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Diagnosis of Iron Deficiency and Iron Deficiency Anemia with Reticulocyte Hemoglobin Content among Children Aged 6-18 Years

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ABSTRACT

Background: Iron deficiency (ID) is the most common micronutrient deficiency in the world. If left untreated, ID will lead to iron deficiency anemia (IDA) and other irreversible consequences. The American Academy of Pediatrics recommended reticulocyte hemoglobin content (Ret-He) as an alternative laboratory examination to screen and detect ID. We aimed to compare Ret-He with other laboratory parameters to screen for iron status in healthy children.
Methods: This is a cross-sectional study comprising 207 children aged 6-18 years in Jakarta, Indonesia. Children were divided according to their iron status. Ret-He was compared with hemoglobin (Hb), mean corpuscular volume (MCV), ferritin, and transferrin saturation (TS) to assess iron status in children. Receiver operating characteristic (ROC) curve was performed to determine the optimal cut-off value for Ret-He using SPSS software.
Results: Ret-He had a positive correlation with MCV (r=0.690, n=207, P<0.001), Hb (r=0.491, n=207, P<0.01), and ferritin (r=0.336, n= 207, P<0.001). Ret-He can not be used to detect iron depletion with the cut-off value of 30.3 pg with 100% sensitivity, 19.7% specificity, 100% negative predictive value (NPV), and 5.4% positive predictive value (PPV). A Ret-He cut-off value of 28.9 pg was established as optimal to identify ID (78.9% sensitivity, 56.2% specificity, 92.2% NPV, and 28.9% PPV) and 27 pg to detect IDA (75% sensitivity, 80% specificity, 98.1% NPV, and 18.7% PPV).
Conclusion: Ret-He can be used as an alternative screening parameter to detect ID and IDA in children aged 6-18 years. Screening for IDA with Ret-He has to be done with other parameters, such as Hb examination.

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Introduction

Iron deficiency (ID) is the most common micronutrient deficiency and the most common cause of anemia worldwide (1–3). Children in the developing world are especially susceptible to ID primarily because of their rapid growth and diet low in bioavailable iron (4, 5). A study in East Kalimantan, Indonesia, showed that the prevalence of ID and IDA in school-aged children were 7.6% and 28%, respectively (6). Another study by South East Asian Nutrition Survey Indonesia among 3,600 school-aged children revealed low iron intake below the Indonesian recommended dietary allowances (65.3%) (7).

ID in children, especially during critical stages of development can cause cognitive and behavioral deficits, impair psychomotor development, increase morbidity of infectious diseases, and reduce intelligent quotient (IQ) test scores (5, 8–10). Studies have shown that raising hemoglobin to normal levels results in significant improvement in IQ scores and attention (10). Therefore, it is essential to screen and detect ID at the earliest stage to prevent unwanted consequences.

Diagnosis of ID is somewhat complex and using several

iron indicators in combination seems to provide the best assessment for iron status. There are multiple biomarkers available to assess ID, but none of them are sufficiently validated in children (4, 11). Using Hb concentrations to assess iron status lacks sensitivity and specificity, so it needs to be combined with other measurements of iron status (2). Until now, serum ferritin is the most specific test that correlates with total body iron stores and is universally available and standardized; however, it is an acute phase reactant that could be increased in the presence of inflammation (3, 11). Transferrin saturation (TS) is also affected diurnally and prandially, whereas serum soluble transferrin receptors (sTfR) lack a standard reference material and are not routinely used in daily practice (3, 12).

The American Academy of Pediatrics (AAP) considers IDA in children as Hb concentration of <11 g/dL and one of the two criteria: (i) ferritin and C-reactive protein (CRP) measurement or (ii) reticulocyte Hb content (Ret-He) measurement. It is also recommended measuring either (i) ferritin and CRP or (ii) Ret-He to screen for ID (2). Ret-He directly measures the recent functional availability of iron in the erythrocyte (2, 13). Prior studies showed that Ret-He is an early and sensitive marker for diagnosis of ID and is not affected by inflammation, diurnal variation, malignancy, or anemia of chronic disease (2). Unlike other biochemical assays, Ret-He can be measured together with a complete peripheral blood test; therefore, it does not require additional blood tubes and is provided with no additional cost (14).

