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Original Article

Reticulocyte hemoglobin equivalent for diagnosing iron deficiency anemia in children

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Abstract

Background The prevalence of iron deficiency anemia (IDA) remains high in Indonesian children. When anemia is detected in a patient, the physician's task is to identify the cause, address it, provide iron therapy, and prevent recurrence. However, prevention is best done by early detection. The reticulocyte hemoglobin equivalent (Ret-He) is a direct measurement of iron level in reticulocytes recently produced in the bone marrow. The Ret-He measurement may be an early indicator of iron deficiency, as it is sensitive at the initial stage of the condition.

Objective To assess for a relationship between Ret-He and IDA as well as to evaluate the usefulness of Ret-He for diagnosing IDA in children.

Methods This analytic, observational study with cross-sectional approach included 50 children aged 6-12 years and was performed from November 2013 to March 2014. The subjects were divided into IDA or non-IDA groups, based on ferritin levels. A correlation analysis using logistic regression was performed and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and odds ratio (OR) were calculated. Results were considered to be statistically significant for P values <0.05.

Results A low Ret-He level was significantly associated with IDA in children (P=0.005). The Ret-He cut-off point of 27.8 pg/L had sensitivity of 43.8%, specificity 85.3%, PPV 58.3%, and NPV 76.3%, with OR 4.5 (95%CI 1.1 to 17.7).

Conclusion We find a significant positive relationship between Haemoglobin (Hb) and Ret-He in children, A low level of Ret-He is associated with greater risk of IDA in children. The Ret-He has a high specificity. As such, Ret-He may be useful as a screening tool for early detection of IDA in children. **[Paediatr Indones. 2016;56:90-4.]**.

Keywords: *iron deficiency anemia, reticulocyte hemoglobin equivalent, diagnosis*

Iron deficiency is one of the most common micronutrient deficiencies in the world, especially in developing countries where half the children/cases of IDA reside. In Indonesia, anemia was found in 40.5% of children under five, 47.2% of school-aged children, 57.1% of girls, and 50.9% of pregnant mothers.[1] Iron deficiency anemia has long-term effects on psychomotor and cognitive development and possibly irreversible declines in immunity to infection. In addition, even if the iron deficiency has not reached the stage of anemia, it may have a permanent impact on neurocognitive development. Iron deficiency anemia (IDA) is associated with changes in neurotransmitter receptors and the signal delivery process of the nervous system.[2,3] Primary prevention of iron deficiency can be achieved by eating a varied diet or a diet containing enough iron. Secondary prevention includes screening programs and early treatment of iron deficiency.[4] Iron deficiency can be diagnosed by evaluations of hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell distribution width (RDW), total iron binding capacity

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(TIBC), zinc protoporphyrin, serum ferritin, serum transferrin receptor (TFR), as well as bone marrow biopsy. However, these tests have their own limitations. Hemoglobin can be low in prolonged iron deficiency and influenced by health status; ferritin levels can be elevated in a variety of circumstances; examination of erythrocyte indices (MCV, MCHC, and RDW) assess the availability of iron in mature red blood cells, so they are slow and insensitive as early indicators; zinc protoporphyrin and serum transferrin receptor (TFR) examination fees are often prohibitively expensive; and bone marrow biopsy is too highly invasive to be performed as a routine examination.[5-7]

The purpose of this study was to analyze the relationship between Ret-He and IDA and to determine the sensitivity and specificity of Ret-He for diagnosing IDA in children. Reticulocyte hemoglobin equivalent could be a sensitive indicator of the early stages of iron deficiency (iron-restricted erythropoiesis) prior to anemia, and it is a direct measurement of iron content in reticulocytes.[8-10] To our knowledge, there has been little research on Ret-He in children, so we aimed to study reticulocyte hemoglobin levels for the diagnosis of iron deficiency anemia, using ferritin level as a gold standard.

Methods

This analytic, observational study conducted with cross-sectional approach was performed in elementary school children in Minahasa from November 2013 to March 2014. Subjects were included by consecutive sampling. Inclusion criteria were healthy children aged 6-12 years, and whose parents provided informed consent. Exclusion criteria were the presence of infection characterized by high fever within the 3 days prior to sampling, received iron supplements or other drugs within the 1 month prior to the study, had kidney, liver, or any disease that required long-term treatment, bleeding disorders, poor nutrition, or obesity.

