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Iron deficiency screening with content hemoglobin reticulocyte (chr) in children aged 6 months to 5 years

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ABSTRACT

Anemia is a major health problem around the world and iron deficiency is the major cause. In children under 5 years old iron deficiency cause disruption in their growth and development. For that reasons iron deficiency in children should be detected as early as possible. The gold standard for the detection of iron deficiency is hemosiderin examination from bone marrow aspiration, but this examination is invasive and difficult to implement. Ferritin is often used to detect iron deficiency but it has various limitations. Reticulocyte hemoglobin content (CHr) was introduced as a new parameter for the identification of iron deficiency. This parameter measures the levels of hemoglobin in the reticulocytes which is newly released from bone marrow and expected to reflect the actual conditions in the bone marrow, therefore it can detect early iron deficiency. It is expected that CHr can identify iron deficiency with cheaper, easy, and applicative method. The aim of this study was to determine the diagnostic performance of CHr in screening of iron deficiency in children aged 6 months to 5 years old. The present study was a cross sectional study to determine the diagnostic performance of CHr and use ferritin as gold standard. The subjects of this study were healthy children aged 6 months-5 years old taken from Posyandu in Yogyakarta district and previously had obtained the consent of their parent/guardian. The CHr examination used a flowcytometry method by Advia 120 Hematology Analyzer. The ferritin examination used an electrochemiluminescens method with Elecsys 1010. ROC curve analysis was performed using SPSS version 17. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and likelihood ratio were calculated using a 2x2 TABLE. Youden's index was used to choose the proper cutoff level of CHr for diagnosing iron deficiency. The study was conducted on 104 subjects. Cutoff level of CHr was $\leq$27.65 pg and the sensitivity, specificity, diagnostic accuracy, positive, and negative predictive values were 91.7%,78.3%, 79.8%, 35.4%, 98.63% respectively. Positive and negative results of likelihood ratios were 4.21 and 0.1. CHr is expected to be used to screen iron deficiency in children under 5 years old.

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ABSTRAK

Anemia telah menjadi masalah kesehatan utama di seluruh dunia dan kekurangan besi merupakan penyebab utama. Pada anak di bawah 5 tahun, kekurangan besi menyebabkan gangguan pertumbuhan dan perkembangan. Untuk alasan ini kekurangan besi pada anak harus didiagnosis sedini mungkin. Baku emas deteksi kekurangan besi adalah pemeriksaan hemosiderin dari aspirasi sumsum tulang, tetapi pemeriksaan ini invasif dan sulit dilakukan. Feritin sering digunakan untuk deteksi kekurangan besi tetapi mempunyai beberapa kelemahan. Kandungan retikulosit hemoglobin (CHr) tela diperkenalkan sebagai parameter baru untuk deteksi kekurangan besi. Parameter ini mengukur kadar hemoglobin di retikulosit yang baru dilepaskan dari sumsum tulang dan diharapkan menggambarkan kondisi sebenarnya sumsum tulang sehingga bisa digunakan untuk mendeteksi kekurangan besi sedini mungkin. Diharapkan CHr dapat digunakan sebagai marker mendeteksi kekurangan besi yang murah, mudah dan aplikatif. Penelitian ini bertujuan untuk menentukan kemampuan CHr sebgai marker diagnosis kekurangan besi dibandingkan dengan baku emas menggunakan ferritin pada anak 6 bulan hingga 5 tahun. Penelitian ini merupakan penelitian potong lintang melibatkan anak sehat dari Posyandu di Kota Yogyakarta yang sebelumnya telah mendapatkan peretujuan dari pengasuh atau orang tuanya. Pemeriksaan CHr menggunakan metode flowsitometri (Advia120 Hematology Analyzer) dan pemeriksaan ferritin menggunakan metode elektrokemiluminescen (Elecsys 1010). Analisis kurva ROC dilakukan dengan SPSS versi 17. Sensitivitas, spesifisitas, nilai prediktif positif dan negative, akurasi dan rasio *likehood* dihitung dengan table 2x2. Indeks Youden digunakan untuk menentukan nilai batas yang tepat CHr untuk diagnosis kekurangan besi. Penelitian dilakukan terhadap 104 subjek. Nilai batas CHr adalah ≤ 27,65 pg dan sensitivitas, spesifisitas, akurasi, nilai prediktif positif dan negatif berturut-turut adalah 91,7%; 78,3%; 79,8%; 35,4%; 98,63%. Hasil positif dan negatif rasio *likehood* 4,21 dan 0,1. CHr diharapkan dapat digunakan sebagai alat skrining kekurngan besi pada anak di bawah 5 tahun.

