **Results**

The mean age of the subjects at the time of study was  $27.95 \pm 5.08$  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).



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157	Variables	Value
58	Age (years)	$27.95 \pm 5.08$
59	MUAC at third trimester (cm)	25.0 (19.8–35.0)
60	Level of education:	
61	Primary	25 (31.6%)
162	Secondary or higher	54 (68.4%)
63	Occupation:	
64	Housewife	52 (65.8%)
65	Laborer	8 (10.1%)
66	Entrepreneur	7 (8.9%)
67	Private employee	4 (5.1%)
68	Civil servant	8 (10.1%)
69	Nutritional status:	
70	CEM	1 (1.3%)
71	Normal	78 (98.7%)
72	Lactation stage:	
73	Colostrum (g/L)	28 (35.4%)
74	Transition (g/L)	24 (30.4%)
75	Mature $(g/L)$	27 (34.2%)

#### Table 1. Characteristics distribution of subjects

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).



184	Variables	Value	Min.	Max.
185	Energy (kcal)	$2,595.58 \pm 401.88$		
186	Carbohydrate (g)	$283.98 \pm 60.49$		
187	Protein (g)	$113.81 \pm 23.65$		
188	Dietary cholesterol (mg)	377.0	0.0	1,314.0
189	Fat (g)	$77.42 \pm 26.61$		
190	Dietary fiber (g)	37.2	31.9	51.0
191	Vitamin A (RE)	967.0	49.0	9,462.0
192	Thiamin (mg)	1.8	1.1	2.9
193	Riboflavin (mg)	$2.13 \pm 1.29$		
194	Niacin (mg)	$12.01 \pm 4.66$		
195	Vitamin B6 (mg)	1.4	0.6	4.6
196	Folic acid (µg)	716.0	545.0	1,346.0
197	Vitamin B12 (µg)	6.1	0.1	65.4
198	Vitamin C (mg)	93.0	51.1	388.0
199	Vitamin E (µg)	15.0	15.0	16.2
200	Iron (mg)	40.5	26.3	47.3
201	Zinc (mg)	$20.09 \pm 3.15$		

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

Table 3. Nut	tritional intake a	nd its correlation	to maternal	hemoglobin,	serum
transferrin	receptor, and lact	toferrin concentra	ation		

204			<i>p</i> -value	
205	Variables	Hemoglobin	Serum transferrin receptor	Lactoferrin
206		(g/dL)	(nmol/L)	(g/L)
207	Energy (kcal)	0.001	0.125	0.783
208	Carbohydrate (g)	0.073	0.692	0.405
209	Protein (g)	0.002	0.072	0.848
210	Dietary cholesterol (mg)	0.001	0.235	0.665
211	Fat (g)	0.009	0.516	0.342
212	Dietary fiber (g)	0.517	0.929	0.708
213	Vitamin A (RE)	0.078	0.312	0.278
214	Thiamin (mg)	0.002	0.036	0.952
215	Riboflavin (mg)	0.012	0.098	0.986
216	Niacin (mg)	0.022	0.046	0.099
217	Vitamin B6 (mg)	0.021	0.171	0.599
218	Folic acid (µg)	0.001	0.024	0.768
219	Vitamin B12 (µg)	0.015	0.192	0.750
220	Vitamin C (mg)	0.001	0.001	0.969
221	Vitamin E (µg)	0.337	0.133	0.701
222	Iron (mg)	0.001	0.001	0.443
223	Zinc (mg)	0.001	0.207	0.460



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225	Mothers did not suffer from anemia, which was shown through Hb concentration above 11
226	g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in
227	human milk was 1.52 (0.38-2.94) g/L (Table 4). There was no correlation between both Hb
228	and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5).
229	However, Hb and sTfR concentration showed a significant inverse correlation ( $p = 0.001$ , r =
230	-0.438).

# Table 4. Hemoglobin, serum transferrin receptor, and lactoferrin concentration ofbreastfeeding mothers

233	Variables	Median	Min.	Max.
234	Hemoglobin (g/dL)	11.3	8.9	14.3
235	Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
236	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

239	Variables	Lactoferrin con	centration (g/L)
200	v ariables	R	<i>p</i> -value
240	Hemoglobin (g/dL)	0.054	0.636
241	Serum transferrin receptor (nmol/L)	0.046	0.686

## 242 **Discussion**

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Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third 243 trimester was adequate. This was reflected in the MUAC, which was equal or more than 23.5 244 245 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not 246 have anemia which could be a result of adequate iron intake as well as other important nutrients 247 that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or 248 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 249 250 native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role 251 in the formation of red blood cells [22].



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<sup>253</sup> Hb and sTfR were found to have an inverse correlation. It can be interpreted that, in the case <sup>254</sup> of anemia, Hb concentration became lower while sTfR concentration rose. This result was <sup>255</sup> 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].

260 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. 261 Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin 262 263 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 264 265 iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between 266 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 267 268 and used Hb and hematocrit value to determine anemic status [16]. In India, a study in nonanemic and anemic breastfeeding mothers found that maternal Hb did not correlate with 269 lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27]. 270

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.



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## 277 Conclusions

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

#### 291 **References**

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298

- <sup>292</sup> [1] Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology,
   <sup>293</sup> mechanisms, and lifelong effect. *Lancet* 2016; 387: 475–490.
- [2] Mikšić Š, Uglešić B, Jakab J, et al. Positive effect of breastfeeding on child development,
   anxiety, and postpartum depression. *Int J Environ Res Public Health* 2020; 17(8): 2725.
   DOI: 10.3390/ijerph17082725.
  - [3] World Health Organization, UNICEF. *Global strategy for infant and young child feeding*. Geneva: World Health Organization; 2003.
- Eglash A, Simon L. ABM clinical protocol #8: human milk storage information for home use for full-term infants, revised 2017. *Breastfeed Med* 2017; 12: 390–395.
- <sup>301</sup> [5] Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: a review on its



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303		composition and bioactivity. Early Hum Dev 2015; 91: 629-6	535.
304 305	[6]	Mosca F, Giannì ML. Human milk: composition and health b 2017; 39: 155.	penefits. Pediatr Med Chir
306 307 308	[7]	Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of lactoferrin maternal haemoglobin percentage among lactating wo socioeconomic status. <i>Pak J Physiol</i> 2017; 13: 30–33.	levels in breast milk with men of low and high
309 310 311	[8]	Wang B, Timilsena YP, Blanch E, et al. Lactoferrin: structu and digestion. <i>Crit Rev Food Sci Nutr</i> ; 8398. Epub ahe 10.1080/10408398.2017.1381583.	
312 313	[9]	Manzoni P. Clinical benefits of lactoferrin for infants and child S43–S52.	dren. JPediatr 2016; 173:
314 315	[10]	Abu-Ouf NM, Jan MM. The impact of maternal iron defici anemia on child's health. <i>Saudi Med J</i> 2015; 36: 146–149.	ency and iron deficiency
316 317	[11]	World Health Organization. Prevalence of anemia among Available from URL: https://data.worldbank.org/indicator/SH	1 0
318 319	[12]	National Institute of Health Research and Development Minis Indonesia. <i>Main result of basic health research 2018</i> . Jakarta	• -
320 321	[13]	Di Renzo GC, Spano F, Giardina I, et al. Iron deficiency anem <i>Heal</i> 2015; 11: 891–900.	ia in pregnancy. Women's
322 323 324	[14]	Fujita M, Paredes Ruvalcaba N, Wander K, et al. Buffered or in inflammation and breast milk macronutrients in northern Ke 2019; 168: 329–339.	-
325 326 327	[15]	World Health Organization/Centers for Disease Control a Consultation. Assessing the iron status of population. 2nd e Organization; 2004.	
328 329	[16]	Zavaleta N, Nombera J, Rojas R, et al. Iron and lactoferrin i given iron supplements. <i>Nutr Res</i> 1995; 15(5): 681–690.	n milk of anemic mothers
330 331	[17]	Rahfiludin MZ, Pangestuti DR. Lactoferrin association with and lactation stages. <i>Curr Res Nutr Food Sci J</i> 2020; 8: 174–	
332 333	[18]	Ministry of Health Republic of Indonesia. The 2019 Republic Health regulation no. 28 regarding Indonesian recommended	
334 335	[19]	Par'i HM, Wiyono S, Harjatmo TP. Assessment of nutritional of Health of Republic of Indonesia; 2017.	<i>ıl status</i> . Jakarta: Ministry
336 337 338	[20]	Khambalia AZ, Collins CE, Roberts CL, et al. Iron deficiency serum ferritin and soluble transferrin receptor concentration pregnancy and birth outcomes. <i>Eur J Clin Nutr</i> 2016; 70: 358	ons are associated with
339 340	[21]	Abbaspour N, Hurrell R, Kelishadi R. Review on iron and health. <i>J Res Med Sci</i> 2014; 19: 164–174.	its importance for human



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342	[22]	Bhardwaj A, Kumar D, Raina SK, et al. Rapid assessment for coexistence of vitamin
343		B12 and iron deficiency anemia among adolescent males and females in northern
344		Himalayan state of India. Anemia; 2013. Epub ahead of print. DOI:
345		10.1155/2013/959605.