Patients underwent history-taking, physical examinations, and laboratory tests. Venous blood sampling (±3 mL) was performed for Ret-He and serum ferritin examinations. A total of 0.5 mL was placed in a microcontainer containing EDTA for routine blood tests, including the *Ret-He Sysmex XE-2100* analyzer. Hemoglobin (Hb) measurement is expressed in g% and Ret-He in pg/L. The rest of the blood was inserted into a glass tube containing no EDTA, allowed to clot for over 2 hours at room temperature, then sent to the laboratory for serum ferritin examination. The ferritin results are expressed in μq/L. Specimens which showed absolute hemolysis or were refrozen were rejected. A diagnosis of iron deficiency anemia was made in subjects whose serum ferritin levels were <12 μq/L.[11,12]

Pearson's correlation and linear regression tests were used to analyze the relationship between Hb and Ret-He, while logistic regression analysis was used to analyze the relationship between Ret-He and IDA, and to get a cut-off point for Ret-He. Calculations of sensitivity, specificity, PPV, NPV, and odds ratios (OR) were done with a 2x2 table. Data were processed using *SPSS version 21* software. Results with P values ≤0.05 were considered to be statistically significant. This study was approved by the Ethics Committee of the University of Sam Ratulangi Medical School, Manado.

Results

During the period of the study, 50 children met the inclusion criteria. Of these, 16 (32%) children had IDA and 34 (68%) children had normal iron status. Characteristics of the study subjects are shown in **Table 1**.

Table 1. Characteristics of the study subjects

	IDA (n=16)	Non-IDA (n=34)
Gender, n		
Male	11	22
Female	5	12
Mean age (SD), years	8.6 (1.6)	10.6 (1.1)
Mean birth weight/height (SD), %	90.1 (3.8)	91.2 (2.5)
Mean Ret- He (SD), pg/L	25.84 (4.87)	29.82 (1.30)
Mean ferritin (SD), μq/L	16.70 (17.51)	51.61 (23.22)

Linear regression analysis revealed a significant positive correlation between Hb levels and Ret-He in children (r = 0.806; P <0.001) (**Figure 1**). In addition, logistic regression analysis revealed that lower levels of Ret-He increased the risk of greater the chance IDA occurence in children (P=0.005) (**Figure 2**), as well as a Ret-He cut-off of 27.8 pg/L. At this cut-off point, sensitivity was 43.8%, specificity 85.3%, PPV 58.3%, and NPV 76.3%. Chi-square test resulted in OR 4.5 (95%CI 1.1 to 17.7), indicating that children with Ret-He $\leq$27.8 pg/L had a 4.5 times greater risk of IDA than children with Ret-He >27.8 pg/L.

