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Original Article

Reticulocyte hemoglobin content as a predictor of iron deficiency anemia

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Abstract

Background Iron deficiency anemia (IDA) is the most common form of anemia in developing countries, such as Indonesia. Iron deficiency anemia in children is a serious problem because it affects their growth and development. Early detection of IDA and subsequent treatment in childhood may prevent future health problems.

Objective To assess the use of reticulocyte hemoglobin content (CHr) to detect IDA in children aged 6-60 months.

Methods We performed a cross-sectional study to measure the sensitivity and specificity of CHr compared to serum ferritin which is considered to be the gold standard for IDA diagnosis. The study was conducted from September 2011 to March 2013 in children aged 6-60 months who visited the Pediatric Outpatient Clinic, Sanglah Hospital, and Puskesmas II in West Denpasar. Data analysis was performed by 2x2 table. The results were assessed by area under the curve (AUC) and receiver operating characteristic (ROC).

Results Of 121 children underwent blood testing during the study period, 69 children were excluded because they did not have hypochromic microcytic anemia, leaving 52 subjects eligible for the study. The prevalence of IDA in this study was 31%. Reticulocyte hemoglobin content (CHr) ≤ 23.1 pg had 88% (95%CI 71 to 100%) sensitivity and 25% (95%CI 11 to 39%) specificity.

Conclusion Reticulocyte hemoglobin content < 23.1 pg may be a good predictor of IDA. **[Paediatr Indones. 2015;55:171-5.]**

Keywords: *diagnostic test, iron deficiency anemia, reticulocyte hemoglobin content*

Iron deficiency anemia (IDA) is the most common form of anemia in the world, arising from the lack of iron in the body and disturbed hemoglobin synthesis. Based on a 1995 household survey in Indonesia, the prevalence of IDA was 40.5%. It increased to 48.1% in 2001 among children under the age of five years.[1-3]

Anemia remains a public health problem. The contribution of anemia to public health is considered to be low if the prevalence in a country is less than 15%, moderate if the prevalence is 15-40%, and high if the prevalence is 40%.[4] Iron deficiency anemia is, therefore, a major public health problem with a prevalence of more than 40% in Indonesia.[5] Anemic children experience inhibited growth and intellectual development, and are more susceptible to disease due to immune deficiency. If the anemia goes untreated, it will impact the development of intelligence.[6-10]

In order to mitigate the effects of IDA, accurate and sensitive tools are needed.[11-13] As such, the aim of this study was to assess the diagnostic capability of reticulocyte hemoglobin content (CHr) to detect IDA in children aged 6-60 months.

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Methods

This cross-sectional study was undertaken to assess the sensitivity and specificity of CHr for early detection of iron deficiency anemia compared to the standard reference of serum ferritin. Subjects were children aged 6-60 months from the Pediatric Outpatient Clinic at Sanglah Hospital and Puskesmas II, West Denpasar from September 1, 2011 to March 31, 2013. The inclusion criteria were microcytic hypochromic anemia and parental consent. We excluded anemic children with leukopenia, thrombocytopenia, organomegaly with suspected malignancy, severe infection, and those taking iron therapy. Subjects were collected by consecutive sampling.

The minimum required sample size was calculated with a single sample proportion each for sensitivity and specificity of CHr 93.3% and 83.2%, respectively; Z score for α 0.05 = 1.96.[14,15] The estimated minimum required sample size was 52 for this study.

Anemia was defined as low hemoglobin status for age and gender according to the WHO criteria as measured by *ADVIA 2120* machine.[16] Our study population consisted of children aged 6-60 months with hemoglobin levels below 11 g/dL, which is considered to be an anemic state according to WHO criteria.[16] The CHr is a measurement of hemoglobin level in the youngest red blood cells or reticulocytes and performed with the *ADVIA 2120* machine. The CHr cut-off point for IDA was < 26 pg. Serum ferritin is an iron compound formed in the intestine and stored in the liver, spleen, and bone marrow for eventual incorporation into hemoglobin. Serum ferritin level is an indicator of the body's iron stores, and measured by the *Elecsys 2010* machine. Serum ferritin levels <12 μg/dL indicate IDA. Subjects provided 5 mL blood specimens. Routine blood examinations to assess hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) require 3 mL of blood. Analysis of CHr and serum ferritin was performed if hypochromic microcytic anemia was found from routine blood examination, which required 2 mL blood specimens. Blood tests were performed in the Laboratory of Clinical Pathology at Sanglah Hospital, Denpasar.

