ORIGINAL ARTICLE

Reticulocyte Hemoglobin Equivalent (Ret-He) as a Potential Diagnostic Marker of Iron Deficiency Anemia among Bangladeshi Adults

M Morsed Zaman Miah[1], Md Enayet Ali Pramanik[2], M Abdur Rafi[3], Mira Akhter[4]

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Abstract

Anemia involving a variety of etiological sources constitutes a common side effect of long-term liver diseases. Anemia caused by an iron deficiency (IDA) is a prominent kind of anemia among various other types. Blood ferritin levels and other iron-related indicators can be used to identify anemia. On the other hand, it is now possible to quantify reticulocyte hemoglobin equivalent (Ret-He), which indicates the reticulocyte iron concentration. It would be useful to diagnose IDA immediately if Ret-He could evaluate the ID. The effectiveness of Ret-He to diagnose ID in Bangladeshi patients was investigated in an ongoing study. Whole bloodstream numbers, blood ferritin phases, and Ret-He concentrations were measured in a cohort of 215 Bangladeshi people. Hemoglobin (Hb) values less than 12 gm/dL were considered anemia. An individual was classified as iron deficient if their blood ferritin concentration was below 12 ng/mL. Participants were split into four groups for this study: non-ID groups with anemia, IDA, ID, and control groups. In comparison to patients with IDA and ID, the concentrations of Ret-He showed a downward tendency. Serum Ret-He levels were correlated with ferritin levels in the subjects. The measurement of the area around the intercept (AUC) for Ret-He on the ROC curve was 0.906, suggesting a correlation with diagnosis. The study's results provide optimism for the therapeutic use of Ret-He value as an indicator for identification in Bangladeshi patients.

Keywords: Anemia, Iron deficiency anemia, Ret-He, Sysmex.

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Introduction

Generally speaking, anemia represents a common health problem, especially in the developing and resource-constrained countries. It is estimated that there may be about one anemia patient out of four general population (the figure may be around 1.74 billion).[1] The incidence varies significantly according to factors, such as age, geography, and socioeconomic status. Pregnant women and children under five had the greatest prevalence. Nonetheless, women who are not pregnant are the demographic group most impacted.[2] The regions with the largest loads were Western Sub-Saharan Africa, South Asia, and Central Sub-Saharan Africa. In 2019, anemia was the cause of 58.6 million years spent disabled.[1] Anemia incidence of about 40% was observed in Bangladesh, a lower middle-income South Asian nation, across all age and sex categories.[3,4]

Numerous etiologies may cause anemia; they can be distinguished from one another; however, they most often coexist. Iron deficiency, vitamin deficiencies, and hemoglobin abnormalities such as thalassemia rank as the top three contributing causes worldwide.[1] Nonetheless, almost half of all anemia cases globally are caused by iron deficiency anemia (IDA) itself.[5] The primary risk factors for IDA are insufficient dietary iron intake or poor absorption, as well as times of life when iron needs are particularly high, such as growth and pregnancy in women.[1,5]

An accurate diagnosis of the cause of anemia is necessary for the efficacy of a therapy intervention. A variety of additional hematological markers, including the mean volume of cells and hematocrit, plus many indicators of iron metabolism, including ferritin level, total iron-binding capability (TIBC), and circulating iron stage, are necessary for the diagnosis of IDA. Serum ferritin is an extremely reliable screening for identifying iron shortage because it is a widely accessible, standardized evaluation of the overall amount of iron accumulated in the body.[6] But diagnosing IDA with all of these features is not always feasible, especially in countries of low and middle-income with limited resources like Bangladesh.

New hematological markers, such as reticulocyte Hb content (CHr) and reticulocyte hemoglobin equivalent (Ret-He), which are thought to represent reticulocyte iron content and cellular iron availability, are demonstrating encouraging outcomes in the diagnosis of IDA.[7,8] Immature red blood cells called reticulocytes,

[1]Department of Haematology, Rajshahi Medical College, Rajshahi, Bangladesh

[2]On-Farm Research Division, Bangladesh Agricultural Research Institute, Rajshahi, Bangladesh

[3]Department of Medicine, Rajshahi Medical College, Rajshahi, Bangladesh

[4]Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh

Corresponding Author: Md Enayet Ali Pramanik, On-Farm Research Division, Bangladesh Agricultural Research Institute, Rajshahi, Bangladesh, Phone: +01744530588, e-mail: enayet.bari.bd@gmail.com

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which may remain in peripheral circulation for 1–2 days, are a reliable predictor of the iron and hemoglobin content of newly generated red blood cells.[9] Reticulocyte hemoglobin equivalent has shown a strong correlation with anemia and is a valid marker of anemia in a number of populations.[6,8,10–13] However, at this time, there is insufficient data to validate Ret-He's use in identifying iron insufficiency in adult Bangladeshi patients. The current study is to assess Ret-He's usefulness in identifying Bangladeshi persons with low iron levels in their bodies.

