DIAGNOSIS OF IRON DEFICIENCY ANEMIA IN THAI FEMALE ADOLESCENTS USING RETICULOCYTE HEMOGLOBIN EQUIVALENT

Natthawadi Thimthong*, Apichat Photi-a**, Chanchai Traivaree**, Dollapak Apipongrat***, Oytip Nathalang*

*Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand
**Division of Hematology/Oncology, Department of Pediatrics, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand
***Division of Hematology, Department of Medicine, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand

Abstract

Background: Female adolescents aged 10 to 19 years are at remarkable risk of iron deficiency anemia (IDA). Reticulocyte hemoglobin equivalent (Ret-He) is an initial indicator of iron incorporation in red blood cells (RBCs) hemoglobin and reflects the iron functional availability in the RBCs.

Objective: This study aimed to assess the diagnostic performance of Ret-He to identify IDA and determine a specific cut-off value for Thai female adolescents.

Methods: Blood samples of 191 Thai female adolescents, ages ranging from 12 to 18 years, were included. Patients underwent complete blood count, reticulocyte count, Ret-He, serum iron (SI), total iron-binding capacity (TIBC), and transferrin saturation (TSAT). The correlation of Ret-He with other parameters and the diagnostic performance to identify IDA were evaluated.

Results: Among 191 patients, 89 and 102 were defined as IDA and non-IDA groups. Ret-He value in the IDA group was significantly lower than that in the non-IDA group (p<0.001). Strong positive correlations were observed between Ret-He and RBC indices and SI and TSAT (p<0.001). A Ret-He value of ≤27.0 pg could distinguish IDA from non-IDA with a sensitivity of 91.2% and a specificity of 100.0% (area under the curve, AUC of 0.99, 95% CI: 0.98-0.99; p<0.001).

Conclusion: This study confirmed that Ret-He is a cost-effective parameter representing an advantage over other traditional iron markers. A specific Ret-He cut-off value of ≤27.0 pg is suitable for distinguishing IDA from non-IDA with excellent diagnostic performance among Thai female adolescents.

Keywords: Iron deficiency anemia, Reticulocyte, Hemoglobin, Reticulocyte hemoglobin equivalent, Thai female adolescents

https://doi.org/10.55374/jseamed.v6i0.126

Correspondence to:
Nathalang O, Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani 12120, Thailand
Email: oytipntl@hotmail.com

Received: 26 May 2022
Revised: 11 August 2022
Accepted: 14 August 2022
Introduction

Iron deficiency anemia (IDA) is the most common cause of disorders of nutritional anemia, especially among reproductive-age women and people with low iron intake. Untreated iron deficiency (ID) can affect growth and development, especially in children. Because of rapid growth, menstrual blood loss, and inadequate iron intake, female adolescents aged 10 to 19 years are at remarkable risk of iron ID. Patients with IDA may experience effects on cognitive function, audiovisual reaction time, physical capacity, and work performance. In clinical practice, symptoms improve promptly with iron supplementation.

Laboratory testing consisting of hemoglobin (Hb) and mean corpuscular volume (MCV) is used to screen for anemia owing to its widespread availability and ease of interpretation. According to the high prevalence of thalassemia in Thailand and Southeast Asian countries, further testing such as iron study, Hb typing, and molecular studies to identify mutations of globin genes are required to differentiate between IDA and thalassemia.

Conventional biomarkers such as serum ferritin (SF), serum iron (SI), total iron-binding capacity (TIBC), and transferrin saturation (TSAT) are used to define the iron status of clinically anemic individuals. However, those biomarkers are influenced by several factors of diurnal variation and dietary intake.

Reticulocyte hemoglobin equivalent (Ret-He) is a reticulocyte-derived parameter available on Sysmex-XN series analyzers. It constitutes an initial indicator of iron incorporation in red blood cells (RBCs) Hb and reflects the iron functional availability in the RBCs. Related studies have demonstrated the applications of Ret-He in IDA and various clinical settings. Notably, Ret-He provided speedy results to indicate iron status and was not affected by other chronic diseases. Therefore, Ret-He is convenient in the diagnosis and follow-up treatment of IDA among infants, pregnant women, and patients with chronic renal failure.