- Yokus O, Yilmaz B, Albayrak M, et al. The significance of serum transferrin receptor [23] levels in the diagnosis of the coexistence of anemia of chronic disease and iron deficiency anemia. Eurasian J Med 2011; 43: 9-12.
- Yoon SH, Kim DS, Yu ST, et al. The usefulness of soluble transferrin receptor in the 349 [24] diagnosis and treatment of iron deficiency anemia in children. Korean J Pediatr 2015; 350 351 58: 15–19.
  - Rai D, Adelman AS, Zhuang W, et al. Longitudinal changes in lactoferrin concentrations [25] in human milk: a global systematic review. Crit Rev Food Sci Nutr 2014; 54: 1539-1547.
- 355 Yang Z, Jiang R, Chen Q, et al. Concentration of lactoferrin in human milk and its [26] 356 variation during lactation in different Chinese populations. Nutrients 2018; 10: 1-10.
- Shashiraj, Faridi MMA, Singh O, et al. Mother's iron status, breastmilk iron and 357 [27] 358 lactoferrin – are they related? Eur J Clin Nutr 2006; 60: 903–908.
- 359 [28] França EL, Silva VA, Volpato RMJ, et al. Maternal anemia induces changes in 360 immunological and nutritional components of breast milk. J Matern Neonatal Med 361 2013; 26: 1223-1227.

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**Editorial Comments Revised 2** 



### Authors:

Mohammad Rahfiludin, Dina Pangestuti, Suyatno Suyatno, Suroto Suroto

## **Decision letter:**

September 14, 2020 FAMILY-00831-2020-02 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 (<u>https://meshb.nlm.nih.gov</u>): 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



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

#### Туре

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

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**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.

# 28 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



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<sup>35</sup> birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia <sup>36</sup> even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based <sup>37</sup> on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) <sup>38</sup> recommendations, children should be exclusively breastfed for the first six months; <sup>39</sup> breastfeeding should then continue for up to two years while also being provided with <sup>40</sup> 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, 41 which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, 42 and immune-boosting properties in addition to nutrients [4]. It contains biologically active 43 components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 44 different proteins in which the concentration differs according to the child's age and other 45 characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a 46 protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main 47 whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide 48 chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. 49 Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor 50 (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many 51 other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and 52 whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. 53 Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, 54 excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin 55 concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic 56 activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, 57 58 lactoferrin may impact gut health and gut-immune development and functioning, decreases the 59 risk of lower respiratory tract illness, and decreases the burden of colonization by some



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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 64 develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores 65 66 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 67 an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women 68 suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence 69 of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 70 48.9% in 2018 [12]. 71

<sup>72</sup> Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with <sup>73</sup> perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, <sup>74</sup> birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected <sup>75</sup> by IDA considering that maternal nutritional status is closely associated with the quality of <sup>76</sup> human milk; therefore, impairment in human milk content may occur due to maternal anemia. <sup>77</sup> Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content <sup>78</sup> [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].



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A study on anemic and non-anemic breastfeeding mothers found that, even though Hb 86 concentration increases after iron supplementation, the lactoferrin concentration in both groups 87 was similar at the end of 30 days of supplementation [16]. From our previous study we know 88 that mothers with better nutritional status have a higher lactoferrin concentration in their milk 89 [17]. However, there have been few studies related to maternal iron status and its effect on the 90 quality of human milk, and it is poorly understood. Considering that lactoferrin is the main 91 92 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. 93

#### 94 **Objectives**

<sup>95</sup> This study aimed to analyze the correlation between Hb and sTfR concentration during <sup>96</sup> pregnancy with the lactoferrin concentration of breastfeeding mothers.

97 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.

104 Measurement

<sup>105</sup> The data for this study were collected in two periods. The first data collection was during the <sup>106</sup> third trimester of pregnancy; this included data on subject characteristics, anthropometric <sup>107</sup> measurement, nutrition intake, and blood samples for Hb and sTfR concentration analysis. The



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second data collection was after delivery in which milk samples were collected for lactoferrin

110 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.

116 Nutritional intake data were obtained using a 24-hour recall method for two non-consecutive 117 days, with food pictures to help subjects determine the food portions they consumed. Food 118 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 119 120 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 121 Regulation No. 28 [18]. The anthropometric data used in this study was mid-upper arm 122 circumference (MUAC) measured with a MUAC tape. Subjects with a MUAC of less than 23.5 123 124 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 immunosorb ent 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].





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For lactoferrin analysis, subjects' milk was collected door to door. About 5 mL was taken with 133 a sterilized human milk pump and placed in a sterile glass bottle. Samples were put inside a 134 refrigerator during the visit and further stored in a freezer at  $-20^{\circ}$ C. Storage time both in the 135 refrigerator and the freezer was recorded and considered during analysis to ensure it did not 136 affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed 137 to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding 138 mothers was confirmed by the day breastfeeding began and the infants' age at the time of 139 140 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 141 142 of 1.1 nanograms/ml.

#### <sup>143</sup> 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.

#### 148 **Results**

The mean age of the subjects at the time of study was  $27.95 \pm 5.08$  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).



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57	Variables	Value
58	Age (years)	$27.95 \pm 5.08$
59	MUAC at third trimester (cm)	25.0 (19.8–35.0)
60	Level of education:	
61	Primary	25 (31.6%)
62	Secondary or higher	54 (68.4%)
63	Occupation:	
64	Housewife	52 (65.8%)
65	Laborer	8 (10.1%)
66	Entrepreneur	7 (8.9%)
67	Private employee	4 (5.1%)
68	Civil servant	8 (10.1%)
69	Nutritional status:	
70	CEM	1 (1.3%)
71	Normal	78 (98.7%)
72	Lactation stage:	
73	Colostrum (g/L)	28 (35.4%)
74	Transition (g/L)	24 (30.4%)
75	Mature (g/L)	27 (34.2%)

#### Table 1. Characteristics distribution of subjects

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).



184	Variables	Value	Min.	Max.
185	Energy (kcal)	$2,595.58 \pm 401.88$		
186	Carbohydrate (g)	$283.98 \pm 60.49$		
187	Protein (g)	$113.81 \pm 23.65$		
188	Dietary cholesterol (mg)	377.0	0.0	1,314.0
189	Fat (g)	$77.42 \pm 26.61$		
190	Dietary fiber (g)	37.2	31.9	51.0
191	Vitamin A (RE)	967.0	49.0	9,462.0
192	Thiamin (mg)	1.8	1.1	2.9
193	Riboflavin (mg)	$2.13 \pm 1.29$		
194	Niacin (mg)	$12.01 \pm 4.66$		
195	Vitamin B6 (mg)	1.4	0.6	4.6
196	Folic acid (µg)	716.0	545.0	1,346.0
197	Vitamin B12 (µg)	6.1	0.1	65.4
198	Vitamin C (mg)	93.0	51.1	388.0
199	Vitamin E (µg)	15.0	15.0	16.2
200	Iron (mg)	40.5	26.3	47.3
201	Zinc (mg)	$20.09 \pm 3.15$		

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

Table 3. Nu	itritional in	take and its c	orrelation	to maternal	hemoglobin,	serum
transferrin	receptor, a	and lactoferrin	concentra	ation		

204		<i>p</i> -value		
205	Variables	Hemoglobin	Serum transferrin receptor	Lactoferrin
206		(g/dL)	(nmol/L)	(g/L)
207	Energy (kcal)	0.001	0.125	0.783
208	Carbohydrate (g)	0.073	0.692	0.405
209	Protein (g)	0.002	0.072	0.848
210	Dietary cholesterol (mg)	0.001	0.235	0.665
211	Fat (g)	0.009	0.516	0.342
212	Dietary fiber (g)	0.517	0.929	0.708
213	Vitamin A (RE)	0.078	0.312	0.278
214	Thiamin (mg)	0.002	0.036	0.952
215	Riboflavin (mg)	0.012	0.098	0.986
216	Niacin (mg)	0.022	0.046	0.099
217	Vitamin B6 (mg)	0.021	0.171	0.599
218	Folic acid (µg)	0.001	0.024	0.768
219	Vitamin B12 (µg)	0.015	0.192	0.750
220	Vitamin C (mg)	0.001	0.001	0.969
221	Vitamin E (µg)	0.337	0.133	0.701
222	Iron (mg)	0.001	0.001	0.443
223	Zinc (mg)	0.001	0.207	0.460



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225	Mothers did not suffer from anemia, which was shown through Hb concentration above 11
226	g/dL and sTfR concentration below 21.0 nmol/L. The median lactoferrin concentration in
227	human milk was 1.52 (0.38-2.94) g/L (Table 4). There was no correlation between both Hb
228	and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5).
229	However, Hb and sTfR concentration showed a significant inverse correlation ( $p = 0.001$ , r =
230	-0.438).

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

3	Variables	Median	Min.	Max.
4	Hemoglobin (g/dL)	11.3	8.9	14.3
5	Serum transferrin receptor (nmol/L)	15.06	8.6	34.9
5	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

239	Variables	Lactoferrin concentration (g/L)		
200	v anables	R	<i>p</i> -value	
240	Hemoglobin (g/dL)	0.054	0.636	
241	Serum transferrin receptor (nmol/L)	0.046	0.686	

#### 242 **Discussion**

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243 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 23.5 244 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not 245 246 have anemia which could be a result of adequate iron intake as well as other important nutrients 247 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, 248 249 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 250 251 in the formation of red blood cells [22].



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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].

260 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. 261 Although Hb and sTfR concentration met the standard for pregnant mothers, lactoferrin 262 263 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 264 265 iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between 266 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 267 268 and used Hb and hematocrit value to determine anemic status [16]. In India, a study in nonanemic and anemic breastfeeding mothers found that maternal Hb did not correlate with 269 lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27]. 270

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.



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### 277

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### 277 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.
- <sup>290</sup> Conflicts of interest: The authors declare no conflicts of interest.