Serum ferritin and CHr findings in the subjects were evaluated for their standard value using 2x2 tables. From the tables, we determined the sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy (Ac), positive likelihood ratio (LR+), and negative likelihood ratio (LR-). We performed a ROC curve analysis using *SPSS 16* software, and determined the AUC for CHr. This study was approved by the Research Ethics Committee of Udayana Medical School, Sanglah Hospital, Denpasar and Puskesmas II, West Denpasar.

Results

During the study, 121 children aged 6-60 months underwent blood testing. Sixty-nine children were not anemic and 52 children had hypochromic microcytic anemia **(Figure 1).**

Characteristics of subjects are shown in **Table 1**. Most subjects were male (63.5%) and aged 12-36 months (50%). Most subjects had good nutritional status (76.9%). We found that 67.3% of subjects had exclusively breastfed.

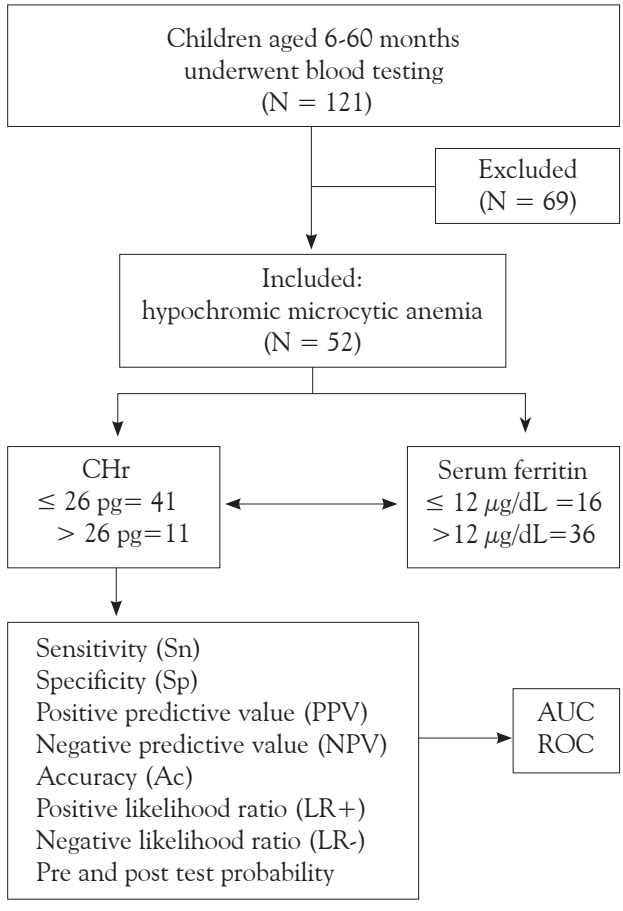

Figure 1. Study outline

Table 1. Subjects' characteristics

Characteristics	(N = 52)
Gender, n (%)	
Male	33 (63.5)
Age, n (%)	
6-12 months	19 (36.5)
13-36 months	26 (50.0)
37-60 months	7 (13.5)
Mean body weight (SD), kg	10.8 (2.84)
Nutritional status, n (%)	
Malnourished	8 (15.4)
Well-nourished	40 (76.9)
Overweight	4 (7.7)
Exclusively breastfed, n (%)	35 (67.3)
Mean hemoglobin (SD), g/dL	9.9 (0.95)
Mean MCV (SD), pg	69.8 (7.07)
Mean MCHC (SD), g/dL	31.9 (2.29)
Mean CHr (SD), pg	23.4 (3.35)
Mean SI (SD), mcg/dL	29.0 (1.83)
Mean TIBC (SD), mcg/dL	345.7 (106.6)
Mean ferritin (SD), ng/mL	36.8 (32.5)

SI=serum iron, TIBC=total iron binding capacity

Figure 2 shows the ROC curve of CHr and **Table 2** shows the AUC value of the ROC curve for CHr. Reticulocyte hemoglobin content was 60.8% (95%CI 43.4 to 78.1%) of AUC. The prevalence of IDA was 31%, this value come from sample with IDA based on ferritin is divided overall sample. **Figure 3** shows the CHr cut-off point.

