Paediatrica Indonesiana

VOLUME 54 March • 2014 NUMBER 2

Original Article

Correlations between hemoglobin, serum ferritin, and soluble transferrin receptor levels in children aged 6-59 months

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Abstract

Background Early detection of iron deficiency is important in young children to prevent iron deficiency anemia, which may cause permanent neurocognitive development disorders. Hemoglobin level is an insensitive tool for detecting iron deficiency without anemia, while serum ferritin levels may be influenced by infection and inflammation. However, soluble transferrin receptor (sTfR) is a sensitive marker for detecting iron deficiency, yet not widely used in daily practice.

Objective To assess for correlations between hemoglobin, serum ferritin, and soluble transferrin receptor levels in children aged 6-59 months.

Methods We performed a cross-sectional study in the Yogyakarta and Bantul Districts involving 85 children aged 6-59 months who visited integrated health posts (*posyandu*) and who met the inclusion criteria. Subjects were chosen by cluster random sampling. Blood specimens were collected to examine hemoglobin, serum ferritin, and sTfR levels.

Results Spearman's correlation test revealed weak negative correlations between hemoglobin and serum ferritin levels, as well as between hemoglobin and sTfR levels, with coefficient correlations of r=-0.220, (P=0.043) and r=-0.317, (P=0.003), respectively. There was no correlation between serum ferritin and sTfR levels (r=-0.033; P=0.767).

Conclusion Hemoglobin levels has weak negative correlations with serum ferritin and sTfR, but serum ferritin does not correlate with sTfR. **[Paediatr Indones. 2014;54:122-6.]**.

Keywords: *hemoglobin, serum ferritin, soluble transferrin receptor, correlation, iron deficiency*

Iron deficiency is a form of micronutrient deficiency causing anemia, and one-third of children in the world suffer from iron deficiency anemia (IDA).[1] The 2004 *Indonesian Household Health Survey* (SKRT) indicates the incidence of IDA to be 39%. Other reports showed that IDA prevalence in children had dropped to 9.8% in 2007.[2,4] In Banjarbaru, South Kalimantan, the incidences of IDA, iron deficiency, and iron depletion in infants aged 0-6 months were 40.8%, 27%, and 28%, respectively.[3] *The Indonesian Basic Health Research* (RISKESDAS) in 2007 reports that the prevalence of IDA in children in the *Daerah Istimewa* Yogyakarta (DIY) Province was 10.4%.[4]

Detection of iron deficiency prior to the occurence of anemia is important, because iron deficiency in early stage may cause permanent neurocognitive developmental disorders, especially in the high-risk population of children aged 1-5 years.[5-7] Bone marrow aspiration with Prussian blue staining as the definitive diagnosis is not routinely done due to the invasiveness

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of the procedure. Hemoglobin level is a sensitive tool for detecting iron deficiency anemia, but not for iron deficiency.[8] Iron, is one important micronutrient. If iron reserves are insufficient for erythrocyte formation, the erythrocyte numbers will then decline, resulting in low hemoglobin level or anemia.[9]

Serum ferritin is a good indicator for iron deficiency in the absence of increasing reactants from acute process. In normal condition, ferritin stores iron intracellularly for later removal and reuse. Serum ferritin levels of <12μg/L reflect an iron deficient state, but increased serum ferritin has been found in people with excess iron and inflammation. Serum ferritin levels are strongly influenced by infection and inflammation.[10] Soluble transferrin receptor (sTfR) is a good indicator for iron deficiency in infants, as it has a specificity of 84% and a sensitivity of 94%. However, its use in clinical practice has been limited and mostly for research purposes.[10,11] Soluble transferrin receptor expression rises when there is inadequate iron supply for tissue demand, or increased need for iron associated with the process of erythropoiesis.[12] This study aimed to assess for correlations between hemoglobin and serum ferritin levels, hemoglobin and sTfR levels, as well as serum ferritin and sTfR levels in children aged 6-59 months.

Methods

We conducted a cross-sectional study from November 2010 to April 2011. Inclusion criteria were healthy-appearing children aged 6-59 months who visited integrated health posts (*posyandu*) in the districts of Yogyakarta and Bantul, and were selected by cluster random sampling. Subjects' parents consented to participate in the study. Children who received iron therapy within the 3 months prior to the study were excluded.