Nevertheless, Ret-He measurement is still limited in certain laboratories in Indonesia. The reference range and cut-offvalues for ID screening have not been universally established and still vary between studies in children. A recent study in Indonesian children aged 6 months to 5 years established a cut-offvalue of 27.65 pg to detect ID with 91.7% sensitivity and 78.3% specificity (15), whereas another study conducted in 50 school-aged Indonesian children established a cut-offvalue of 27.8 pg with 43.8% sensitivity and 85.3% specificity to screen for IDA (16).

We aimed to determine the performance of Ret-He as a new parameter to assess iron status in children compared with other laboratory parameters. This study also aimed to establish Ret-He cut-offvalues with the best performance to identify iron-deficient state in this setting.

Materials and Methods

Study Population

This is a cross-sectional study conducted on school-aged children in the suburbs of Jakarta, Indonesia, between March and November 2016. A total of 242 children aged 6–18 years were recruited as subjects in this study. Children who (i) had hematological or systemic diseases such as infection, inflammation, malignancy, and other chronic diseases that could affect the parameters analyzed, (ii) had history of blood transfusion in the past 3 months, (iii) received iron therapy, or (iv) presented with a high value of high sensitivity CRP (hs-CRP) were excluded from this study. All written consent was obtained from the subjects or parents and legal guardians of the subjects. The examiners did history taking and physical examination on the subjects before taking their blood samples. The protocol of this study was in agreement with the standards set by the Faculty of Medicine Universitas Indonesia Ethics Committee.

Clinical Laboratory Parameters

The following indices were measured: Hb, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), Ret-He, ferritin, serum iron (SI), and total iron-binding capacity (TIBC). TS was calculated using SI and TIBC [TS=(SI/TIBC)×100] (17).

Laboratory Method and Statistical Analysis

Venous blood samples were analyzed at two different laboratories using Sysmex XT 2000i and ADVIA 2120 to measure Ret-He. Data analysis was performed using Statistical Package for Social Sciences version 22.0. Normality was confirmed using the Kolmogorov–Smirnov test. Data with normal distribution were presented as mean and standard deviation, whereas abnormally distributed data were presented as median (min–max). Spearman's correlation was calculated to determine the extent of association between variables. Receiver operating characteristic (ROC) analysis was used to assess the overall discriminative power of Ret-He for detection of iron depletion, ID without anemia, and IDA at different cut-offvalues. Diagnostic accuracy of Ret-He was compared with other laboratory parameters to determine iron status. The optimal cut-offvalue was chosen using Youden's index, where sensitivity- (1-specificity) reached the maximum value (18). Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), likelihood ratio (LR) and accuracy for each cut-offvalue were calculated with 95% confidence interval (CI). A p-value of <0.05 was considered statistically significant.

Definition of Iron Status

IDA is defined by World Health Organization criteria as low Hb value according to age: Hb < 11.5 g/dL in 6–11 years old children and Hb<12 g/dL in 12-18 years old children (19) with one out of the two criteria: TS<15% and/or ferritin <15 µg/L (20). ID without anemia is diagnosed if the subjects have normal Hb according to age and one of the two criteria mentioned earlier (20, 21). Diagnostic criteria of iron depletion are normal Hb according to age, normal TS, and ferritin <15 µg/L (21).

Results

A total of 242 children were enrolled in this study. Of these subjects, 29 were excluded because of illnesses and high hs-CRP levels and 6 subjects for incomplete data entry. This study analyzed a total of 207 subjects. The median age of the subjects was 11 years old (7-18 years), with 103 boys (49.8%) and 104 girls (50.2%).

Iron Status in School-Aged Children

A total of 133 subjects (64.2%) had normal iron status,

9 (4.3%) were iron depleted, 38 (18.4%) had ID, and 12 (5.8%) had IDA. The overall prevalence of anemia was 13.0% (27 subjects).