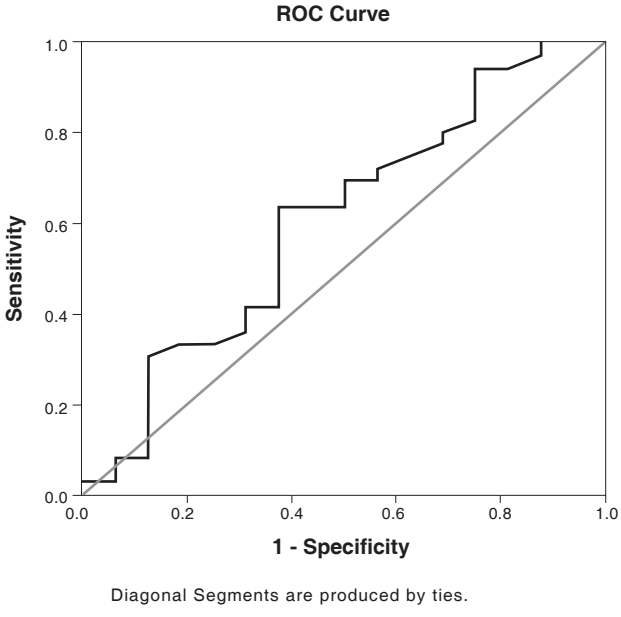

Figure 2. ROC curve for CHr

Table 2. AUC score

Area	Std. error	Asymptotic sig.	Asymptotic 95%CI	
			Lower	Upper
0.608	0.089	0.219	0.434	0.781

Figure 3 as a sensitivity and specificity curve which showed the optimal of CHr cut off point. To obtain the cut off point was to draw a vertical line from the intersection point. The optimal cut off point is a between 19 and 20. When we viewed in statistical tables microsoft office excel, number 19 with a value 23.1 pg had sensitivity 65% and specificity of 64%, while the number 20 is 23.25 pg had sensitivity of 62% and a specificity of 64%. Cut off point 23.1 pg appears to be better, so it was concluded that the cut off point CHr was 23.1 pg.

Reticulocyte hemoglobin content $\leq$ 23.1 pg had a sensitivity of 88% (95%CI 71 to 100%), specificity 25% (95%CI 11 to 39%), PPV 34% (95%CI 20 to 49%), NPV 82% (95%CI 59 to 100%), accuracy 44%, LR+ 1.17 (95%CI 0.99 to 1.52%), and LR- of 0.5 (95%CI

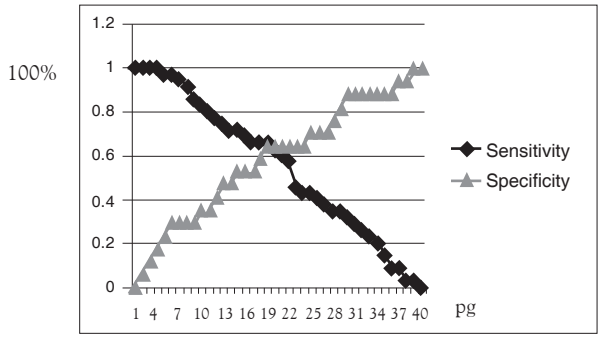

Figure 3. Cut-off point for CHr

0.12 to 2.06%). Pretest odds were 1.03; post-test odds were 1.2; and post-test probability was 0.55.

Discussion

A 1995 Indonesian household survey showed that the prevalences of anemia among children were < 34% in boys and 48% in girls.[2] In contrast, we found IDA to be more common in boys than in girls, under five years of age. Because, in our study, number of boys was bigger than girls.