Keywords: iron deficiency - ferritin - CHr - diagnostic performance - screening

INTRODUCTION

Anemia is a global health problem that affects both developed and developing countries. Anemia occurs at any age, but is more common in pregnant women and children. Household Health Survey in 1995 showed that the prevalence of anemia in children under five years old was 40.5%. A study by Sekartini et al. in Jakarta has shown that 38.2% of infants aged 4-12 months were anemic and 71.4% of them had iron deficiency anemia. Another study in Bali in children aged 6-59 months has show that the prevalence of iron deficiency or anemia was 63.5%.[1-5]

Iron deficiency can affect the development of cognitive, psychomotor, language skills, motor skills, coordination, and intelligence of all ages. Although anemia caused by iron deficiency can easily be treated with iron supplementation, the change in cognitive function in children with iron deficiency may not be corrected completely. Consequently, early diagnosis of iron deficiency is important.[6,7]

Laboratory tests play a role in the identification and monitoring of iron deficiency. Hematological examinations are easy, inexpensive, and widely available but can only detect iron deficiency at an advanced stage, while biochemical examinations are not widely available yet and

the cost is still relatively expensive. The iron status, TIBC (total iron binding capacity), and ferritin are strongly influenced by the presence of infection and inflammation. The gold standard for the examination of iron deficiency is iron staining of bone marrow, but this examination is invasive, risky, while its difficult interpretation makes it impractical to use.[6-9]

In the mid-1990s a reticulocyte analysis with automatic flow cytometry method was introduced, thus there are new parameters such as volume, hemoglobin concentration, and reticulocyte maturity. The examination of reticulocyte indices is relatively easy and its use has been evaluated. CHr is one of reticulocyte indices that has been developed for the identification of iron deficiency.[10-12] Several studies have been conducted to determine the diagnostic performance of CHr to identify iron deficiency but there were many variations in the results. The aim of the present study was to determine the diagnostic performance and cutoff of CHr that can be used to screen iron deficiency in children aged 6 months to 5 years old in Yogyakarta.

MATERIALS AND METHODS

The present study was a cross-sectional study with a diagnostic test designed to assess the diagnostic performance of CHr for iron deficiency screening in children aged 6 months to 5 years old in Yogyakarta. Samples were taken from local clinics (posyandu) in Yogyakarta and the laboratory examination was performed at the Department of Clinical Pathology Laboratory, Dr. Sardjito General Hospital Yogyakarta.

A complete blood count and CHr were examined using Advia 120 hematology analyzer. Ferritin level was measured using the Elecsys 1010 with electrochemiluminescens method. Iron deficiency in children under five years old was described when ferritin levels were <12 ug/L, as stated by the WHO guideline. CRP and morphological examination of peripheral blood were performed to determine the presence of infection and anemia due to other causes.

Data were displayed in descriptive characteristics of the subjects. Diagnostic performance of CHr values was calculated at various levels with ferritin as gold standard, while cutoff calculation used ROC curves, and the data were processed using SPSS version 17.

RESULTS

The data were obtained from 151 subjects from local health clinics or posyandu in Gemawang and Gondokusuman District who agreed to follow this study. Forty seven subjects were excluded because they did not meet the criteria, had incomplete data and ineligible condition.