The minimum required sample size was calculated to be 85, based on 80% power and 5% type I error. We collected baseline data on subjects' age, gender, weight, height, and nutritional status. Blood specimens were collected for examinations of hemoglobin and serum ferritin levels at the Parahita Laboratory, Yogyakarta, and for sTfR levels at the SEAMEO Laboratory, Jakarta. Hemoglobin (Hb) levels were assessed using flow cytometry. Serum ferritin and sTfR levels were assessed using enzyme-linked fluorescent assays (ELFA). Iron deficiency is a condition characterized by reduced iron reserves in the body as indicated by sTfR levels ≥8.3 μg/mL, using the ELFA method.

Correlations between levels of hemoglobin, serum ferritin, and sTfR were analyzed using Pearson's correlation test. Spearman's correlation test was used for non-normal data distribution. We used iron deficiency (sTfR levels >8.3 μg/mL) as the control variable in partial correlation sub-analysis. Data normality was analyzed using the Kolmogorov-Smirnov test.

Results

Eighty five children aged 6-59 months involved in this study. The basic characteristics of subjects are shown in **Table 1.**

Kolmogorov-Smirnov test was used to analyze the linearity between pairs of variables as follows: hemoglobin-sTfR, serum ferritin-sTfR, and hemoglobin-serum ferritin. Statistical analyses yielded a linear relationship between hemoglobin-sTfR (F=14.95; P=0.002). However, there was no linear relationship between serum ferritin-sTfR (F=1.079; P=0.319) or hemoglobin-serum ferritin (F=3.063; P=0.155).

Based on normality and linearity tests, there were variables with abnormal distribution. Variables with non-linear correlations were analyzed using the Spearman's correlation and partial correlation tests to analyze the relationship between two variables and a

Table 1. Baseline characteristics of study subjects

Characteristics	n	%
Age, months		
6–12	4	4.7
12–24	13	15.3
24–59	68	80.0
Gender		
Male	46	54.1
Female	39	45.9
Nutritional status		
Good	54	63.5
Underweight	31	36.5
Iron deficiency		
Yes (sTfR ≥8.3 μg/mL)	26	30.6
No (sTfR <8.3 μg/mL)	59	69.4
Anemia		
Yes (Hb <11.0 g/dL)	5	5.9
No (Hb ≥11.0 g/dL)	80	94.1

single control variable. Spearman's correlation test results are shown in the scatter plots in **Figure 1**.

Spearman's correlation test showed that there were negative relationships between hemoglobin and serum ferritin levels (r=-0.220, P=0.043), as well as hemoglobin and sTfR levels (r=-0.317, P=0.003), indicating that increased levels of hemoglobin were associated weakly with decreased levels of serum ferritin and sTfR, or vice versa. There was no correlation between serum ferritin and sTfR levels (r=-0.033; P=0.767) (**Table 2**).

Partial correlation analysis using iron deficiency as the control variable showed similar results between hemoglobin and serum ferritin (**Table 3**). This means in children with severe iron deficiency, those with decreased hemoglobin levels tended to increase serum ferritin. The correlation between hemoglobin and sTfR levels controlled by iron deficiency was not statistically significant (P>0.05). Serum ferritin and sTfR levels controlled by iron deficiency were not significantly correlated (P >0.05).

Multivariate analysis included variables with P values <0.25 by bivariate analysis. **Table 4** shows that

Table 2. The relationships between hemoglobin and serum ferritin levels, hemoglobin and sTfR levels, as well as serum ferritin and sTfR levels (Spearman's correlation)

Variables	Coefficient correlation (*r*)	P value
Hemoglobin - serum ferritin levels	- 0.220	0.043*
Hemoglobin – sTfR levels	- 0.317	0.003**
Serum ferritin – sTfR levels	- 0.033	0.767