## 291 **References**

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- <sup>292</sup> [1] Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology,
   <sup>293</sup> mechanisms, and lifelong effect. *Lancet* 2016; 387: 475–490.
- [2] Mikšić Š, Uglešić B, Jakab J, et al. Positive effect of breastfeeding on child development,
   anxiety, and postpartum depression. *Int J Environ Res Public Health* 2020; 17(8): 2725.
   DOI: 10.3390/ijerph17082725.
  - [3] World Health Organization, UNICEF. *Global strategy for infant and young child feeding*. Geneva: World Health Organization; 2003.
- [4] Eglash A, Simon L. ABM clinical protocol #8: human milk storage information for home use for full-term infants, revised 2017. *Breastfeed Med* 2017; 12: 390–395.
- <sup>301</sup> [5] Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: a review on its



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303		composition and bioactivity. Early Hum Dev 2015; 91: 629-635.
304 305	[6]	Mosca F, Giannì ML. Human milk: composition and health benefits. <i>Pediatr Med Chir</i> 2017; 39: 155.
306 307 308	[7]	Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of lactoferrin levels in breast milk with maternal haemoglobin percentage among lactating women of low and high socioeconomic status. <i>Pak J Physiol</i> 2017; 13: 30–33.
309 310 311	[8]	Wang B, Timilsena YP, Blanch E, et al. Lactoferrin: structure, function, denaturation and digestion. <i>Crit Rev Food Sci Nutr</i> ; 8398. Epub ahead of print 2017. DOI: 10.1080/10408398.2017.1381583.
312 313	[9]	Manzoni P. Clinical benefits of lactoferrin for infants and children. <i>J Pediatr</i> 2016; 173: S43–S52.
314 315	[10]	Abu-Ouf NM, Jan MM. The impact of maternal iron deficiency and iron deficiency anemia on child's health. <i>Saudi Med J</i> 2015; 36: 146–149.
316 317	[11]	World Health Organization. Prevalence of anemia among pregnant women. 2016. Available from URL: https://data.worldbank.org/indicator/SH.PRG.ANEM.
318 319	[12]	National Institute of Health Research and Development Ministry of Health Republic of Indonesia. <i>Main result of basic health research 2018</i> . Jakarta; 2018.
320 321	[13]	Di Renzo GC, Spano F, Giardina I, et al. Iron deficiency anemia in pregnancy. <i>Women's Heal</i> 2015; 11: 891–900.
322 323 324	[14]	Fujita M, Paredes Ruvalcaba N, Wander K, et al. Buffered or impaired: maternal anemia, inflammation and breast milk macronutrients in northern Kenya. <i>Am J Phys Anthropol</i> 2019; 168: 329–339.
325 326 327	[15]	World Health Organization/Centers for Disease Control and Prevention Technical Consultation. <i>Assessing the iron status of population</i> . 2nd ed. Geneva: World Health Organization; 2004.
328 329	[16]	Zavaleta N, Nombera J, Rojas R, et al. Iron and lactoferrin in milk of anemic mothers given iron supplements. <i>Nutr Res</i> 1995; 15(5): 681–690.
330 331	[17]	Rahfiludin MZ, Pangestuti DR. Lactoferrin association with maternal nutritional status and lactation stages. <i>Curr Res Nutr Food Sci J</i> 2020; 8: 174–181.
332 333	[18]	Ministry of Health Republic of Indonesia. The 2019 Republic of Indonesia Ministry of Health regulation no. 28 regarding Indonesian recommended dietary allowance; 2019.
334 335	[19]	Par'i HM, Wiyono S, Harjatmo TP. Assessment of nutritional status. Jakarta: Ministry of Health of Republic of Indonesia; 2017.
336 337 338	[20]	Khambalia AZ, Collins CE, Roberts CL, et al. Iron deficiency in early pregnancy using serum ferritin and soluble transferrin receptor concentrations are associated with pregnancy and birth outcomes. <i>Eur J Clin Nutr</i> 2016; 70: 358–363.
339 340	[21]	Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci 2014; 19: 164–174.



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- 342 [22] Bhardwaj A, Kumar D, Raina SK, et al. Rapid assessment for coexistence of vitamin B12 and iron deficiency anemia among adolescent males and females in northern 343 Anemia; 2013. Epub state of India. ahead 344 Himala van of print. DOI: 10.1155/2013/959605. 345
  - Yokus O, Yilmaz B, Albayrak M, et al. The significance of serum transferrin receptor [23] levels in the diagnosis of the coexistence of anemia of chronic disease and iron deficiency anemia. Eurasian J Med 2011; 43: 9-12.
- 349 [24] Yoon SH, Kim DS, Yu ST, et al. The usefulness of soluble transferrin receptor in the diagnosis and treatment of iron deficiency anemia in children. Korean J Pediatr 2015; 350 351 58: 15–19.
  - Rai D, Adelman AS, Zhuang W, et al. Longitudinal changes in lactoferrin concentrations [25] in human milk: a global systematic review. Crit Rev Food Sci Nutr 2014; 54: 1539-1547.
- 355 Yang Z, Jiang R, Chen Q, et al. Concentration of lactoferrin in human milk and its [26] variation during lactation in different Chinese populations. Nutrients 2018; 10: 1-10. 356
- Shashiraj, Faridi MMA, Singh O, et al. Mother's iron status, breastmilk iron and 357 [27] 358 lactoferrin – are they related? Eur J Clin Nutr 2006; 60: 903–908.
- 359 França EL, Silva VA, Volpato RMJ, et al. Maternal anemia induces changes in [28] immunological and nutritional components of breast milk. J Matern Neonatal Med 360 361 2013; 26: 1223-1227.

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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



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

#### Туре

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

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**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.

## 28 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



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<sup>35</sup> birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia <sup>36</sup> even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based <sup>37</sup> on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) <sup>38</sup> recommendations, children should be exclusively breastfed for the first six months; <sup>39</sup> breastfeeding should then continue for up to two years while also being provided with <sup>40</sup> 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, 41 which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, 42 and immune-boosting properties in addition to nutrients [4]. It contains biologically active 43 components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 44 different proteins in which the concentration differs according to the child's age and other 45 characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a 46 protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main 47 whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide 48 chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. 49 Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor 50 (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many 51 other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and 52 whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. 53 Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, 54 excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin 55 concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic 56 activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, 57 58 lactoferrin may impact gut health and gut-immune development and functioning, decreases the 59 risk of lower respiratory tract illness, and decreases the burden of colonization by some



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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 64 develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores 65 66 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 67 an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women 68 suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence 69 of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 70 48.9% in 2018 [12]. 71

<sup>72</sup> Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with <sup>73</sup> perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, <sup>74</sup> birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected <sup>75</sup> by IDA considering that maternal nutritional status is closely associated with the quality of <sup>76</sup> human milk; therefore, impairment in human milk content may occur due to maternal anemia. <sup>77</sup> Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content <sup>78</sup> [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].



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A study on anemic and non-anemic breastfeeding mothers found that, even though Hb 86 concentration increases after iron supplementation, the lactoferrin concentration in both groups 87 was similar at the end of 30 days of supplementation [16]. From our previous study, we know 88 that mothers with better nutritional status have a higher lactoferrin concentration in their milk 89 [17]. However, there have been few studies related to maternal iron status and its effect on the 90 quality of human milk, and it is poorly understood. Considering that lactoferrin is the main 91 92 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. 93

#### 94 **Objectives**

<sup>95</sup> This study aimed to analyze the correlation between Hb and sTfR concentration during
 <sup>96</sup> pregnancy with the lactoferrin concentration of breastfeeding mothers.

97 Material and methods

#### 98 Study Design

<sup>99</sup> This was a quantitative study with an analytical design and a cross-sectional approach.

100 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



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

#### 110 **Participants**

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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.

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

#### 119 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



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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 immunosorb ent 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 142 143 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  $-20^{\circ}$ C. Storage time both in the 144 refrigerator and the freezer was recorded and considered during analysis to ensure it did not 145 146 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 147 mothers was confirmed by the day breastfeeding began and the infants' age at the time of 148 collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin 149 ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit 150 151 of 1.1 nanograms/ml.

#### 152 Statistical Methods

<sup>153</sup> Data were analyzed using SPSS software version 23. The normality of the data was assessed <sup>154</sup> using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using



155	Manuscript body Download source file (74.19 kB)	Family Medicine & Primary Care Review
156	Pearson's product moment test, while the rank Spearman test was use	ed to assess the correlation
157	of variables if the data distribution was not normal.	
158	Ethical consideration	
159	Ethical clearance to conduct this study (No. 252/EC/FKM/2016)	was obtained from the
160	Commission of Ethics of Medical and Public Health Research of the	Faculty of Public Health,
161	Diponegoro University, Semarang, Indonesia. All subjects provided	written informed consent
162	before inclusion.	
163	Results	
164	The mean age of the subjects at the time of study was $27.95 \pm 5.08$ y	years old. During the third
165	trimester of pregnancy, mean MUAC was 25.0 cm. The majority of l	preastfeeding 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).