TABLE 1. Characteristics of subjects

Characteristics	**N (%)**
Sex	
Male	55 (52.9)
Female	49 (47.1)
Body Weight (kg)	12.34±18.19
Age (months old)	31.88±15.43
6 mo - 12 mo	16 (15.4)
>12 mo - 24 mo	21 (20.2)
>24 mo - 36 mo	28 (26.9)
>36 mo- 48 mo	23 (22.1)
>48 mo - 60 mo	16 (15.4)
Term of delivery	
Preterm	6 (5.8)
At term	90 (86.5)
Post-term	8 (7.7)
History of delivery	
Sectio caesaria	14 (13.5)
Spontaneus	83 (79,8)
Spontaneus with problems	7 (6.7)
Parturation helped by	
General practitioner	18 (17.3)
Obstetrician	32 (30.8)
Midwife	54 (51.9)
Iron supplementation in pregnancy	
Yes	97 (93.3)
No	7 (6.7)

A total of 55 (52.9%) subjects were male and 49 (47.1%) subjects were female, with most subjects in the age range of between 24 months and 36 months old. A total of 90 (86.5%) subjects were born at term, which made them more likely to have sufficient iron reserves.

TABLE 2. Laboratory characteristics of subjects (N=104)

Examinations	Mean ± SD/Median (min-max)
Hemoglobin (g/dl)	12.30±0.96*
RBC ($x10^6/mm^3$)	5.0 (4.27-6.47)
MCV (fl)	75.1 (56.8-82.8)
MCH (pg)	24.6 (15.6-27.3)
MCHC (g/dl)	32.65 (25.4-34.7)
RDW (%)	13.7 (12.18- 18.4)
CHr (pg)	28.65 (19.7-32.2)
Ferritin (µg/l)	31.75±18.19*

In this research, the diagnostic performance of CHr for iron deficiency screening used ferritin as gold standard with cutoff 12 ug / L. The determination of optimal cutoff values was done using ROC curves. Area Under the Curve (AUC) of Chr for diagnosis of iron deficiency was 0898 with p = 0.000 (95% CI 0832 to 0.963).

Based on Youden's index, it was gained that the farthest distance in the ROC curve was on CHr cutoff 27.65 pg with Youden index 0699. CHr values ≤ 27.65 pg had 91.7% sensitivity (95% CI 86.4-7), specificity of 78.3%, and positive predictive value, negative predictive value, positive likelihood ratio, as well as negative likelihood ratio of 35.4%, 98.63%, 4:21, and 0.1, respectively.

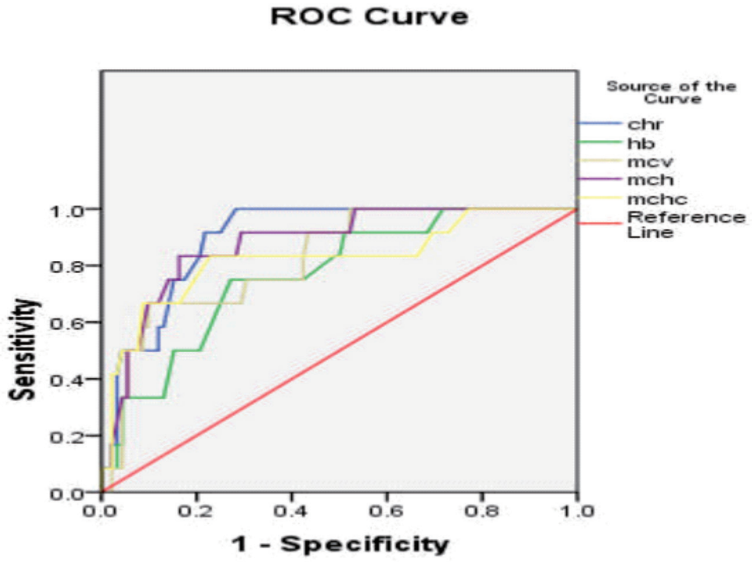

FIGURE 1. The comparison between ROC curve of CHr and hemoglobin and erythrocyte index