Recently, the diagnostic performance using Ret-He cut-off values to identify IDA and non-IDA among various Thai populations has been reported. However, the information on Ret-He levels and a specific cut-off value among Thai female adolescents is currently unavailable. This study aimed to assess the diagnostic performance of Ret-He to identify IDA and determine the cut-off value for Thai female adolescent populations.

Methods

This study was approved by the Committee of the Institutional Review Board, Royal Thai Army Medical Department, Bangkok, Thailand (IRBRTA 984/2564), and the Human Research Ethics Committee of Thammasat University (HREC-TUSc) Pathumthani, Thailand (COE No. 018/2564).

The cross-sectional study enrolled female adolescents, ages ranging from 12 to 18 years attending the Division of Hematology/Oncology, Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand, from September 2021 to February 2022, were recruited. The sample size calculation was based on a single proportion formula, this study was based on the prevalence of anemia in Thai female adolescents of 25.2%, with a confidence interval of 95% and a margin of error of 6.5%. The calculated sample size of 191 participants was sufficient to meet the study objective. The subjects were categorized into two groups; IDA and non-IDA. Anemia was defined as a Hb concentration <12.0 g/dL. IDA was defined as serum iron (SI) <50.0 mg/dL and/or TSAT <16.0%. Informed consent forms were signed by the subjects’ parents. Ethylene-diaminetetraacetic acid (EDTA) and clotted blood samples were collected and analyzed at the Laboratory of Hematology, Department of Pathology, Phramongkutklao Hospital, to perform laboratory tests. The laboratory has received International Organization for Standardization (ISO) 15189:2012 and ISO 15190:2003 certifications.

Complete blood count (CBC), reticulocyte count, and Ret-He were analyzed using a Sysmex XN-9000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Serum from clotted blood samples was analyzed for iron markers, including SI and unsaturated iron-binding capacity.
(UIBC) using COBAS INTEGRA Iron Gen.2 reagent (Roche Diagnostics GmbH, Germany) and COBAS INTEGRA UIBC reagent and analyzed by a Cobas 8000 series c502 Chemistry Analyzer (Roche Diagnostics Ltd., Rotkreuz, Switzerland). TIBC was calculated as a sum of SI and UIBC. In addition, a transferrin saturation (TSAT) was calculated using the formula; TSAT (%) = (SI/TIBC) × 100. All blood samples were assessed within two hours after collecting blood. In addition, quality control samples were run daily to ensure adequate functionality of the analyzers.

Statistical analysis
The IBM SPSS software for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism, Version 9 (GraphPad Software, CA, USA) were used for statistical analysis. Demographic characteristics were analyzed using descriptive statistics, and continuous variables were presented as mean and standard deviation (SD). The comparison between the study groups was carried out using the Student’s t-test. The correlation (r) between the Ret-He and other blood parameters was evaluated using Pearson’s correlation coefficient with a 95% confidence interval (95% CI). The diagnostic performance of RET-He to detect IDA was determined, including sensitivity, specificity, predictive values, and the area under the receiver operating characteristic (ROC) curve (AUC), and compared with the reference assays, SI and TSAT. A p<0.05 was considered statistically significant.

Results
The study population included 191 female participants, 89 IDA, and 102 non-IDA patients. No difference was found between the two groups regarding age and body mass index (BMI). The RBC parameters and iron biomarkers included in this study are summarized in Table 1. All red blood cell indices, including Hb, Hct, and RBC indices, except RDW, in the IDA group, were significantly lower than those of the non-IDA group (p<0.001). For iron biomarkers, lower SI and TSAT and higher TIBC were observed in the IDA group compared with those of the non-IDA group (p<0.001). There is no significant difference in the reticulocyte count between the two groups (p>0.05). However, Ret-He was significantly lower than that in the non-IDA group (p<0.001) (Figure 1).