172	Variables	Value
173	Age (years)	$27.95 \pm 5.08$
174	MUAC <sup>a</sup> at third trimester (cm)	25.0 (19.8-35.0)
175	Level of education:	
176	Primary	25 (31.6%)
177	Secondary or higher	54 (68.4%)
178	Occupation:	
179	Housewife	52 (65.8%)
180	Laborer	8 (10.1%)
181	Entrepreneur	7 (8.9%)
182	Private employee	4 (5.1%)
183	Civil servant	8 (10.1%)
184	Nutritional status:	
185	CEM <sup>b</sup>	1 (1.3%)
186	Normal	78 (98.7%)
187	Lactation stage:	
188	Colostrum (g/L)	28 (35.4%)
189	Transition (g/L)	24 (30.4%)
190	Mature (g/L)	27 (34.2%)
191	<sup>a</sup> Mid-upper arm circumference; <sup>b</sup> Chronic end	ergy deficiency

#### Table 1. Characteristics distribution of subjects 171

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). 192 193 Nutritional intake that had a significant relationship to Hb concentration was protein, dietary 194 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 195 intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, 196 197 the rank Spearman test found no correlation with lactoferrin concentration (Table 3).



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200	Variables	Value	Min.	Max.
201	Energy (kcal)	$2,595.58 \pm 401.88$		
202	Carbohydrate (g)	$283.98 \pm 60.49$		
203	Protein (g)	$113.81 \pm 23.65$		
204	Dietary cholesterol (mg)	377.0	0.0	1,314.0
205	Fat (g)	$77.42 \pm 26.61$		
206	Dietary fiber (g)	37.2	31.9	51.0
207	Vitamin A (RE)	967.0	49.0	9,462.0
208	Thiamin (mg)	1.8	1.1	2.9
209	Riboflavin (mg)	$2.13 \pm 1.29$		
210	Niacin (mg)	$12.01 \pm 4.66$		
211	Vitamin B6 (mg)	1.4	0.6	4.6
212	Folic acid (µg)	716.0	545.0	1,346.0
213	Vitamin B12 (µg)	6.1	0.1	65.4
214	Vitamin C (mg)	93.0	51.1	388.0
215	Vitamin E (µg)	15.0	15.0	16.2
216	Iron (mg)	40.5	26.3	47.3
217	Zinc (mg)	$20.09 \pm 3.15$		

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

# Table 3. Nutritional intake and its correlation to maternal hemoglobin, serumtransferrinreceptor, and lactoferrinconcentration

220			<i>p</i> -value	
221	Variables	Hemoglobin	Serum transferrin receptor	Lactoferrin
222		(g/dL)	(nmol/L)	(g/L)
223	Energy (kcal)	0.001	0.125	0.783
224	Carbohydrate (g)	0.073	0.692	0.405
225	Protein (g)	0.002	0.072	0.848
226	Dietary cholesterol (mg)	0.001	0.235	0.665
227	Fat (g)	0.009	0.516	0.342
228	Dietary fiber (g)	0.517	0.929	0.708
229	Vitamin A (RE)	0.078	0.312	0.278
230	Thiamin (mg)	0.002	0.036	0.952
231	Riboflavin (mg)	0.012	0.098	0.986
232	Niacin (mg)	0.022	0.046	0.099
233	Vitamin B6 (mg)	0.021	0.171	0.599
234	Folic acid (µg)	0.001	0.024	0.768
235	Vitamin B12 (µg)	0.015	0.192	0.750
236	Vitamin C (mg)	0.001	0.001	0.969
237	Vitamin E (µg)	0.337	0.133	0.701
238	Iron (mg)	0.001	0.001	0.443
239	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



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243	human milk was 1.52 (0.38-2.94) g/L (Table 4). There was no correlation between both Hb
244	and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5).
245	However, Hb and sTfR concentration showed a significant inverse correlation ( $p = 0.001$ , r =
246	-0.438).

#### 247 Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers 248

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

#### 253 Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with 254 lactoferrin concentration of breastfeeding mothers

255	Variables	Lactoferrin concentration (g/L)		
200	v allables	R	<i>p</i> -value	
256	Hemoglobin (g/dL)	0.054	0.636	
257	Serum transferrin receptor (nmol/L)	0.046	0.686	

#### 258 **Discussion**

#### 259 **Key Results**

260 Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third 261 trimester was adequate. This was reflected in the MUAC, which was equal or more than 23.5 262 cm in the majority of subjects. The median maternal Hb and sTfR showed that mothers did not 263 have anemia which could be a result of adequate iron intake as well as other important nutrients 264 that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or 265 ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, 266 polyphenols, calcium, and proteins in milk products, and will increase the absorption of both 267 native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role 268 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].

#### 277 Limitations

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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.

#### 282 Interpretation

283 In our previous study, we found that maternal nutritional status was a predictor of lactoferrin 284 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 285 concentration was low and they were not significantly correlated. Another study supported this 286 result, which found that lactoferrin concentration in human milk did not depend on maternal 287 iron status or iron supplementation [25]. Zavaleta et al. reported no correlation between 288 289 maternal iron status and lactoferrin concentration in human milk at birth and during early 290 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-291 292 anemic and anemic breastfeeding mothers found that maternal Hb did not correlate with 293 lactoferrin concentration in human milk on day 1, 14 weeks, and six months after delivery [27].



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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.

#### 300 Generalisability

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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.

#### 303 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.
- <sup>315</sup> Conflicts of interest: The authors declare no conflicts of interest.



316		Jscript body load source file (74.19 kB)	Family Medicine & Primary Care Review
317	Refe	erences	
318 319	[1]	Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in Epidemiology, Mechanisms, and Lifelong Effect. Lance	•
320 321 322	[2]	Mikšić Š, Uglešić B, Jakab J, et al. Positive Effect of Bi Development, Anxiety, and Postpartum Depression. <i>Int</i> 17. Epub ahead of print 2020. DOI: 10.3390/ijerph1708	J Environ Res Public Health;
323 324	[3]	World Health Organization, UNICEF. <i>Global Strategy</i> , <i>Feeding</i> . Geneva: World Health Organization, 2003.	for Infant and Young Child
325 326	[4]	Eglash A, Simon L. ABM Clinical Protocol #8: Human Home Use for Full-Term Infants, Revised 2017. Breast	0
327 328	[5]	Andreas NJ, Kampmann B, Mehring Le-Doare K. Hum Its Composition and Bioactivity. <i>Early Hum Dev</i> 2015;	
329 330	[6]	Mosca F, Gianni ML. Human Milk: Composition and F Chir 2017; 39: 155.	Iealth Benefits. Pediatr Med
331 332 333	[7]	Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of Lac With Maternal Haemoglobin Percentage Among Lactati Socioeconomic Status. <i>Pak J Physiol</i> 2017; 13: 30–33.	
334 335 336	[8]	Wang B, Timilsena YP, Blanch E, et al. Lactoferrin : St and Digestion. <i>Crit Rev Food Sci Nutr</i> ; 8398. Epub aher 10.1080/10408398.2017.1381583.	
337 338	[9]	Manzoni P. Clinical Benefits of Lactoferrin for Infants 173: S43–S52.	and Children. <i>J Pediatr</i> 2016;
339 340	[10]	Abu-Ouf NM, Jan MM. The Impact of Maternal Iron D Anemia on Child's Health. <i>Saudi Med J</i> 2015; 36: 146-	
341 342	[11]	World Health Organization. Prevalence of Anemia Amon https://data.worldbank.org/indicator/SH.PRG.ANEM (2	6 6
343 344	[12]	National Institute of Health Research and Development Indonesia. <i>Main Result of Basic Health Research 2018</i> .	<b>v</b> 1
345 346	[13]	Di Renzo GC, Spano F, Giardina I, et al. Iron Deficiency Women's Heal 2015; 11: 891–900.	y Anemia in Pregnancy.
347 348 349	[14]	Fujita M, Paredes Ruvalcaba N, Wander K, et al. Buffer Anemia, Inflammation and Breast Milk Macronutrients <i>Anthropol</i> 2019; 168: 329–339.	-
350 351 352	[15]	World Health Organization/Centers for Disease Control Consultation. Assessing The Iron Status of Population. Organization, 2004.	
353 354	[16]	Zavaleta N, Nombera J, Rojas R, et al. Iron and Lactofe Mothers Given Iron Supplements. <i>Nutr Res</i> ; 15, https://d	



