Paediatrica Indonesiana

VOLUME 53 May • 2013 NUMBER 3

Original Article

# Serum ferritin to detect iron deficiency in children below five years of age

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## Abstract

***Background*** Iron deficiency (ID) anemia impacts the cognitive and motor development of children until the age of 10 years, despite receiving iron therapy. Early detection of ID is recommended and serum ferritin has been proposed as an alternative indicator for ID detection.

***Objective*** To assess the diagnostic accuracy of serum ferritin for detecting ID in children below five years of age.

***Methods*** This cross-sectional, diagnostic study was conducted in primary health care centers in Yogyakarta and Bantul. Hemoglobin (Hb), serum ferritin and soluble transferrin receptor (sTfR) levels were performed on children aged 6–59 months. A sTfR level of ≥ 8.2 mg/L was used to define iron deficiency. The best cut off point for serum ferritin level use as a diagnostic tool was determined by receiver operator curve.

***Results*** The prevalence of ID was 32%. Mean hemoglobin levels in iron deficient and healthy children were 11.7 (SD 0.5) g/dL and 12.2 (SD 0.7) g/dL, respectively. The sensitivity, specificity, and positive predictive value (PPV) of serum ferritin (<12 ug/L) were 17%, 93%, and 56%, respectively. Using a cut off of <32.4 ug/L, serum ferritin had sensitivity of 62.1% and specificity of 50.8%.

***Conclusions*** The diagnostic value of serum ferritin levels is modestly capable of detecting ID. Therefore, serum ferritin should not be used as an alternative indicator for detecting ID in children below five years of age. **[Paediatr Indones. 2013;53:150-4.]**

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

Iron deficiency (ID) continues to be the most common cause of anemia worldwide.[1] In children below five years of age, the prevalence of iron deficiency anemia (IDA) was reported to be 48.1% in Indonesia and 38 – 73% in Yogyakarta.[2,3] Iron deficiency anemia impairs the cognitive and motor development of children until 10 years of age, even if iron therapy was given.[4] Therefore, detection and treatment of iron deficiency before anemia occurs may improve later developmental outcomes.

Iron staining of bone marrow aspiration has been the gold standard for ID diagnosis, however, this procedure is invasive.[5] The sTfR is the best alternative indicator to detect ID. Its sensitivity and specificity are 84% and 94%, respectively, for detecting ID in infants.[6] The levels of sTfR increase when ID occurs.[7] Nevertheless, this test, too, has limitations as it is expensive and often unavailable in routine settings.[8] Serum ferritin has been suggested as another alternative indicator. It reflects iron body stores. Moreover, it is less expensive than sTfR, available in most health care settings, and has a high specificity for IDA diagnosis.[5]

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World Health Organization recommends using a serum ferritin level of <12 ug/L in combination with a hemoglobin level of <11 g/L for IDA diagnosis in children aged 6 months to 5 years.[8,9] However, this cut off has not been used to screen for ID. Diagnostic levels of serum ferritin to detect iron deficiency in children below five years of age has not been determined. The aim of the study was to assess serum ferritin diagnostic levels and the best cutoff point to detect ID in children below five years of age.

## Methods

We conducted a cross-sectional, diagnostic study from November 2010 to April 2011 at primary health care centers in Yogyakarta and Bantul. We estimated a required sample size of 199 subjects, based on $\alpha$ = 0.05 and power = 90%. The inclusion criteria were healthy children aged 6 – 59 months whose parents consented to participate. Children who had infection, inflammation, anemia (Hb level <11 g/dL), serum ferritin level > 140 ug/L, or received iron therapy within the prior 3 months were excluded. Patients were classified as having infection and inflammation when we found fever with or without a focal infection. Iron deficiency was defined as serum ferritin <12 $\mu$g/L. As the gold standard for detecting iron deficiency, we measured sTfR, with a value of ≥8.2 mg/L considered to be iron-deficient.[10]

An assistant of the study interviewed parents to collect data on every child at their first visit. Data included age, sex, birth weight, gestational age, diet recall, socioeconomic status, and iron therapy. The criteria for good recalled diet were exclusive breastfeeding, and age-appropriate diet without the consumption of cow's milk in the first year of life. If these criteria were not met, subjects were considered to have poor recalled diet. Socio-economic status was classified based on monthly family income as high (more than IDR 2,001,000), middle (IDR 701,000 to IDR 2,000,000), and low (less than IDR. 700,000).[11]

Physical examinations were performed to assess for infection and inflammation, as well as anthropometric status by measurement of weights (kg) and heights (cm). Based on Z-score, nutritional status was classified as well-nourished (weight for height index -2SD to 2SD) or undernourished (weight for height index -3SD to -2SD). Venous blood samples (2.5 mL) were drawn by laboratory staff to check Hb, serum ferritin and sTfR levels. The Hb level was measured with cyanmethemoglobin and serum ferritin level was measured with enzyme-linked immunosorbent assay (ELISA) in the Parahita Laboratory, Yogyakarta. We sent eligible samples to the Southeast Asian Ministry of Education Organization (SEAMEO) laboratory in Jakarta to measure sTfR levels using a sandwich ELISA technique.