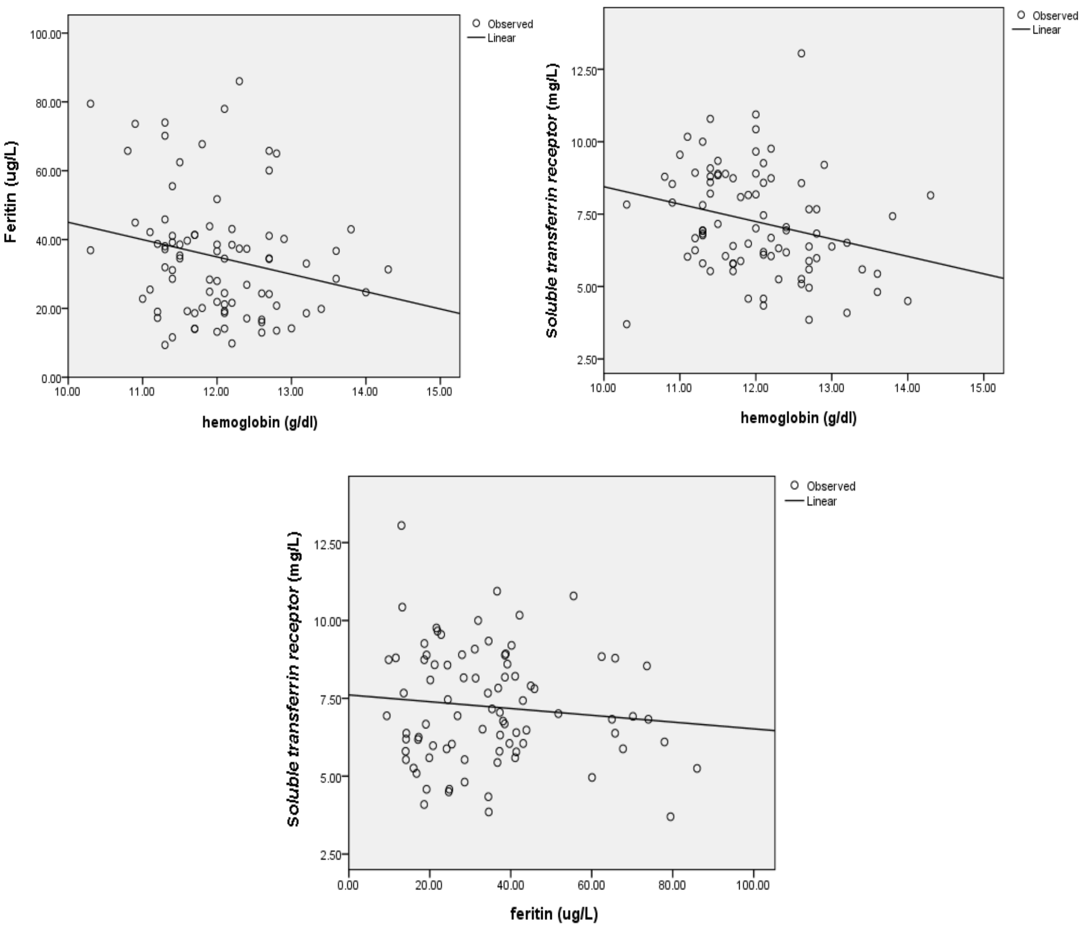

Figure 1. **A**. Scatter plot of correlation between hemoglobin and serum ferritin levels, **B**. Scatter plot of correlation between hemoglobin and sTfR levels, **C**. Scatter plot of correlation between serum ferritin and sTfR levels.

Table 3. The relationships between hemoglobin and serum ferritin levels, hemoglobin and sTfR levels, as well as sTfR and serum ferritin levels using iron deficiency as the control variables (partial correlation)

Variables	Coefficient correlation (*r*)	P value
Hemoglobin - serum ferritin levels	- 0.258	0.018*
Hemoglobin – sTfR levels	- 0.086	0.435
Serum ferritin – sTfR levels	- 0.044	0.690

stores and functional deficiency. Iron deficiency in our study was confirmed by sTfR levels. Van den Bosch *et al*. and Phiri *et al*. explained that normal children have sTfr values of < 8.3 μg/mL.[13,14] Among the 85 subjects in our study, 26 children (30.6%) were iron deficient, while 59 (69.4%) children had no iron deficiency.