355		Iscript body load source file (74.19 kB) 14
356		5317(95)00035-Н (1995).
357 358	[17]	Rahfiludin MZ, Pangestuti DR. Lactoferrin Association with Maternal Nutritional Status and Lactation Stages. <i>Curr Res Nutr Food Sci J</i> 2020; 8: 174–181.
359 360 361	[18]	Ministry of Health Republic of Indonesia. The 2019 Republic of Indonesia Ministry of Health Regulation No. 28 Regarding Indonesian Recommended Dietary Allowance. 2019.
362 363	[19]	Par'i HM, Sugeng Wiyono, Titus Priyo Harjatmo. Assessment of Nutritional Status. Jakarta: Ministry of Health of Republic of Indonesia, 2017.
364 365 366	[20]	Khambalia AZ, Collins CE, Roberts CL, et al. Iron Deficiency in Early Pregnancy Using Serum Ferritin and Soluble Transferrin Receptor Concentrations are Associated with Pregnancy and Birth Outcomes. <i>Eur J Clin Nutr</i> 2016; 70: 358–363.
367 368	[21]	Abbaspour N, Hurrell R, Kelishadi R. Review on Iron and Its Importance for Human Health. <i>J Res Med Sci</i> 2014; 19: 164–174.
369 370 371 372	[22]	Bhardwaj A, Kumar D, Raina SK, et al. Rapid Assessment for Coexistence of Vitamin B12 and Iron Deficiency Anemia Among Adolescent Males and Females in Northern Himalayan State of India. <i>Anemia</i> ; 2013. Epub ahead of print 2013. DOI: 10.1155/2013/959605.
373 374 375	[23]	Yokus O, Yilmaz B, Albayrak M, et al. The Significance of Serum Transferrin Receptor Levels in the Diagnosis of the Coexistence of Anemia of Chronic Disease and Iron Deficiency Anemia. <i>Eurasian J Med</i> 2011; 43: 9–12.
376 377 378	[24]	Yoon SH, Kim DS, Yu ST, et al. The Usefulness of Soluble Transferrin Receptor in The Diagnosis and Treatment of Iron Deficiency Anemia in Children. <i>Korean J Pediatr</i> 2015; 58: 15–19.
379 380 381	[25]	Rai D, Adelman AS, Zhuang W, et al. Longitudinal Changes in Lactoferrin Concentrations in Human Milk: A Global Systematic Review. <i>Crit Rev Food Sci Nutr</i> 2014; 54: 1539–1547.
382 383	[26]	Yang Z, Jiang R, Chen Q, et al. Concentration of Lactoferrin in Human Milk and its Variation during Lactation in Different Chinese Populations. <i>Nutrients</i> 2018; 10: 1–10.
384 385	[27]	Shashiraj, Faridi MMA, Singh O, et al. Mother's Iron Status, Breastmilk Iron and Lactoferrin - Are They Related? <i>Eur J Clin Nutr</i> 2006; 60: 903–908.
386 387 388	[28]	França EL, Silva VA, Volpato RMJ, et al. Maternal Anemia Induces Changes in Immunological and Nutritional Components of Breast milk. <i>J Matern Neonatal Med</i> 2013; 26: 1223–1227.



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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

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

#### Туре

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

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**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.

## 28 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



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<sup>35</sup> birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia <sup>36</sup> even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based <sup>37</sup> on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) <sup>38</sup> recommendations, children should be exclusively breastfed for the first six months; <sup>39</sup> breastfeeding should then continue for up to two years while also being provided with <sup>40</sup> 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, 41 which is rich in nutrients. Human milk has many antioxidant, antibacterial, prebiotic, probiotic, 42 and immune-boosting properties in addition to nutrients [4]. It contains biologically active 43 components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 44 different proteins in which the concentration differs according to the child's age and other 45 characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a 46 protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main 47 whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide 48 chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. 49 Based on its structure, lactoferrin has a similarity concentration to serum transferrin receptor 50 (sTfR) of 60%. Lactoferrin is present in higher concentrations in milk and colostrum, and many 51 other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and 52 whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. 53 Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, 54 excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin 55 concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic 56 activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, 57 58 lactoferrin may impact gut health and gut-immune development and functioning, decreases the 59 risk of lower respiratory tract illness, and decreases the burden of colonization by some



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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 64 develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores 65 66 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 67 an increasing trend every year. In 2016, the data showed that up to 40.1% of pregnant women 68 suffer from anemia [11]. According to Indonesia Basic Health Research 2018, the prevalence 69 of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 70 48.9% in 2018 [12]. 71

<sup>72</sup> Iron deficiency anemia (IDA) is harmful during pregnancy because it is associated with <sup>73</sup> perinatal outcomes including premature labor, intrauterine growth retardation, low birth weight, <sup>74</sup> birth asphyxia, and neonatal anemia [13]. Breastfeeding mothers can also be negatively affected <sup>75</sup> by IDA considering that maternal nutritional status is closely associated with the quality of <sup>76</sup> human milk; therefore, impairment in human milk content may occur due to maternal anemia. <sup>77</sup> Maternal anemia can alter the quality of human milk both in nutrient and non-nutrient content <sup>78</sup> [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].



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A study on anemic and non-anemic breastfeeding mothers found that, even though Hb 86 concentration increases after iron supplementation, the lactoferrin concentration in both groups 87 was similar at the end of 30 days of supplementation [16]. From our previous study, we know 88 that mothers with better nutritional status have a higher lactoferrin concentration in their milk 89 [17]. However, there have been few studies related to maternal iron status and its effect on the 90 quality of human milk, and it is poorly understood. Considering that lactoferrin is the main 91 92 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. 93

#### 94 **Objectives**

<sup>95</sup> This study aimed to analyze the correlation between Hb and sTfR concentration during <sup>96</sup> pregnancy with the lactoferrin concentration of breastfeeding mothers.

97 Material and methods

#### 98 Study Design

<sup>99</sup> This was a quantitative study with an analytical design and a cross-sectional approach.

100 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



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

#### 110 **Participants**

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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.

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

#### 119 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



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- 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 immunosorb ent 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 142 143 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  $-20^{\circ}$ C. Storage time both in the 144 refrigerator and the freezer was recorded and considered during analysis to ensure it did not 145 146 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 147 mothers was confirmed by the day breastfeeding began and the infants' age at the time of 148 collection. Analysis of lactoferrin concentration in human milk used a Human Lactoferrin 149 ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit 150 151 of 1.1 nanograms/ml.
- 152 Statistical Methods
- <sup>153</sup> Data were analyzed using SPSS software version 23. The normality of the data was assessed <sup>154</sup> using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using



155	Manuscript body Download source file (75.7 kB)	Family Medicine & Primary Care Review
156	Pearson's product moment test, while the rank Spearman test was u	used to assess the correlation
157	of variables if the data distribution was not normal.	
158	Ethical consideration	
159	Ethical clearance to conduct this study (No. 252/EC/FKM/201	6) was obtained from the
160	Commission of Ethics of Medical and Public Health Research of t	he Faculty of Public Health,
161	Diponegoro University, Semarang, Indonesia. All subjects provide	ed written informed consent

163 **Results** 

before inclusion.

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The mean age of the subjects at the time of study was  $27.95 \pm 5.08$  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).

172	Variables	Value
173	Age (years)	$27.95 \pm 5.08$
174	MUAC <sup>a</sup> at third trimester (cm)	25.0 (19.8-35.0)
175	Level of education:	
176	Primary	25 (31.6%)
177	Secondary or higher	54 (68.4%)
178	Occupation:	
179	Housewife	52 (65.8%)
180	Laborer	8 (10.1%)
181	Entrepreneur	7 (8.9%)
182	Private employee	4 (5.1%)
183	Civil servant	8 (10.1%)
184	Nutritional status:	
185	CEM <sup>b</sup>	1 (1.3%)
186	Normal	78 (98.7%)
187	Lactation stage:	
188	Colostrum (g/L)	28 (35.4%)
189	Transition (g/L)	24 (30.4%)
190	Mature (g/L)	27 (34.2%)
191	<sup>a</sup> Mid-upper arm circumference; <sup>b</sup> Chronic ene	ergy deficiency

#### Table 1. Characteristics distribution of subjects 171

All subjects met the nutritional need for pregnant women based on Indonesian RDA (Table 2). 192 193 Nutritional intake that had a significant relationship to Hb concentration was protein, dietary 194 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 195 intake of thiamin, niacin, folic acid, vitamin C, and iron. In all of the nutritional intake studied, 196 197 the rank Spearman test found no correlation with lactoferrin concentration (Table 3).



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200	Variables	Value	Min.	Max.
201	Energy (kcal)	$2,595.58 \pm 401.88$		
202	Carbohydrate (g)	$283.98 \pm 60.49$		
203	Protein (g)	$113.81 \pm 23.65$		
204	Dietary cholesterol (mg)	377.0	0.0	1,314.0
205	Fat (g)	$77.42 \pm 26.61$		
206	Dietary fiber (g)	37.2	31.9	51.0
207	Vitamin A (RE)	967.0	49.0	9,462.0
208	Thiamin (mg)	1.8	1.1	2.9
209	Riboflavin (mg)	$2.13 \pm 1.29$		
210	Niacin (mg)	$12.01 \pm 4.66$		
211	Vitamin B6 (mg)	1.4	0.6	4.6
212	Folic acid (µg)	716.0	545.0	1,346.0
213	Vitamin B12 (µg)	6.1	0.1	65.4
214	Vitamin C (mg)	93.0	51.1	388.0
215	Vitamin E (µg)	15.0	15.0	16.2
216	Iron (mg)	40.5	26.3	47.3
217	Zinc (mg)	$20.09 \pm 3.15$		

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

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

220		<i>p</i> -value		
221	Variables	Hemoglobin	Serum transferrin receptor	Lactoferrin
222		(g/dL)	(nmol/L)	(g/L)
223	Energy (kcal)	0.001	0.125	0.783
224	Carbohydrate (g)	0.073	0.692	0.405
225	Protein (g)	0.002	0.072	0.848
226	Dietary cholesterol (mg)	0.001	0.235	0.665
227	Fat (g)	0.009	0.516	0.342
228	Dietary fiber (g)	0.517	0.929	0.708
229	Vitamin A (RE)	0.078	0.312	0.278
230	Thiamin (mg)	0.002	0.036	0.952
231	Riboflavin (mg)	0.012	0.098	0.986
232	Niacin (mg)	0.022	0.046	0.099
233	Vitamin B6 (mg)	0.021	0.171	0.599
234	Folic acid (µg)	0.001	0.024	0.768
235	Vitamin B12 (µg)	0.015	0.192	0.750
236	Vitamin C (mg)	0.001	0.001	0.969
237	Vitamin E (µg)	0.337	0.133	0.701
238	Iron (mg)	0.001	0.001	0.443
239	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

> **es** Editorial System

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243	human milk was 1.52 (0.38-2.94) g/L (Table 4). There was no correlation between both Hb
244	and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5).
245	However, Hb and sTfR concentration showed a significant inverse correlation ( $p = 0.001$ , r =
246	-0.438).