Correlation of Ret-He with Other Hematological Parameters

We found that Ret-He had a positive moderate degree significant correlation with MCV (r=0.690, n=207, P<0.01) and Hb level (r=0.491, n=207, P<0.001). We also found a positive low degree significant correlation between Ret-He and ferritin (r=0.336, n=207, P<0.001).

Diagnostic Performance of Ret-He

The ROC curve is designed to find the cut-off value of Ret-He for iron depletion, ID, and IDA. Analysis with ROC showed that Ret-He was an unreliable diagnostic parameter for iron depletion with an area under the curve (AUC) of 0.395 (P=0.289, 95% CI 0.284–0.506) as seen in Figure 1. The optimal cut-off value for iron depletion was 30.3 pg with 100% sensitivity and 19.7% specificity. The NPV and PPV were 100% and 5.4%, respectively. LR for positive and negative value were 1.25 and 0, respectively with accuracy of 0.819.

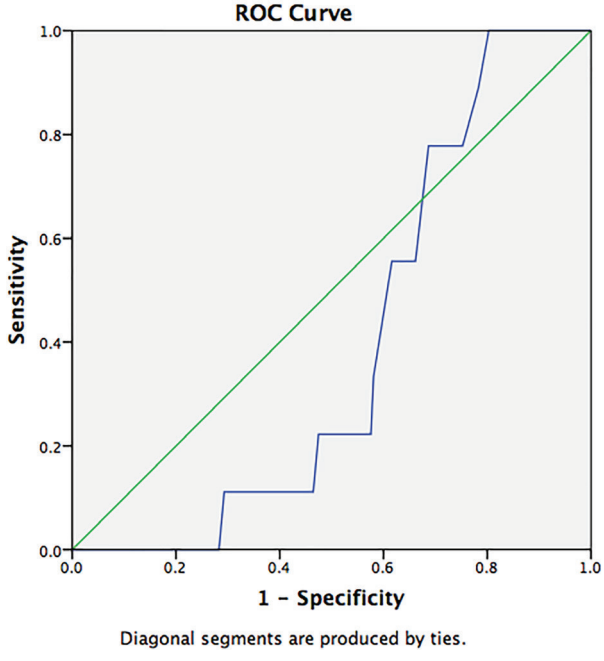

Figure 1: Diagnostic performance of reticulocyte hemoglobin content to diagnose iron depletion.

Figure 2 shows the comparison of AUC between Ret-He and other hematological parameters that are often used to detect ID. Ret-He was compared with Hb, MCV, and ferritin. The AUC for Ret-He was the highest compared with other parameters (AUC=0.676, P=0.001, 95% CI 0.592–0.760), followed by ferritin (AUC=0.672, P=0.001, 95% CI 0.572–0.773) and MCV (AUC=0.645, P=0.005, 95% CI 0.555–0.735). Hb was the most unreliable diagnostic parameter with AUC of 0.517 (P=0.744, 95% CI 0.430–0.604). According to Youden's index, a Ret-He cut-off value of 28.9 pg was established as optimal to identify ID with an overall mean sensitivity of 78.9% and specificity of 56.2%. The NPV was 92.2%, PPV 28.9%,

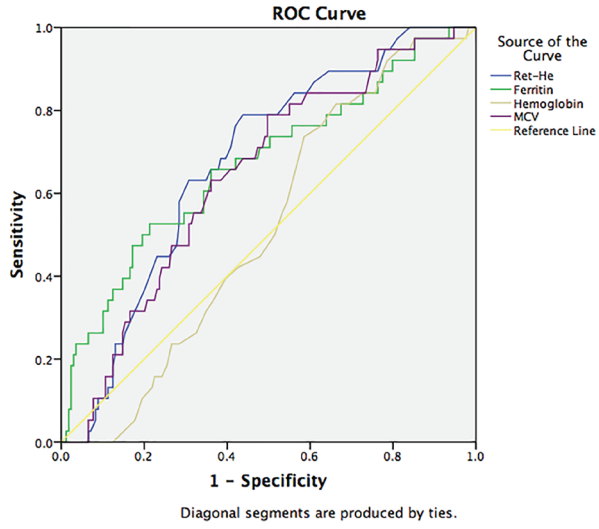

Figure 2: Diagnostic performance of reticulocyte hemoglobin content (Ret-He) compared with hemoglobin, ferritin, and mean corpuscular volume (MCV) to detect iron deficiency.