Kolmogorov-Smirnov test was used for the assessment of normality. To compare the distribution of Hb, serum ferritin and sTfR levels between the ID and normal groups, the T-test or Mann-Whitney test was used. A P value of <0.05 was considered to be statistically significant.

Diagnostic values of serum ferritin was determined by calculating the sensitivity, specificity, prevalence (pre-test probability), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, negative likelihood ratio, pre-test odds, post-test odds, and post-test probability. Receiver operator curve (ROC) was performed to choose the best serum ferritin concentration cut off

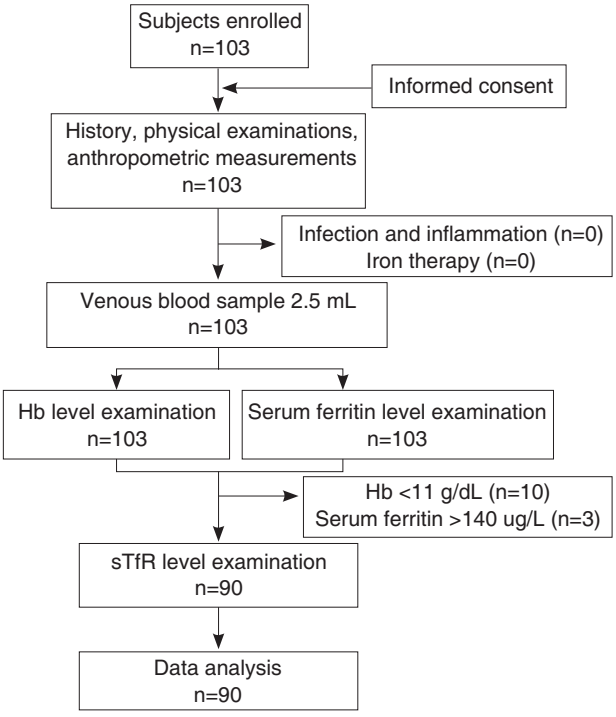


**Figure 1.** Study profile flow chart

point for ID diagnosis. This study was approved by the Ethics Committee of the Gadjah Mada University Medical School, Yogyakarta.

## Results

There were 103 children initially enrolled in this study. From these children, 13 children were excluded for anemia (10 children) and serum ferritin level >140 ug/L (3 children) (**Figure 1**).

We found that the prevalence of ID in our subjects was 32%. The highest prevalence was found in the 25 – 59 months age group (23 out of 29). All children from the ID group had normal birth weights and gestational ages. There were 14 males and 15 females detected to have ID (**Table 1**).

We found that iron deficient children had significant lower Hb level and higher sTfR level compared to normal children, but no significant difference in median of serum ferritin levels between the groups (**Table 2**).

**Table 1.** Baseline characteristics of subjects

| Characteristics | Iron deficient children (n = 29) | Normal children (n = 61) |
|---|---|---|
| Age, n (%) | | |
| 6 – 24 months | 6 | 13 (21) |
| 25 – 59 months | 23 | 48 (79) |
| Sex, n (%) | | |
| Male | 14 | 34 (56) |
| Female | 15 | 27 (44) |
| Birth weight, n (%) | | |
| <2500 grams | 0 | 2 (3) |
| 2500 – 4000 grams | 29 | 59 (97) |
| Gestational age, n (%) | | |
| <37 weeks | 0 | 0 |
| 37 – 42 weeks | 29 | 61 (100) |
| Nutritional status, n (%) | | |
| Well-nourished | 13 | 42 (69) |
| Undernourished | 16 | 19 (31) |
| Recalled diet, n (%) | | |
| Good | 13 | 37 (61) |
| Poor | 16 | 24 (39) |
| Socioeconomic status, n (%) | | |
| High | 0 | 2 (3) |
| Middle | 13 | 28 (46) |
| Low | 16 | 31 (51) |

**Table 2.** Hb, sTfR, and serum ferritin levels by group

| Variables | Iron deficient children (n = 29) | Normal children (n = 61) | P value |
|---|---|---|---|
| Mean Hb (SD), g/dL | 11.7 (0.5) | 12.2 (0.7) | 0.001* |
| Mean sTfR (SD), mg/L | 9.3 (1.1) | 6.2 (1.1) | <0.001* |
| Median serum ferritin (SD), ug/L | 24.3 (2.4 – 121.5) | 33 (4.5 – 114.2) | 0.228# |

*t-test; #Mann-Whitney

**Table 3.** Crosstabulation between serum ferritin and sTfR values

| | | sTfR | | |
|---|---|---|---|---|
| | | ron deficient children | Normal children | Total |
| Serum ferritin | Iron deficient | 5 | 4 | 9 |
| | Normal | 24 | 57 | 81 |
| | Total | 29 | 61 | 90 |

Sensitivity = 17%; specificity = 93%; prevalence (pre-test probability) = 32%; PPV = 56%; NPV = 70%; positive likelihood ratio = 2.63; negative likekihood ratio= 0.89; pre-test odds = 0.47; post-test odds = 1.23; post-test probability = 55%

Cross-tabulation between serum ferritin and sTfR values are shown in **Table 3**. According to ROC, the best serum ferritin level cut off point was <32.4 ug/L, increasing its sensitivity to 62.1% and specificity to 50.8%.