#### 247 Table 4. Concentrations of hemoglobin and transferrin receptor in the blood of pregnant women and the concentration of lactoferrin in milk of breastfeeding mothers 248

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

#### 253 Table 5. Correlation between maternal hemoglobin and serum transferrin receptor with 254 lactoferrin concentration of breastfeeding mothers

255	Variables	Lactoferrin con	Lactoferrin concentration (g/L)		
200		R	<i>p</i> -value		
256	Hemoglobin (g/dL)	0.054	0.636		
257	Serum transferrin receptor (nmol/L)	0.046	0.686		

#### 258 **Discussion**

Based on the Indonesian RDA, the nutritional intake of pregnant mothers during their third 259 260 trimester was adequate. This was reflected in the MUAC, which was equal to or more than 23.5 261 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 262 263 that enhance iron absorption, such as vitamin C, vitamin B12, and folic acid. Vitamin C or 264 ascorbic acid overcome the negative effect of iron-absorption inhibitors such as phytate, 265 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 266 267 in the formation of red blood cells [22].



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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].

279 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 280 lactoferrin concentration in mature human milk with various food intake of mothers [28]. 281 282 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 283 meat, soy, or milk was not necessarily associated with lactoferrin concentration [27]. This was 284 285 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]. 286

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



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maternal iron status and lactoferrin concentration in human milk at birth and during early 294 lactation. Their study was conducted in both anemic and non-anemic breastfeeding mothers 295 and used Hb and hematocrit value to determine anemic status [16]. In India, a study in non-296 297 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]. 298 A study in Brazil found that total protein levels in human milk were higher in anemic mothers 299 regardless of their lactation stage (colostrum, transition, mature) [32]. Since lactoferrin is the 300 301 main whey protein in human milk, and all mothers in the present study were non-anemic, we 302 assumed this might be the cause of the low concentration of lactoferrin. Nevertheless, further 303 studies are needed to explain the mechanism.

#### <sup>304</sup> 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.

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.



- <sup>318</sup> Source of funding: This work was financed by the Directorate of Community Nutrition from
- the Ministry of Health, Republic of Indonesia.
- 320 Conflicts of interest: The authors declare no conflicts of interest.

## 321 **References**

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- [1] Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in The 21st Century: Epidemiology, Mechanisms, and Lifelong Effect. *Lancet* 2016; 387: 475–490.
- [2] Mikšić Š, Uglešić B, Jakab J, et al. Positive Effect of Breastfeeding on Child Development, Anxiety, and Postpartum Depression. *Int J Environ Res Public Health*; 17. Epub ahead of print 2020. DOI: 10.3390/ijerph17082725.
- <sup>327</sup> [3] World Health Organization, UNICEF. *Global Strategy for Infant and Young Child* <sup>328</sup> *Feeding*. Geneva: World Health Organization, 2003.
- <sup>329</sup> [4] Eglash A, Simon L. ABM Clinical Protocol #8: Human Milk Storage Information for <sup>330</sup> Home Use for Full-Term Infants, Revised 2017. *Breastfeed Med* 2017; 12: 390–395.
- Andreas NJ, Kampmann B, Mehring Le-Doare K. Human Breast Milk: A Review on Its
   Composition and Bioactivity. *Early Hum Dev* 2015; 91: 629–635.
- Mosca F, Gianni ML. Human Milk: Composition and Health Benefits. *Pediatr Med Chir* 2017; 39: 155.
- 335[7]Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of Lactoferrin Levels in Breast Milk336With Maternal Haemoglobin Percentage Among Lactating Women of Low and High337Socioeconomic Status. Pak J Physiol 2017; 13: 30–33.
  - [8] Wang B, Timilsena YP, Blanch E, et al. Lactoferrin : Structure, Function, Denaturation and Digestion. *Crit Rev Food Sci Nutr*; 8398. Epub ahead of print 2017. DOI: 10.1080/10408398.2017.1381583.
- [9] Manzoni P. Clinical Benefits of Lactoferrin for Infants and Children. J Pediatr 2016;
   173: S43–S52.
  - [10] Abu-Ouf NM, Jan MM. The Impact of Maternal Iron Deficiency and Iron Deficiency Anemia on Child's Health. *Saudi Med J* 2015; 36: 146–149.
- World Health Organization. Prevalence of Anemia Among Pregnant Women, https://data.worldbank.org/indicator/SH.PRG.ANEM (2016).
  - [12] National Institute of Health Research and Development Ministry of Health Republic of Indonesia. *Main Result of Basic Health Research 2018*. Jakarta, 2018.
- <sup>349</sup> [13] Di Renzo GC, Spano F, Giardina I, et al. Iron Deficiency Anemia in Pregnancy.
   <sup>350</sup> Women's Heal 2015; 11: 891–900.
- <sup>351</sup> [14] Fujita M, Paredes Ruvalcaba N, Wander K, et al. Buffered or Impaired: Maternal
   <sup>352</sup> Anemia, Inflammation and Breast Milk Macronutrients in Northern Kenya. *Am J Phys*



353	<u>Down</u>	load source file (75.7 kB) 14
354		Anthropol 2019; 168: 329–339.
355 356 357	[15]	World Health Organization/Centers for Disease Control and Prevention Technical Consultation. <i>Assessing The Iron Status of Population</i> . 2nd ed. Geneva: World Health Organization, 2004.
358 359 360	[16]	Zavaleta N, Nombera J, Rojas R, et al. Iron and Lactoferrin in Milk of Anemic Mothers Given Iron Supplements. <i>Nutr Res</i> ; 15, https://doi.org/10.1016/0271-5317(95)00035-H (1995).
361 362	[17]	Rahfiludin MZ, Pangestuti DR. Lactoferrin Association with Maternal Nutritional Status and Lactation Stages. <i>Curr Res Nutr Food Sci J</i> 2020; 8: 174–181.
363 364 365	[18]	Ministry of Health Republic of Indonesia. The 2019 Republic of Indonesia Ministry of Health Regulation No. 28 Regarding Indonesian Recommended Dietary Allowance. 2019.
366 367	[19]	Par'i HM, Sugeng Wiyono, Titus Priyo Harjatmo. Assessment of Nutritional Status. Jakarta: Ministry of Health of Republic of Indonesia, 2017.
368 369 370	[20]	Khambalia AZ, Collins CE, Roberts CL, et al. Iron Deficiency in Early Pregnancy Using Serum Ferritin and Soluble Transferrin Receptor Concentrations are Associated with Pregnancy and Birth Outcomes. <i>Eur J Clin Nutr</i> 2016; 70: 358–363.
371 372	[21]	Abbaspour N, Hurrell R, Kelishadi R. Review on Iron and Its Importance for Human Health. J Res Med Sci 2014; 19: 164–174.
373 374 375 376	[22]	Bhardwaj A, Kumar D, Raina SK, et al. Rapid Assessment for Coexistence of Vitamin B12 and Iron Deficiency Anemia Among Adolescent Males and Females in Northern Himalayan State of India. <i>Anemia</i> ; 2013. Epub ahead of print 2013. DOI: 10.1155/2013/959605.
377 378	[23]	Khambalia A, Latulippe ME, Campos C, et al. Milk folate secretion is not impaired during iron deficiency in humans. <i>J Nutr</i> 2006; 136: 2617–2624.
379 380 381	[24]	Yokus O, Yilmaz B, Albayrak M, et al. The Significance of Serum Transferrin Receptor Levels in the Diagnosis of the Coexistence of Anemia of Chronic Disease and Iron Deficiency Anemia. <i>Eurasian J Med</i> 2011; 43: 9–12.
382 383 384	[25]	Yoon SH, Kim DS, Yu ST, et al. The Usefulness of Soluble Transferrin Receptor in The Diagnosis and Treatment of Iron Deficiency Anemia in Children. <i>Korean J Pediatr</i> 2015; 58: 15–19.
385 386 387	[26]	Rai D, Adelman AS, Zhuang W, et al. Longitudinal Changes in Lactoferrin Concentrations in Human Milk: A Global Systematic Review. <i>Crit Rev Food Sci Nutr</i> 2014; 54: 1539–1547.
388 389	[27]	Yang Z, Jiang R, Chen Q, et al. Concentration of Lactoferrin in Human Milk and its Variation during Lactation in Different Chinese Populations. <i>Nutrients</i> 2018; 10: 1–10.
390 391	[28]	Cai X, Duan Y, Li Y, et al. Lactoferrin level in breast milk: a study of 248 samples from eight regions in China. <i>Food Funct</i> 2018; 9: 4216–4222.

Manuscript body

<sup>392</sup> [29] Motil KJ, Thotathuchery M, Bahar A, et al. Marginal dietary protein restriction reduced



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2013; 26: 1223-1227.

394 395		nonprotein nitrogen, but not protein nitrogen, components of human milk. J Am Coll Nutr 1995; 14: 184–191.
396 397 398	[30]	Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation. <i>Nutrition During Lactation</i> . Washington (DC): National Academies Press (US), https://www.ncbi.nlm.nih.gov/books/NBK235590/#ddd00119 (1991).
399 400	[31]	Shashiraj, Faridi MMA, Singh O, et al. Mother's Iron Status, Breastmilk Iron and Lactoferrin - Are They Related? <i>Eur J Clin Nutr</i> 2006; 60: 903–908.
401 402	[32]	França EL, Silva VA, Volpato RMJ, et al. Maternal Anemia Induces Changes in Immunological and Nutritional Components of Breast milk. J Matern Neonatal Med



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**Editorial Comments Revised 5** 



### 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

#### Туре

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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**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.