In the IDA group, the baseline Ret-He level was positively correlated with baseline Hb level (r=0.72, p<0.001), MCV level (r=0.84, p<0.001), MCH level (r=0.88, p<0.001) and MCHC level (r=0.45, p<0.001) (Figure 2). In addition, the baseline Ret-He level was correlated with SI level (r=0.74, p<0.001) and TSAT (r=0.71, p<0.001).

The reference iron biomarkers to define IDA, SI <50.0 mg/dL, and TSAT <16.0% and the ROC analysis among female adolescents with Hb levels of less than 12.0 g/dL is shown in Figure 3. The sensitivity and specificity of those biomarkers were calculated (Table 2). By ROC analysis, the optimal Ret-He cut-off for IDA detection was generated using the best combination of sensitivity and specificity. The ROC curve revealed the area under the curve of 0.99 (95% CI: 0.98-0.99; p<0.001) at cut-off ≤27.0 pg (Figure 3), at which IDA was distinguished with a sensitivity of 91.2%, a specificity of 100.0% specificity, a positive predictive value (PPV) of 92.0%, and a negative predictive value (NPV) of 100.0% (Table 2).
### Table 1. Demographics and clinical findings of the patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>IDA (n=89)</th>
<th>Non-IDA (n=102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.83±2.10</td>
<td>15.20±2.32</td>
<td>0.258</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.46±5.70</td>
<td>21.74±5.50</td>
<td>0.373</td>
</tr>
<tr>
<td>RBC (×10⁶/L)</td>
<td>4.51±0.85</td>
<td>4.84±2.70</td>
<td>0.262</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.35±1.94</td>
<td>12.60±1.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>30.71±5.53</td>
<td>38.53±5.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>68.94±8.33</td>
<td>84.71±5.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.11±3.59</td>
<td>27.72±2.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.36±2.18</td>
<td>32.32±3.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>19.23±4.75</td>
<td>16.11±18.60</td>
<td>0.125</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>1.48±2.43</td>
<td>2.06±2.71</td>
<td>0.213</td>
</tr>
<tr>
<td>Ret-He (pg)</td>
<td>21.03±4.19</td>
<td>30.40±3.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SI (µg/dL)</td>
<td>22.86±10.49</td>
<td>78.87±28.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TIBC (µg/mL)</td>
<td>300.24±113.92</td>
<td>78.73±28.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>8.50±4.16</td>
<td>29.10±12.42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; Hb, hemoglobin; HCT, hematocrit; IDA, iron deficiency anemia; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Ret-He, reticulocyte hemoglobin equivalent; SI, serum iron; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

![Figure 1. Differences in the Ret-He level among IDA and non-IDA patients](image-url)
Figure 2. Relationship of Ret-He and Hb, MCV, MCH, MCHC, SI, and TSAT

Figure 3. Receiver operating characteristic (ROC) curve analyses of Ret-He, SI, and TSAT to determine IDA among female adolescents

Table 2. Diagnostic performance of Ret-He, SI and TSAT to determine IDA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-off</th>
<th>AUC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ret-He (pg)</td>
<td>≤27.0</td>
<td>0.99</td>
<td>91.18</td>
<td>100.00</td>
<td>92.00</td>
<td>100.00</td>
</tr>
<tr>
<td>SI (mg/dL)</td>
<td>&lt;50.0</td>
<td>0.98</td>
<td>91.18</td>
<td>96.63</td>
<td>85.00</td>
<td>96.00</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>&lt;16.0</td>
<td>0.99</td>
<td>99.18</td>
<td>100.00</td>
<td>98.87</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; Ret-He, reticulocyte hemoglobin equivalent; SI, serum iron; TSAT, transferrin saturation.
Discussion