FIGURE 1 shows the comparison of AUC between CHr and other haematological examinations that were often used to identify iron deficiency. CHr was compared with the hemoglobin and erythrocyte indices MCV, MCH, and MCHC. Area under curve for MCV was 0823 (95% CI, 0711-0936), while MCH had AUC of 0877 (95% CI, 0.785 to 0969), and MCHC with the AUC was 0824 (95% CI, 06780970). Significance value for the parameters of MCV, MCH, and MCHC was 0.000. Hemoglobin had 0768 AUC (95% CI, 0638-0899) with p = 0.003. CHr had the widest AUC 0898 with p = 0.000 (95% CI 0832 to 0.963).

DISCUSSION

Iron deficiency is a major cause of anemia and many children suffered from it. Many previous studies have indicated that children with iron deficiency showed an impaired motor function, cognitive dysfunction, emotional mental developmental disorders, and disorders of the nervous system. Early detection of iron deficiency is required to be done quickly.[1]

A study by Ullrich *et al.*[13] which aimed to screen for iron deficiency in healthy infants aged between 9-12 months old obtained the sensitivity of 83% and specificity of 72% at 27.5 pg cut off, while this research used transferrin saturation as gold standard. Brugnara *et al.*[6] who examined CHr to identify iron deficiency in 210 children with a mean age of 2.9 years old and transferrin saturation as gold standard obtained a sensitivity of 70% and specificity of 78% with a CHr cut off 26 pg.

Kiudeliene *et al.*[14] with a cut off value of CHr 28.55 pg obtained a sensitivity of 76.6% and a specificity of 78.4%. The study was conducted on 180 children aged 6-24 months old and the gold standard used was the fulfilment of two of the four parameters, namely ferritin, transferrin, transferrin saturation and sTfR. Mateos Gonzalez *et al.*[15] examined 237 children aged 6 months to 14 years old, using transferrin saturation <20% as gold standard, a sensitivity of 94% and specificity of 94% obtained with a CHr cutoff 25 pg for diagnosis of iron deficiency anemia. The study also analyzed the diagnostic performance of CHr for iron deficiency without anemia by using a combination of ferritin <12 ng / mL and transferrin saturation <15% and obtained 88% sensitivity and 86% specificity at cut off 25 pg.

In this study the cut off CHr ≤ 27.65 pg the negative predictive value was obtained at 98.63%. These results are consistent with the research by Ulrich *et al.*[13] which produces positive predictive value (PPV) 28% (95% CI, 17% -40%) and the negative predictive value (NPV) 97% (95% CI, 92% -99%) at CHr cutoff 27.5 pg. Based on the NPV at CHr cutoff 27.65 pg, it can be concluded that in the group with negative test results, 98.63% of it was actually negative when it was examined with the gold standard. When it was combined with the sensitivity values that were also high (91.7%), the CHr examination can be used as a screening for iron deficiency in children.

The AUC comparison was conducted between CHr with other hematological examinations that often used to identify iron deficiency. The research by Ulrich *et al.*[13] yieldeds AUC of 0.73 MCH, hemoglobin with AUC 0.73, and 0.85 for CHr AUC in the screening of iron deficiency in children. Based on these results CHr had the largest AUC that CHr is a more accurate marker than hemoglobin and MCH. FIGURE 1 shows a comparison of CHr AUC with hemoglobin and the erythrocyte indices MCV, MCH, and MCHC. In this study, CHr has the widest AUC 0.898 compared to hemoglobin and red cell indices with p = 0.000 (95% CI 0.832 to 0.963).

CONCLUSION

CHr with a cutoff ≤ 27.65 pg can be used to screen iron deficiency because it has sensitivity, specificity, accuracy, positive predictive value (NRP), negative predictive value (NRN), positive likelihood ratio (LR +), and negative likelihood ratio of (LR-) 91.7%, 78.3%, 79.8%, 35.4%, 98.63%, 4:21, and 0.1, respectively.

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