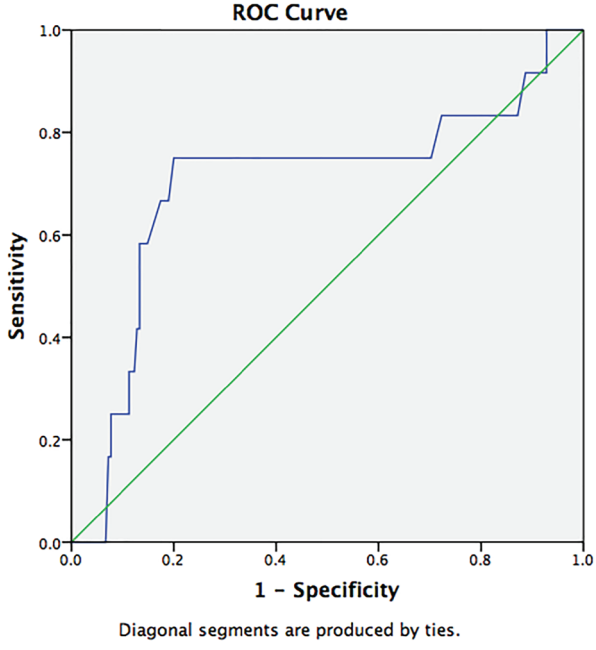

Figure 3: Diagnostic performance of reticulocyte hemoglobin content to detect iron deficiency anemia.

LR + 1.80, and LR- 0.38 with accuracy of 0.604.

A Ret-He cut-off value of 27 pg was established as the most optimal to detect IDA with AUC of 0.700 (P=0.01, 95% CI 0.517–0.883) as seen in Figure 3. Overall mean sensitivity and specificity was 75% and 80%, respectively. The NPV was 98.1%, PPV 18.7%, LR+ 3.75, and LR - 0.31 with accuracy of 0.797. The AUC for Ret-He to detect IDA was inferior to Hb (AUC=0.941, P<0.01, 95% CI 0.902–0.980) and ferritin (AUC=0.763, P=0.02, 95% CI 0.602–0.925) but higher than MCV (AUC=0.614, P=0.184, 95% CI 0.416–0.769).

Discussion

The overall prevalence of anemia was 13% in our sample of school-aged children in the suburbs of Jakarta. In a previous study, we showed a prevalence of 14% in two schools of these areas (22). Both these rates (the current

and our previous study) seems lower than the reports of other studies in other districts of Indonesia, such as that reported in East Kalimantan Province that reported a prevalence of about 54% for anemia in children and adolescents (23). Also the prevalence of IDA was 5.8% in our study, which was again less than the study in East Kalimantan that reported a prevalence of 16% in children aged 5–12 years and 15% in children aged 12–18 years (23). This difference can be related to the decreasing trend of anemia in children of Indonesia, with the greatest decline in children (24).

There are three stages of ID before anemia develops: iron depletion, ID without anemia, and IDA (20). Even the early stages of ID can cause irreversible consequences, especially to growth and development; therefore, it is important to detect ID in the early stages to prevent unwanted complications (25-27). Currently, no universal screening test is recommended for diagnosis of ID and IDA. The AAP recommends a screening program to detect IDA in children aged 9–12 months who are at risk of developing anemia (2), whereas the Centers for Disease Control and Prevention recommends Hb and Hct screening in 5–12 years old children with a history of IDA (28). Because of the high prevalence of IDA in Indonesia, the Indonesian Pediatric Society recommends a screening program for IDA using Hb in children aged 2 years and to be repeated every year until adolescence (29).