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**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.
- <sup>27</sup> **Key words:** human milk, lactoferrin, hemoglobins, transferrin receptors.

## 28 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



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<sup>35</sup> birth spacing, and possibly a reduced risk of diabetes and ovarian cancer [1]. A study in Croatia <sup>36</sup> even found that breastfeeding can lower the risk of depression in postpartum mothers [2]. Based <sup>37</sup> on World Health Organization (WHO) and United Nations Children's Fund (UNICEF) <sup>38</sup> recommendations, children should be exclusively breastfed for the first six months; <sup>39</sup> breastfeeding should then continue for up to two years while also being provided with <sup>40</sup> 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, 41 42 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 43 components, non-protein nitrogen, immunoglobulin, lipids, carbohydrates, and over 400 44 different proteins in which the concentration differs according to the child's age and other 45 characteristics, to reflect their need [5]. Among the nutrients contained in human milk is a 46 protein that is beneficial for infants' health, namely lactoferrin. Lactoferrin is one of the main 47 whey proteins in human milk, with significant quantities [6]. Lactoferrin is a single polypeptide 48 chain glycoprotein with a molecular weight of around 78 kDa and consists of 691 amino acids. 49 50 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 51 other secretions like tears, saliva, urine, and gastric fluid. Meanwhile, in plasma or serum and 52 whole blood, lactoferrin concentration is low, varying from 0.02 µg/ml to 1.52 µg/ml. 53 Pregnancy and menstrual cycle affect lactoferrin concentration in plasma. On the contrary, 54 excessive iron intake, tumor growth, infection, and inflammation increases lactoferrin 55 concentration [7]. Lactoferrin has antimicrobial, anti-inflammatory, and anti-carcinogenic 56 activities, highlighting the therapeutic values of this multifunctional protein [8]. In infants, 57 lactoferrin may impact gut health and gut-immune development and functioning, decreases the 58 risk of lower respiratory tract illness, and decreases the burden of colonization by some 59



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 64 develops slowly over time, and may not be symptomatic or clinically obvious. Once iron stores 65 are completely depleted, iron accessibility to the tissues declines, leading to symptomatic 66 anemia [10]. Since 2011, the global prevalence of anemia among pregnant mothers has shown 67 68 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 69 of anemic pregnant mothers increased over the previous five years, from 37.1% in 2013 to 70 48.9% in 2018 [12]. 71

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].



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A study on anemic and non-anemic breastfeeding mothers found that, even though Hb 86 87 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 88 that mothers with better nutritional status have a higher lactoferrin concentration in their milk 89 [17]. However, there have been few studies related to maternal iron status and its effect on the 90 quality of human milk, and it is poorly understood. Considering that lactoferrin is the main 91 protein of human milk and has many benefits for infants' growth and development during the 92 breastfeeding period, this study aims to analyze its correlation with iron status. 93

#### 94 **Objectives**

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

97 Material and methods

### 98 Study Design

<sup>99</sup> This was a quantitative study with an analytical design and a cross-sectional approach.

100 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



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108	were collected for lactoferrin concentration analysis. All subjects	participated in both stages of
109	data collection.	
110	Participants	
111	The subjects were 79 pregnant mothers who were selected using	ng purposive sampling. The
112	sample size was determined with Slovin's formula. The inclusion	n criteria were willingness to
113	participate in the study, giving birth in September 2017, breastfee	eding mothers, had singleton
114	child, had children born at a normal weight (> 2500 g) and had ch	nildren without abnormalities
115	that made suckling difficult.	
116	Variables	
117	The variables assessed in this study were maternal hemoglobin, se	rum transferrin receptor, and
118	lactoferrin concentration.	
119	Data Sources/ Measurement	
120	Data on subject characteristics, such as education level and occup	ation, were obtained through
121	interviews with the subjects. Education level was categorized in	nto two groups: (a) primary
122	which was six years in elementary school and three years in	junior high school; and (b)
123	secondary or higher, which was three years in senior high school	and about three to four years
124	at college.	
125	Nutritional intake data were obtained using a 24-hour recall met	hod for two non-consecutive
126	days, with food pictures to help subjects determine the food po	rtions they consumed. Food
127	intake was recorded in the form of household portions such as ta	ablespoons, teaspoons, cups
128	etc. This was then converted into grams and analyzed using Nutri	Survey software to calculate
129	the nutrition intake. The data were then compared with the Indo	nesian recommended dietary
130	allowance (RDA) which is based on the 2019 Republic of In	donesia Ministry of Health



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- 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 142 143 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  $-20^{\circ}$ C. Storage time both in the 144 refrigerator and the freezer was recorded and considered during analysis to ensure it did not 145 affect lactoferrin concentration. Data on milk collection time was also recorded and analyzed 146 to avoid diurnal variation during milk collection. The lactation stage of the breastfeeding 147 148 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 149 ELISA (Biovendor-Laboratorni medivina a.s, Karasek, Czech Republic) with a detection limit 150 151 of 1.1 nanograms/ml.
- 152 Statistical Methods
- <sup>153</sup> Data were analyzed using SPSS software version 23. The normality of the data was assessed <sup>154</sup> using the Kolmogorov–Smirnov test. Data with a normal distribution were analyzed using



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156	Pearson's product moment test, while the rank Spearman test was used to assess the correlation
157	of variables if the data distribution was not normal.
158	Ethical consideration
159	Ethical clearance to conduct this study (No. 252/EC/FKM/2016) was obtained from the
160	Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health,
161	Diponegoro University, Semarang, Indonesia. All subjects provided written informed consent
162	before inclusion.
163	Results
164	The mean age of the subjects at the time of study was $27.95 \pm 5.08$ years old. During the third
165	trimester of pregnancy, mean MUAC was 25.0 cm. The majority of breastfeeding mothers had
166	secondary or higher education levels (68.4%), were housewives (65.8%), and had normal
167	nutritional status, as indicated by the MUAC (98.7%). The distribution of lactation stage among
168	breastfeeding mothers was almost even: colostrum (35.4%), transition (30.4%), and mature

(34.2%) (Table 1).

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172	Variables	Value
173	Age (years)	$27.95\pm5.08$
174	MUAC <sup>a</sup> at third trimester (cm)	25.0 (19.8–35.0)
175	Level of education:	
176	Primary	25 (31.6%)
177	Secondary or higher	54 (68.4%)
178	Occupation:	
179	Housewife	52 (65.8%)
180	Laborer	8 (10.1%)
181	Entrepreneur	7 (8.9%)
182	Private employee	4 (5.1%)
183	Civil servant	8 (10.1%)
184	Nutritional status:	
185	CEM <sup>b</sup>	1 (1.3%)
186	Normal	78 (98.7%)
187	Lactation stage:	
188	Colostrum (g/L)	28 (35.4%)
189	Transition (g/L)	24 (30.4%)
190	Mature (g/L)	27 (34.2%)
191	<sup>a</sup> Mid-upper arm circumference; <sup>b</sup> Chronic ene	rgy deficiency

#### 171 **Table 1. Characteristics distribution of subjects**

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).



amily Medicine Manuscript body & Primary Care Review 198 Download source file (79.04 kB) Table 2. Nutritional intake of subjects during the third trimester of pregnancy 199 Variables Value Min. 200 Max. Energy (kcal)  $2.595.58 \pm 401.88$ 201 Carbohydrate (g)  $283.98 \pm 60.49$ 202 203 Protein (g)  $113.81 \pm 23.65$ 204 Dietary cholesterol (mg) 377.0 0.0 1,314.0 Fat (g)  $77.42 \pm 26.61$ 205 206 Dietary fiber (g) 37.2 31.9 51.0 207 Vitamin A (RE) 967.0 49.0 9,462.0 208 Thiamin (mg) 1.8 1.1 2.9 209 Riboflavin (mg)  $2.13 \pm 1.29$ 210 Niacin (mg)  $12.01 \pm 4.66$ Vitamin B6 (mg) 211 1.4 0.6 4.6 Folic acid  $(\mu g)$ 716.0 212 545.0 1,346.0 Vitamin B12 (µg) 65.4 213 6.1 0.1

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

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 $20.09 \pm 3.15$ 

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Vitamin C (mg)

Vitamin E ( $\mu g$ )

Iron (mg)

Zinc (mg)

220			<i>p</i> -value	
221	Variables	Hemoglobin	Serum transferrin receptor	Lactoferrin
222		(g/dL)	(nmol/L)	(g/L)
223	Energy (kcal)	0.001	0.125	0.783
224	Carbohydrate (g)	0.073	0.692	0.405
225	Protein (g)	0.002	0.072	0.848
226	Dietary cholesterol (mg)	0.001	0.235	0.665
227	Fat (g)	0.009	0.516	0.342
228	Dietary fiber (g)	0.517	0.929	0.708
229	Vitamin A (RE)	0.078	0.312	0.278
230	Thiamin (mg)	0.002	0.036	0.952
231	Riboflavin (mg)	0.012	0.098	0.986
232	Niacin (mg)	0.022	0.046	0.099
233	Vitamin B6 (mg)	0.021	0.171	0.599
234	Folic acid (µg)	0.001	0.024	0.768
235	Vitamin B12 (µg)	0.015	0.192	0.750
236	Vitamin C (mg)	0.001	0.001	0.969
237	Vitamin E (µg)	0.337	0.133	0.701
238	Iron (mg)	0.001	0.001	0.443
239	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



243	human milk was 1.52 (0.38–2.94) g/L (Table 4). There was no correlation between both Hb
244	and sTfR concentration with lactoferrin concentration of breastfeeding mothers (Table 5).
245	However, Hb and sTfR concentration showed a significant inverse correlation ( $p = 0.001$ , r =
246	-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

255	Variables	Lactoferrin concentration (g/L)	
200	v arrables	R	<i>p</i> -value
256	Hemoglobin (g/dL)	0.054	0.636
257	Serum transferrin receptor (nmol/L)	0.046	0.686

## 258 Discussion

259 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 23.5 260 261 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 262 263 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, 264 polyphenols, calcium, and proteins in milk products, and will increase the absorption of both 265 native and fortification iron [21]. Meanwhile, vitamin B12 and folic acid play an important role 266 in the formation of red blood cells [22]. 267



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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].