The WHO criteria (2007) for anemia in children aged 6 months-5 years, is having Hb <11.0 g/dL. In our study, 5 children (5.9%) had anemia and 80 (94.1%) children did not. These results were similar to the reported low incidence of nutritional anemia in children in Yogyakarta, i.e., 10.4%.[4] None of our subjects had malnutrition, overweight, or obesity. The RISKESDAS in Daerah Istimewa Yogyakarta (DIY) at 2010 reported that 80% of infants had good nutritional status. The DIY Province manages almost 100% of all malnourished children in its area.[4]

Table 4. Hemoglobin, serum ferritin, and sTfR levels to predict iron deficiency

Step	Variable	b	r	P value
Step 1	Hemoglobin	0.040	0.069	0.339
	Ferritin	0.001	0.033	0.633
	sTfR	- 0.196	- 0.776	0.001*
	constanta	2.598		0.001
Step 2	Hemoglobin	0.035	0.060	0.386
	sTfR	- 0.197	- 0.782	0.001*
	constanta	2.700		0.001
Step 3	sTfR	0.201	- 0.797	0.001*
	constanta	3.148		0.001

Note: b = regression coefficient, r = correlation coefficient. Based on the regression equation $y = a + b_1x_1 + b_2x_2 + ... + b_ix_i$ (y = dependent variable; a = constanta; b1, b2, bi = regression coefficient of each variable; x1, x2, xi = independent variables)

sTfR may be best used to predict iron deficiency, as it gave the highest correlation coefficient (r=-0.797). Soluble transferin receptor (sTfR) levels were a strong predictor for iron deficiency in children aged 6-59 months (P<0.05).

Based on the regression equation, iron deficiency in children can be predicted by the levels of hemoglobin, serum ferritin and sTfR as follows: iron deficiency = 2.598 + 0.040 hemoglobin serum + 0.001 ferritin serum - 0.196 sTfR. The mean regression coefficient of 0.040 in our study indicated that an increase of 1 g/dL of hemoglobin levels at Hb <11.0 g/dL can predict iron deficiency by 4%. The 0.001 regression coefficient indicated that any increase 1 ug/L serum ferritin levels of serum ferritin levels at <12.0 ug/L could predict iron deficiency by 0.1%, while the -0.196 regression coefficient indicated that each decrease of 1 mg/L levels of sTfR at sTfR levels ≥8.3 ug/ml could predict iron deficiency by 19.6%.

Discussion

There is a high incidence of iron deficiency in children, especially those aged 1-5 years.[1] In our study, we evaluated the diagnostic efficiency of a variety of conventional laboratory tests to identify deficient iron

A negative correlation between hemoglobin and sTfR levels in our study is similar with those of Schiza *et al.* in children aged 6 weeks-12 months, but their result was not statistically significant. It is in contradictive with our results, so does the correlation results between sTfR and serum ferritin levels. They also showed that at any age, hemoglobin and sTfR levels weakly correlated in a negative direction.[15] The correlation between hemoglobin and serum ferritin levels was different from the results of Van den Bosch *et al.* which showed a significant correlation between ferritin and hemoglobin levels toward a negative correlation (r=0.5522; P<0.0001).[13] This difference is due to age, inflammation, and infection, especially worm infestation which can increase serum ferritin levels.[16]

Serum ferritin has been widely used as an iron marker in individuals without inflammatory conditions. Conversely, sTfR is considered to reflect the degree of tissue iron need, and there is evidence that it is a good indicator of iron status when the iron stores are depleted. The reciprocal relationship between sTfR and serum ferritin describes a good relationship over a wide range of normal and depleted iron stores states.[14] In our study, this relationship has been described with scatter plot graphs.

In contrast to our results, Yokus *et al.* reported a stronger correlation between hemoglobin and sTfR levels ($r=-0.519$; $P<0.05$). However, we used healthy subjects, while they used iron deficiency anemia subjects. Therefore, the narrower range of hemoglobin and serum ferritin levels in their study may have produced a stronger correlation.

A limitation of our study was that we did not assess indicators of inflammation, such as C-reactive protein (CRP), α1-acid glycoprotein, and stool examinations for worm infection. The presence of inflammatory conditions might affect serum ferritin levels in our subjects. These findings suggest that a single tool for detecting iron deficiency in children aged 6-59 months, such as serum ferritin examination alone, is not recommended because it could obscure the diagnosis. Other parameters such as sTfR or another conventional hematological laboratory procedure are needed.

In conclusion, hemoglobin levels has weak negative correlations with serum ferritin and sTfR, but serum ferritin does not correlate with sTfR.

Acknowledgments

We are grateful to the staff of the health posts in Taman and Patehan, Yogyakarta, and also Barongan and Bungas, Bantul for making this study possible. We would particularly like to thank Dr. Zulaela, Dipl. Med. Stats, Msi for his valuable contribution in statistical analysis for this study.

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