## Discussion

Iron deficiency usually occurs in the second year of life, due to decreased iron intake and rapid growth in the first year of life. Normal infants need to absorb approximately 0.8 mg/day of dietary iron (0.6 mg for growth and 0.2 mg to replace ongoing losses). Towards the end of the second year of life, this swift rate of growth begins to slow, so routine diets should include sufficient iron-rich foods to meet demands.[5,12]

In our subjects, we found the highest prevalence of iron deficiency to be in the third year of life. Sixteen of 29 children with ID were undernourished, had poor recalled diet, and low socioeconomic status. Poor recalled diet included children not breastfed exclusively or not getting appropriate diet for their age. No history of cow's milk consumption was found in either group. Low socioeconomic status led to parents being unable to provide appropriate diets, as well as appropriate developmental stimulation for their children.[4,9]

We found similar numbers of ID in males and females. Gender differences reportedly only affect ID in adolescents, as females are at higher risk due to menstruation and rapid growth. In developing countries, ID has also been attributed to chronic blood loss due to parasitic infections.[5,13] A limitation of our study was that we did not perform stool examinations to detect parasitic infections.

We found that the mean Hb level in the iron deficient group was lower than that of the normal group (P=0.01). Even if the Hb level was still within normal limits in iron deficient patients, iron supplementation must be given to prevent anemia based on the Indonesian Pediatric Society recommendations.

Mean sTfR level in the iron deficient group was higher than that of the normal group (P<0.001). Levels of serum ferritin in both groups were within normal ranges. This shows that sTfR is a more sensitive indicator for iron deficiency than serum ferritin. Normal values of serum ferritin for children aged 6 months – 15 years are 12 – 140 ug/L.[14] Interpretation of serum ferritin must be looked at closely. Ferritin is an acute-phase reactant that can become elevated in settings of inflammation, chronic infection, and malignancy.[5,10,15]

C-reactive protein (CRP) and α1-acid glycoprotein (AGP) are common biomarkers of infection and inflammation under field conditions. The WHO and Centers for Disease Control (CDC) stress the need to include CRP and AGP in iron status assessments. CRP rises rapidly after the onset of infection and declines within 24–48 hours. AGP reaches its maximum concentration 48 hours after the onset of inflammation and remains elevated for 120-144 hours.[16] In order to prevent false positives using serum ferritin, we excluded the children who suffered from infection and inflammation based on history-taking, and whose serum ferritin was >140 ug/L. CRP and AGP examinations were not performed in our study.

Both cut off and diagnostic values of serum ferritin to detect iron deficiency in children below five years of age have not yet been identified. The WHO recommends using a serum ferritin level of <12 ug/L in combination with a Hb level of <11 g/dL for IDA diagnosis in children aged 6 months to 5 years.[8,9] Therefore, we used a serum ferritin level of <12 ug/L as an initial cut off point. However, this level resulted in poor diagnostic values, with sensitivity, specificity and PPV of 17%, 93% and 56%, respectively. Positive likelihood ratio was only 2.63, which meant serum ferritin was not a useful differentiator. The poor diagnostic values may have been influenced by the small sample size.

Several studies had similar results. Serum ferritin levels of <15 ug/L and ≤12 ug/L were used to detect ID in adult patients and also had poor diagnostic values.[17,18] However, an Estonian study found serum ferritin level <10.9 ug/L had 83% sensitivity and 80% specificity to detect ID in healthy, term infants aged 9 – 12 months with normal birth weight. Infants with increased CRP concentration (CRP >5 mg/L) were excluded.[19]

We constructed a ROC in order to choose the best cut off point of serum ferritin concentration, which was 32.4 ug/L. Its sensitivity and specificity were 62.1% and 50.8%, respectively, however, this

level was still not sensitive enough to make a confident diagnosis. A good screening tool is defined as having a sensitivity ≥80%, despite the presence of low specificity.[20]

In conclusion, we recommend that serum ferritin not be used as an alternative indicator in detecting ID in children below five years of age. However, further study should be performed with a larger sample size and with examinations of infection or inflammation biomarkers, including CRP or AGP while examining iron status.

## Acknowledgment

We are extremely grateful to Endy Paryanto MD for the special comments during manuscript preparation, Hendro Dwi Wicaksono as our assistant in the study. We also thank the laboratory staff of the Parahita Laboratory in Yogyakarta and the SEAMEO Laboratory in Jakarta.

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