279 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 280 281 lactoferrin concentration in mature human milk with various food intake of mothers [28]. 282 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 283 meat, soy, or milk was not necessarily associated with lactoferrin concentration [27]. This was 284 likely due to the synthesis from maternal stores or body tissues when nutrients in milk were 285 286 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



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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 nonanemic 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.

#### <sup>304</sup> 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.

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.



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318	Sourc	e of funding: This work was financed by the Directorate	of Community Nutrition from	
319	the M	linistry of Health, Republic of Indonesia.		
320	Conflicts of interest: The authors declare no conflicts of interest.			
321	Refe	rences		
322 323	[1]	Victora CG, Bahl R, Barros AJD, et al. Breastfee Epidemiology, Mechanisms, and Lifelong Effect. <i>Lancet</i>	•	
324 325 326	[2]	Mikšić Š, Uglešić B, Jakab J, et al. Positive Effec Development, Anxiety, and Postpartum Depression. <i>Int</i> 17. Epub ahead of print 2020. DOI: 10.3390/ijerph17082	J Environ Res Public Health;	
327 328	[3]	World Health Organization, UNICEF. <i>Global Strategy</i> <i>Feeding</i> . Geneva: World Health Organization, 2003.	v for Infant and Young Child	
329 330	[4]	Eglash A, Simon L. ABM Clinical Protocol #8: Human Home Use for Full-Term Infants, Revised 2017. <i>Breastfe</i>	0	
331 332	[5]	Andreas NJ, Kampmann B, Mehring Le-Doare K. Human Composition and Bioactivity. <i>Early Hum Dev</i> 2015; 91:		
333 334	[6]	Mosca F, Giannì ML. Human Milk: Composition and Hea 2017; 39: 155.	alth Benefits. Pediatr Med Chir	
335 336 337	[7]	Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of Lac With Maternal Haemoglobin Percentage Among Lactat Socioeconomic Status. <i>Pak J Physiol</i> 2017; 13: 30–33.		
338 339 340	[8]	Wang B, Timilsena YP, Blanch E, et al. Lactoferrin : Str and Digestion. <i>Crit Rev Food Sci Nutr</i> ; 8398. Epub 10.1080/10408398.2017.1381583.		
341 342	[9]	Manzoni P. Clinical Benefits of Lactoferrin for Infants 173: S43–S52.	and Children. J Pediatr 2016;	
343 344	[10]	Abu-Ouf NM, Jan MM. The Impact of Maternal Iron D Anemia on Child's Health. <i>Saudi Med J</i> 2015; 36: 146–1		
345 346	[11]	World Health Organization. Prevalence of Anemia https://data.worldbank.org/indicator/SH.PRG.ANEM (20		
347 348	[12]	National Institute of Health Research and Development I Indonesia. <i>Main Result of Basic Health Research 2018</i> . J	•	
349 350	[13]	Di Renzo GC, Spano F, Giardina I, et al. Iron Defice Women's Heal 2015; 11: 891–900.	ciency Anemia in Pregnancy.	
351 352	[14]	Fujita M, Paredes Ruvalcaba N, Wander K, et al. Bu Anemia, Inflammation and Breast Milk Macronutrients	1	



353		<b>Jscript body</b> load source file (79.04 kB)	<b>Family Medicine</b> & Primary Care Review 14
354		Anthropol 2019; 168: 329–339.	
355 356 357	[15]	World Health Organization/Centers for Disease Cont Consultation. Assessing The Iron Status of Population. Organization, 2004.	
358 359 360	[16]	Zavaleta N, Nombera J, Rojas R, et al. Iron and Lactofer Given Iron Supplements. <i>Nutr Res</i> ; 15, https://doi.org/10 (1995).	
361 362	[17]	Rahfiludin MZ, Pangestuti DR. Lactoferrin Associati Status and Lactation Stages. <i>Curr Res Nutr Food Sci J</i> 2	
363 364 365	[18]	Ministry of Health Republic of Indonesia. The 2019 Rep Health Regulation No. 28 Regarding Indonesian Reco 2019.	
366 367	[19]	Par'i HM, Sugeng Wiyono, Titus Priyo Harjatmo. Ass Jakarta: Ministry of Health of Republic of Indonesia, 20	
368 369 370	[20]	Khambalia AZ, Collins CE, Roberts CL, et al. Iron Defici Serum Ferritin and Soluble Transferrin Receptor Conc Pregnancy and Birth Outcomes. <i>Eur J Clin Nutr</i> 2016; 7	entrations are Associated with
371 372	[21]	Abbaspour N, Hurrell R, Kelishadi R. Review on Iron Health. <i>J Res Med Sci</i> 2014; 19: 164–174.	and Its Importance for Human
373 374 375 376	[22]	Bhardwaj A, Kumar D, Raina SK, et al. Rapid Assessme B12 and Iron Deficiency Anemia Among Adolescent M Himalayan State of India. <i>Anemia</i> ; 2013. Epub 10.1155/2013/959605.	Iales and Females in Northern
377 378	[23]	Khambalia A, Latulippe ME, Campos C, et al. Milk for during iron deficiency in humans. <i>J Nutr</i> 2006; 136: 261	<b>•</b>
379 380 381	[24]	Yokus O, Yilmaz B, Albayrak M, et al. The Significance Levels in the Diagnosis of the Coexistence of Anemia Deficiency Anemia. <i>Eurasian J Med</i> 2011; 43: 9–12.	<b>.</b>
382 383 384	[25]	Yoon SH, Kim DS, Yu ST, et al. The Usefulness of Solul Diagnosis and Treatment of Iron Deficiency Anemia i 2015; 58: 15–19.	-
385 386 387	[26]	Rai D, Adelman AS, Zhuang W, et al. Longitud Concentrations in Human Milk: A Global Systematic R 2014; 54: 1539–1547.	0
388 389	[27]	Yang Z, Jiang R, Chen Q, et al. Concentration of Lact Variation during Lactation in Different Chinese Populati	
390 391	[28]	Cai X, Duan Y, Li Y, et al. Lactoferrin level in breast mileight regions in China. <i>Food Funct</i> 2018; 9: 4216–4222.	• •
392	[29]	Motil KJ, Thotathuchery M, Bahar A, et al. Marginal die	etary protein restriction reduced



393		uscript body Noad source file (79.04 kB)	Family Medicine & Primary Care Review 15
394 395		nonprotein nitrogen, but not protein nitrogen, components o <i>Nutr</i> 1995; 14: 184–191.	of human milk. <i>J Am Coll</i>
396 397 398	[30]	Institute of Medicine (US) Committee on Nutritional Statu Lactation. <i>Nutrition During Lactation</i> . Washington (DC): N (US), https://www.ncbi.nlm.nih.gov/books/NBK235590/#ddd	National Academies Press
399 400	[31]	Shashiraj, Faridi MMA, Singh O, et al. Mother's Iron Sta Lactoferrin - Are They Related? <i>Eur J Clin Nutr</i> 2006; 60: 90	
401 402 403	[32]	França EL, Silva VA, Volpato RMJ, et al. Maternal Ane Immunological and Nutritional Components of Breast milk. 2013; 26: 1223–1227.	e



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### Authors:

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## **Decision letter:**

February 04, 2021 FAMILY-00831-2020-06 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





## **Original paper**

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

Mohammad Zen Rahfiludin , Dina Rahayuning Pangestuti 1, Suyatno Suyatno 1, Suroto Suroto 1

1. Faculty of Public Health, Diponegoro University, Semarang, Indonesia

Family Medicine & Primary Care Review 2021; 23(4): 465–469 DOI: https://doi.org/10.5114/fmpcr.2021.110364 Online publish date: 2021/12/30

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- Correlation.pdf [0.45 MB]

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1. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 2016; 387: 475–490.

2. Mikšić Š, Uglešić B, Jakab J, et al. Positive effect of breastfeeding on child development, anxiety, and postpartum depression. Int J Environ Res Public Health 2020; 17(8): 2725, doi: 10.3390/ijerph17082725.

3. World Health Organization, UNICEF. Global strategy for infant and young child feeding. Geneva: WHO; 2003.

4. Eglash A, Simon L. ABM Clinical Protocol #8: Human milk storage information for home use for full-term infants, revised 2017. Breastfeed Med 2017; 12: 390–395.

5. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: a review on its composition and bioactivity. Early Hum Dev 2015; 91: 629–635.

6. Mosca F, Giannì ML. Human milk: composition and health benefits. Pediatr Med Chir 2017; 39: 155.

7. Taqi T, Fatima Qazi A, Qazi S, et al. Correlation of lactoferrin levels in breast milk with maternal haemoglobin percentage among lactating women of low and high socioeconomic status. Pak J Physiol 2017; 13: 30–33.

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