The globally accepted IDA diagnosis, also used in the present study, is by the use of TS and ferritin. However, detecting mild ID in the absence of anemia presents a great challenge, as ferritin, TS, and sTfR are affected by diurnal variation, inflammation, and other conditions (2, 3). Furthermore, these parameters are expensive and need more volume of blood samples taken; therefore, they are not suitable as screening parameters. Using Hb to screen IDA is also neither sensitive nor specific (2). This is while a screening test is a medical test performed on subjects of a defined asymptomatic population to reduce the morbidity of the disease by early detection, such as in asymptomatic iron-deficient children (30) and should be more accessible, less invasive, less expensive, less time consuming, and less psychologically discomforting for the patients, compared to a diagnostic test (31).

The AAP recommends using Ret-He as an alternative laboratory examination to screen for ID and IDA (2, 32). Compared with other parameters of iron status, Ret-He has better credentials as a screening tool because it has the advantage of needing less blood sample and is cheaper, making it more practical for ambulatory screening of ID. This diagnostic parameter is also not affected by inflammation and malignancy. Reticulocyte indices also provide a more real-time view of bone marrow iron status because it only exists in blood circulation for 1–2 days (33).

As a relatively new variable, cut-off values of Ret-He still vary among different studies, especially in the pediatric population. There has not been any universally agreed cut-off value to diagnose ID and IDA in children. A prior study suggests that reference values of Ret-He are influenced by age (34). A study by Mateos et al. (35) proposed a cut-off value of 25 pg in children aged 6 months to 14 years, whereas another study established a cut-off value of 27.6 pg in 6–8 years old and 26.9 pg in 9-11 years old children to detect functional ID (34). Cut-off values also differ on infants (27.5 pg) (14), preschool children (27.65 pg) (15), and adults (27.2 pg) (13).

Our results established that Ret-He is a good predictor of ID in children with an optimal cut-off value of 28.9 pg (overall mean sensitivity of 78.9% and specificity of 56.2%). Our cut-off value is higher than the cut-off values proposed by prior studies. This may be caused by the difference in operational definitions to diagnose ID and population characteristics. As a diagnostic parameter to detect ID, Ret-He is comparable to ferritin with slightly higher AUC. Ret-He was also stronger than Hb and MCV to detect ID. Ret-He also showed a positive correlation with other hematological parameters, such as Hb, MCV, and ferritin.

This study established a Ret-He cut-off value of 27 pg as the most optimal to detect IDA with 75% sensitivity and 80% specificity. This result is similar to another study in elementary school children in Minahasa, Indonesia, which determined a cut-off point of 27.8 pg with 43.8% sensitivity and 85.3% specificity to diagnose IDA in children (16). Currently, there has not been any large-scale study to determine the reference range of Ret-He in Indonesian children, so we cannot compare our proposed cut-off values to healthy children population. The AUC for detecting IDA with Ret-He alone is still inferior to other parameters, such as Hb and ferritin. Thus, diagnosing IDA using Ret-He must be performed in conjunction with other parameters, such as Hb measurement.

This study showed that Ret-He is not a reliable parameter to diagnose iron depletion, with AUC less than 0.5. Therefore, we still recommend diagnosing iron depletion with considering other measures such as serum ferritin. Although the accuracy of Ret-He as a screening test in this study was moderate (moderate sensitivity and specificity), it has the credentials of a good screening test which is a good NPV. Having a high NPV for a screening test allows us to "rule out" people without the disease (31). Although having a low PPV can lead to overtreatment and unnecessary cost requirement for further diagnosis, it can be minimized by using the test in populations at risk to diminish false positive outcomes.