Older children are at greater risk of IDA. Age was significantly associated with anemia due to increased iron needs.[17] This finding was in agreement with several studies that mentioned that IDA events are often found in children aged 1-3 years (21%). The iron need in children aged 0-6 months is only 0.27 mg/day, but it increases to 11 mg/day in children aged 7-12 months, and 7 mg/day for those aged 12-36 months.[18-20] The need for iron is affected by increased blood volume, tissue mass, and iron deposits, therefore IDA occurs more often in children aged 12-36 months.[18] Economic conditions indirectly affect food consumption, both quantitatively and qualitatively. Foods with high iron content such as milk or meat are not easily accessible by most people in Indonesia.[20,21]

Reticulocyte hemoglobin content is one of several hematological markers that can be used to assess reticulocyte hemoglobin levels in cells that are circulating in the bloodstream for 24 to 48 hours, before becoming mature blood cells. As such, CHr can be used to describe current iron conditions in the body.[22-24] Serum ferritin is a biochemical examination based on iron metabolism. Serum ferritin levels correlate to iron stores in the body. When iron stores are decreased, serum ferritin is decreased, a marker for the onset of iron deficiency.[25-27] We found microcytic anemia with CHr levels ≤26 pg in 41 children, and CHr >26 pg in 11 children. Sixteen subjects had serum ferritin levels of ≤12 μg/mL, and 36 children had serum ferritin >12 μg/mL.

The mean AUC in this study was 60.8% (95%CI 43.4 to 78.1%). Mean AUC value of 60.8% mean that if we use CHr to detect IDA on 100 children with hypochromic microcytic anemia the appropriate conclusions will be obtained in 60 patients.

Similar to previous studies, we found an 88% sensitivity (95%CI 71 to 100%). The specificity was low at 25% (95%CI 11 to 39%). The high sensitivity of 88% indicates that children with CHr scores < 23.1 pg have a high possibility of IDA. Positive predictive value and NPV were influenced by disease prevalence that may vary among population settings. Therefore, LR(+) and LR(-) are needed as parameters that are not affected by disease prevalence. LR(+) much greater than one indicates a strong positive result of diagnostic tests and more likely confirms the presence of disease.[28-30]

The positive predictive value was high in this study, meaning that CHr values > 23.1 pg are likely to indicate negative results by 82%. The LR(+) of 1.17 (95%CI 0.99 to 1.52%) means that for CHr scores ≤ 23.1 pg, the ratio of positive results in the positive group compared with the positive results in the negative group is 1.17. Likelihood ratio values in this study were 1-2, meaning that CHr scores contribute moderately in the decision-making to diagnose IDA, and require other diagnostic tests.

The pretest probability (prevalence) of IDA in children aged 6-60 months prior to the diagnostic test was 0.31. The possibility of a child with IDA after the CHr test was 0.55. Post-test probability value is within the range of 0.25 to 0.65 of test treatment threshold which means further tests are needed to determine therapy, based on the post-test. The low post-test probability may be the reason that ferritin tests are recommended along with the CHr.[29] Reticulocyte hemoglobin content had a moderate positive likelihood ratio, therefore, to increase the post-test probability of more than 0.65, other diagnostic tools are needed to strengthen the sensitivity and reduce the false negative diagnosis.[29]

Study limitations were the small age range, as subjects were children aged 6-60 months. Studies with a wider age range, involving children aged 5-18 years are needed to improve the application of this study to a population. Subjects with serum ferritin levels <12 mg/dL in this study were small in number. These may have resulted from undetected inflammation/infection in subjects, even though we excluded subjects with severe infection from the study. Thalassemia patients may also show microcytic anemia, but with ferritin levels > 12 mg/dL, thus muddying the diagnosis of IDA.

In conclusion, CHr may be used to detect IDA in children aged 6-60 months. Further multicenter studies with subjects in a wider age range are required to increase the application of CHr to detect IDA.

Conflict of inetrest

None declared.

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