Measurement of Ret-He has been implemented to detect changes in iron status. This marker is beneficial in monitoring the response to both iron-replacement therapy and bone marrow response after starting treatment.\(^{(26)}\) Related studies revealed that Ret-He constitutes a capable parameter for detecting the early stages of IDA. The reference intervals for Ret-He have already been determined in different patient populations, ranging from 29.8 pg to 38.2 pg.\(^{(27)}\) The proposed 31.2 pg cut-off value of Ret-He was used to differentiate IDA from non-IDA patients.\(^{(28)}\) In Thailand, the differences in Ret-He among school-aged children were evaluated, and the Ret-He cut-off \(\leq 27.0\) pg was suggested to identify IDA in a thalassemia-prevalent area.\(^{(21)}\) Additionally, the diagnostic performance of the Ret-He has been implemented in different Thai patient populations, and the optimal cut-off \(> 30.0\) pg could signify a non-IDA state.\(^{(22)}\) Because the Ret-He cut-off value varies in study populations, the specific cut-off value should be determined before clinical applications.

A total of 191 female adolescents were included in this study. According to their Hb levels, SI, and TSAT, they were divided into two groups, IDA and non-IDA. Ret-He was significantly lower in the IDA group compared with that of the non-IDA group. A positive correlation between Ret-He and CBC parameters, including Hb levels, MCV, MCH, and MCHC found in the target population, was concordant with related studies.\(^{(21,22,29,30)}\) Notably, Ret-He exhibited a strong positive correlation with MCV and MCH, corresponding to the Hb of the young RBCs entirely released from the bone marrow. Hence, Ret-He provides real-time information on the functional availability of iron for effective erythropoiesis and changes in iron status earlier than the Hb content of mature RBCs. In addition, a strong positive correlation between Ret-He and SI and TSAT was also observed.

Concerning Ret-He diagnostic performance assessment among Thai female adolescents (12 to 18 years), a Ret-He value of 27.0 pg and below could distinguish IDA from non-IDA with a sensitivity of 91.2% and a specificity of 100.0%. This result was supported by a recent study in which a Ret-He cut-off value of 27.0 pg was found to predict IDA with a sensitivity of 91.7% and a specificity of 81.0% among school-aged Thai children.\(^{(21)}\) In addition, the diagnostic performance of Ret-He was comparable to SI and TSAT. Therefore, Ret-He constitutes a practical parameter to diagnose IDA. Presently, the advanced hematology analyzer provides information on reticulocyte (RET) and Ret-He, which allows clinicians to determine the quality and quantity of the young RBCs fraction. The test is fast, inexpensive, and practical to perform within two hours after collecting blood.

Even though SI and SF levels and TSAT were routinely used to assess IDA, in this study, only SI levels and TSAT were used as reference methods because SF levels can alter by other non-physiological changes, such as inflammatory disorders and infections.\(^{(31)}\) The disadvantages of using SI and TSAT included increased patient expenses and required additional blood specimens. Further, the limitation of this study involved using the population at a single center in a high thalassemia-prevalent area. Unfortunately, hemoglobin typing and molecular studies were not performed among all subjects. Also, only 12-18-year-old females were included. Hence, the results cannot be generalized to other populations. A larger population study is recommended. Our results clearly showed that Ret-He constitutes a helpful marker in diagnosing IDA and non-IDA among female adolescents. Further multi-center studies in different geographical regions are suggested.

Conclusion

Ret-He constitutes a parameter included in reticulocyte testing using automated analyzers. Its use is cost-effective and does not require more sample collection, illustrating an advantage over other conventional biomarkers. This study was the first to use a Ret-He cut-off value of \(\leq 27.0\) pg to distinguish IDA from non-IDA with excellent diagnostic sensitivity and specificity among Thai female adolescents.
Disclosures
The authors declared they have no conflicts of interest.

References
19. Wirawan R, Tedja AT, Henrika F, Lydia A. Concordance between reticulocyte hemoglobin equivalent and reticulocyte hemoglobin content in CKD patients undergoing