The main limitation of our study was the use of two types of machines to measure Ret-He. The ADVIA machine measures reticulocyte hemoglobin content (CHr), which is older and more studied, whereas Ret-He is a newer parameter measured by the Sysmex machine (32). Although prior studies showed a very good level of agreement and correlation between measurements performed by both tools, the two methods are not identical and can cause slight variations in the results (13, 36). We also did not take into consideration the point that cut-off values may differ between special age groups. Another limitation of this study was the definition of iron status using a combination of diagnostic tests.

Further studies with larger samples need to be done to determine the reference range for Ret-He in Indonesian children.

Conclusion

Our results indicated that Ret-He can be used to screen for ID in populations with a high prevalence of ID. The authors also recommended using Ret-He to screen for IDA in conjunction with other hematological parameters such as Hb measurement. This indicator may be cost effective and more practical for ambulatory settings. Further studies need to be taken with larger subject samples to establish a Ret-He reference range according to age group.

Conflict of Interest: None declared.

References

1. Lanzkowsky P. Iron-Deficiency Anemia. In: Lanzkowsky P, Lipton J, Fish J, editors. Lanzkowsky's Manual of Pediatric Hematology and Oncology. 6th ed. Philadelphia: Elsevier Inc.; 2016. p. 69–83.
2. Baker RD, Greer FR. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). Pediatrics. 2010;126(5):1040–50.
3. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet. 2016;387(10021):907–16.
4. Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. J Pediatr Gastroenterol Nutr. 2014;58(1):119–29.
5. Rothman M, Faber M, Covic N, Matsungo TM, Cockeran M, Kvalsvig JD, et al. Infant development at the age of 6 months in relation to feeding practices, Iron status, and growth in a peri-urban community of South Africa. Nutrients. 2018;10(1):1–13.
6. Ringoringo HP. Insidens defisiensi besi dan anemia defisiensi besi pada bayi berusia 0-12 bulan di Banjarbaru Kalimantan Selatan: studi kohort prospektif. Sari Pediatr. 2009;11(1):8–14.
7. Widodo Y, Sandjaja, Sumedi E. *Dietary intake of Indonesian children 6 month–12 year of age. [Gambaran konsumsi gizi anak umur 6 bulan -12 tahun di Indonesia].* Gizi Indon. 2013;36(2):143–52.
8. McCann JC, Ames BN. An overview of evidence for a causal relationship between iron deficiency during development and deficits in cognitive or behavioral function 1-3. Am J Clin Nutr. 2007;85(4):931–45.
9. Luo R, Shi Y, Zhou H, Yue A, Zhang L, Sylvia S, et al. Micronutrient deficiencies and developmental delays among infants: evidence from a cross-sectional survey in rural China. BMJ Open. 2015;5(10):1–8.
10. Lam LF, Lawlis TR. Feeding the brain–The effects of micronutrient interventions on cognitive performance among school-aged children: A systematic review of randomized controlled trials. Clin Nutr. 2017;36(4):1007–14.
11. Burke RM, Leon JS, Suchdev PS. Identification, prevention and treatment of iron deficiency during the first 1000 days. Nutrients. 2014;6(10):4093–114.
12. Brannon PMPM, Taylor CLCL. Iron supplementation during pregnancy and infancy: Uncertainties and implications for research and policy. Nutrients. 2017;9(12):1327.
13. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. Clin Lab Haematol. 2006;28(5):303–8.
14. Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. J Am Med Assoc. 2005;294(8):924–30.
15. Pramantik DN, Ratnaningsih T, Mulyono B. Iron deficiency screening with content hemoglobin reticulocyte (chr) in children aged 6 months to 5 years. J Med Sci. 2008;47(3):155–121.
16. Rungngu SLP, Wahani A, Mantik MF. Reticulocyte hemoglobin equivalent for diagnosing iron deficiency anemia in children. Paediatr Indones. 2016;56(2):90–4.
17. Eleftheriadis T, Liakopoulos V, Antoniadi G, Stefanidis I. Which is the best way for estimating transferrin saturation. Ren Fail. 2010;32(8):1022–3.
18. Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: The case of tests with continuous results. Biochem Medica. 2016;26(3):297–307.
19. Marks PW. Approach to anemia in adult and children. In: Hoffman R, Benz EJ, Silberstein LE, Heslop H, Weitz J, Anastasi J, editors. Hematology: basic principles and practice. 6th ed. Philadelphia: Elsevier Inc.; 2013. p. 418–26.
20. World Health Organization. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System. World Health Organization. 2011. p. 1–5.
21. Flemming MD. Disorders of iron and cooper metabolism, the sideroblastic anemias, and lead toxicity. In: Orkin SH, Fisher DE, Ginsburg D, Look AT, Lux SE, Nathan DG, editors. Nathan and Oski's hematology of infancy and childhood. 8th ed. Philadelphia: Elsevier Inc.; 2015. p. 344–64.
22. Andriastuti M, Ilmana G, Nawangwulan SA, Kosasih KA. Prevalence of anemia and iron profile among children and adolescent with low socio-economic status. International Journal of Pediatrics and Adolescent Medicine. 2019;19:1-5
23. Widjaja IR, Widjaja FF, Santoso LA, Wonggokusuma E, Oktaviati O. Anemia among children and adolescents in a rural area. Paediatr Indones. 2014;54(2):88-93.
24. Barkley JS, Kendrick KL, Codling K, Muslimatun S, Pachón H. Anaemia prevalence over time in Indonesia: estimates from the 1997, 2000, and 2008 Indonesia Family Life Surveys. Asia Pacific Journal of Clinical Nutrition. 2015;24(3):452.
25. Chang S, Zeng L, Brouwer ID, Kok FJ, Yan H. Effect of iron deficiency anemia in pregnancy on child mental development in rural China. Pediatrics. 2013;131(3):e755–63.
26. Tran TD, Tran T, Simpson JA, Tran HT, Nguyen TT, Hanieh S, et al. Infant motor development in