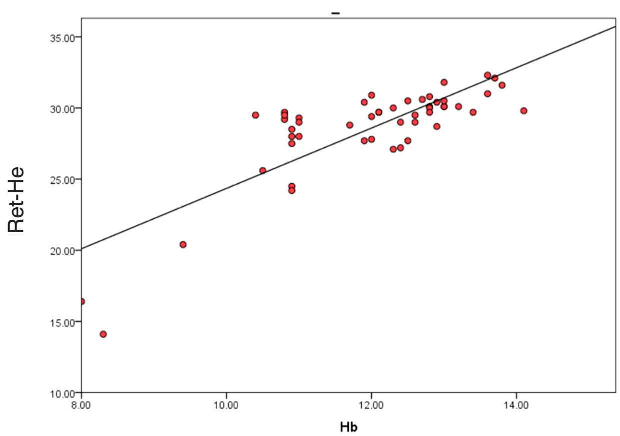

Figure 1. Scatterplot of the relationship between Hb and Ret-He

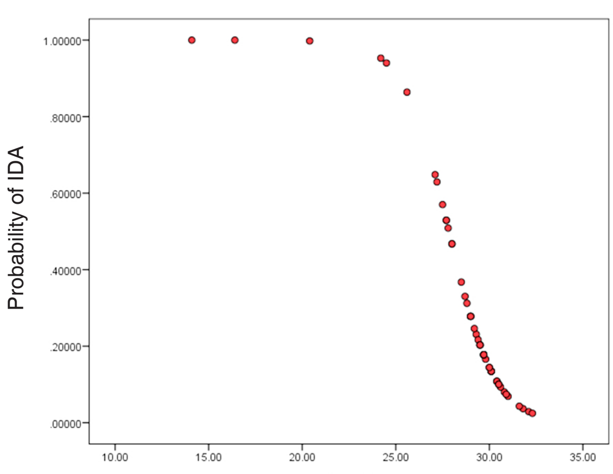

Figure 2. Scatterplot of the relationship between Ret-He and IDA in children

Discussion

The 1995 Indonesian Health Survey reported an IDA prevalence of 40.5% in children under 5 years and 47,2% in school- age children.[13] Furthermore, the Indonesian Pediatrics Society (IDAI) found that the prevalence of anemia was 20-25% in 1,000 school children from 11 provinces.[14] A previous study found that 48% of children aged between 5 and 14 years suffer from IDA.[15] In our 6-12-year-old subject population, we discovered the incidence of IDA to be 32%.

In our study, more boys than girls had IDA [11/16 *vs.* 5/16, respectively], similar to previous studies.[16-18] Iron deficiency anemia is more common in boys at that age (11-12 year old) because they have greater blood volume, muscle mass, and myoglobin than girls.[19] A study in Ghana reported that the most common age group of children with IDA was 6.9 to 11.5 years. At this age, children tended to consume less iron-containing food, because of socioeconomic factors, knowledge, and cultural practices.[20] Previous studies have reported that IDA in school children is mainly due to in adequate iron intake in their daily diet, regardless of their nutritional status.[21,22]

Hemoglobin is a specialized protein in erythrocytes which contains heme prosthetic groups with an iron atom at its center. Hemoglobin is considered to be constant throughout the life span of erythrocytes and reticulocytes, unless there are structural changes that lead to dysfunction and intracellular fragmentation.[8] Examination of reticulocyte hemoglobin content (CHr) in a previous study used the *ADVIA 2120 Hematology Analyzer* (Bayer Diagnostics, Tarrytown, NY, USA) with *Oxazines 750* dye stain for reticulocytes. This technique was used to measure hemoglobin volume and concentration of each reticulocyte, by scattering light from two different angles. The CHr is calculated from the hemoglobin volume and concentration.[23,24] *The Sysmex XE 2100* from Japan is also an automated hematology analyzer but it is used to measure Ret-He, and is not identical to the ADVIA analyzer. Reticulocytes are stained with polymethine dye that specifically binds cytoplasmic RNA/DNA. The reticulocytes then undergo flow cytometry using a semiconductor laser. The flow cytometry data can be immediately and automatically transformed into a Ret-He measurement. [23,24]

Logistic regression analysis revealed a Ret-He cut-off point of ≤ 27.8 pg/L. From Chi-square analysis, we obtained an OR of 4.5 (95%CI 1.1 to 17.7), indicating that children who have ≤27.8 pg/L Ret-He have a 4.5 times higher risk of IDA than those with Ret-He >27.8 pg/L. An adult study reported that a Ret-He cut-off point of 25 pg had sensitivity of 76% and specificity of 81%.[25] Another study in adults with IDA reported that a Ret-He cut-off point of <28 pg had a sensitivity of 65.2% and specificity of 87.6%.[26] A third study showed that a Ret-He cut-off point of 26 pg had a sensitivity of 85% and specificity of 69%.[27] Taken together, these studies illustrate the relationship between low Ret-He and iron deficiency anemia.

In conclusion, higher levels of Hb have a significant association to higher levels of Ret-He, while lower levels of Ret-He increase the risk of IDA. A Ret-He cut-off point of ≤ 27.8 pg/L has 43.8% sensitivity and 85.3% specificity in the diagnosis of IDA in children. The high specificity of Ret-He suggests that it may be useful as a screening tool for IDA in children.

Conflict of interest

None declared.

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