rural Vietnam and intrauterine exposures to anaemia, iron deficiency and common mental disorders: a prospective community-based study. BMC Pregnancy Childbirth. 2014;14(8):1–11.
27. Rahimi E, Behrozian R, Eishi A. Prevalence of Gastrointestinal Tract Lesions in Patients with Iron-Deficiency Anemia. IJBC. 2008; 1 (1) :5-10 URL: http://ijbc.ir/article-1-27-en.html
28. Morey SS. CDC issues guidelines for prevention, detection and treatment of iron deficiency. Am Fam Physician. 1998;58(6):1475–7.
29. Ikatan Dokter Anak Indonesia. *IDAI recommendations for iron supplementation in children. [Rekomendasi IDAI suplementasi besi untuk anak]*. Jakarta: Ikatan Dokter Anak Indonesia; 2011.
30. Maxim LD, Niebo R, Utell MJ. Screening tests: A review with examples. Inhal Toxicol. 2014;26(13):811–28.
31. Trevethan R. Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. Front Public Heal. 2017;5(307):1–7.
32. Hatoun J, Sobota A, Meyers A. Using reticulocyte hemoglobin equivalent to screen for iron deficiency may be problematic. Glob Pediatr Heal. 2014;1–4.
33. Kiudelien R, Grini R, Labanauskas L. Prognostic value of reticulocyte hemoglobin content to diagnose iron deficiency in 6–24-month-old children. Med. 2008;44(9):673–7.
34. López-Ruzafa E, Vázquez-López MA, Lendinez-Molinos F, Poveda-González J, Galera-Martínez R, Bonillo-Perales A, et al. Reference values of reticulocyte hemoglobin content and their relation with other indicators of iron status in healthy children. J Pediatr Hematol Oncol. 2016;38(7):e207–12.
35. Mateos E, De-la-cruz J, Lo E. Reticulocyte hemoglobin content for the diagnosis of iron deficiency. J Pediatr Hematol Oncol. 2008;30(7):539–42.
36. Thomas L, Franck S, Linssen J, Thome M. Reticulocyte hemoglobin measurement–comparison of two methods in the diagnosis of iron-restricted erythropoiesis. Clin Chem Lab Med. 2005;43(11):1193–202.