Materials and Methods

Study Design and Participants

At Rajshahi Medical College Hospital, a 1000-bed tertiary care center in northern Bangladesh, the cross-sectional research investigation was conducted in the outpatient hematology unit. Assuming the specificity of Ret-He to diagnose IDA as 90% as reported by a previous study,[6] sample size was calculated for 5% marginal error from the following formula: $n_{Sp} = \frac{{z_{\alpha/2}}^2 \times Sp \times (1 - Sp)}{d^2 \times (1 - p)}$,

where Sp = predetermined specificity of the test, p stands for illness prevalence, and d for estimate precision (maximum marginal error).[14] Assuming the prevalence of anemia as 40 parcentages,[4] the formula provided that 230 participants would be adequate sample for evaluating the test. Patients having symptoms and signs (e.g., tiredness, weakness, pallor, etc.) as determined by the doctors and who visited the research site between June and December 2020 were included in the trial. Individuals with anemia brought on by any of the many chronic illnesses, such as hemoglobinopathies, cancer, or chronic renal disorders, were not included.

Collecting a Blood Sample and Measuring Various Characteristics

After each person provided their informed permission, blood was drawn using an automated hematology analyzer (XN-2100® manufactured by Sysmex, Kobe, Japan) to determine total blood counts and Ret-He levels. Each participant's blood specimen was taken in ethylenediaminetetraacetic acid dipotassium salt (EDTA) tubes containing 2–3 mL. By applying a chemical analyzer (H-7700P modular®: Hitachi, Tokyo, Japan), the level of serum ferritin was analyzed.

Definition of Anemia and Iron Deficiency

A hemoglobin measurement of less than 12 gm/dL was considered anemic in the current experiment.[2] The criterion utilized in this experiment to determine an iron shortage state was developed from previous research that shown an incredibly reliable indication of an iron deficit was blood ferritin concentrations below this cut-off point.[15] Based on the levels of ferritin in the serum and the hemoglobin levels, patients were divided into four categories. In the IDA group were patients who had hemoglobin and ferritin in the blood levels of below 12 gm/dL or lower than 12 ng/mL, respectively, indicating anemia and low levels of iron. Iron deficiency patients had inadequate blood ferritin and hemoglobin concentrations (12 ng/mL and 12 gm/dL, respectively), but they did not have anemia. The group of patients who did not have ID-related anemia were those with serum ferritin and hemoglobin levels below 12 ng/mL and 12 gm/dL, respectively, and anemic. Both ID and anemia were not present in the control group, as shown by serum ferritin and Hb values of below 12 ng/mL and fewer than 12 gm/dL, respectively.

Table 1: Sociodemographic characteristics of the participants ($n = 215$)

Characteristics	*Participants number (N)*	*Percentage (%)*
Age		
18–30	78	36.3
31–40	63	29.3
41–50	38	17.7
51–60	21	9.8
>60	15	7.0
Sex		
Female	178	82.8
Male	37	17.2
Religion		
Muslim	195	90.7
Hindu	20	9.3
Residence		
Rural	140	65.1
Urban	75	34.9
Educational status		
None	12	5.6
Primary	79	36.7
Secondary/Higher secondary	106	49.3
University graduate	18	8.4
Family income		
Low	62	28.8
Middle	129	60.0
High	24	11.2
BMI		
Underweight	22	10.2
Normal	153	71.2
Overweight	40	18.6

Statistical Analysis

The statistical software SPSS version 24.0 was used for all computations. To compare among groups, the Kruskal–Walli's test was used since the data were not regularly distributed. To assess the sensitivity and specificity of Ret-He as a diagnostic tool, receiver operating characteristic (ROC) plots and the Pearson correlation coefficient were also used. For each study that was done, a p-value of 0.05 or less was accepted as statistically significant.

Results

Participants' Characteristics

The study had 215 participants in all. Almost two-third of them aged between 18 and 40 years and most of them were female (83%). More than 60% of the participants were from rural areas and almost same portion belonged to middle-income families (Table 1).

Among the participants, 99 (46%) were diagnosed as IDA, 23 (10.7%) as ID without anemia, 69 (32.1%) as anemia without ID and 24 (11.2%) as healthy control (Table 2).

Comparison of Ret-He Levels According to the Iron Status

Hemoglobin equivalent level was 25.10 (9.10) pg on average (IQR) for the individuals. During enrolment, Ret-He levels were shown

Table 2: Laboratory parameters of the participants ($n = 215$)

Parameter	*Total, n = 215*	*IDA, n = 99*	*ID, n = 23*	*Anemia without ID, n = 69*	*Control, n = 24*
Hb					
Mean (SD)	10.95 (2.12)	7.85 (1.83)	12.65 (0.82)	9.09 (1.62)	13.41 (0.49)
Median (IQR)	10.50 (3.30)	7.80 (2.90)	12.90 (0.80)	9.7 (1.90)	13.2 (0.80)
MCV					
Mean (SD)	77.38 (13.25)	64.77 (10.80)	69.66 (17.57)	77.37 (15.22)	86.03 (6.47)
Median (IQR)	68.00 (13.00)	65.00 (11.70)	72.5 (10.00)	76.50 (10.90)	87.15 (9.11)
MCH					
Mean (SD)	25.39 (61.45)	28.59 (90.48)	24.29 (3.76)	21.98 (6.84)	23.02 (2.89)
Median (IQR)	20.60 (5.20)	20.00 (5.20)	22.10 (5.10)	20.90 (6.30)	22.10 (5.24)
MCHC					
Mean (SD)	29.28 (3.70)	28.52 (4.07)	30.44 (3.74)	29.60 (3.40)	31.32 (1.98)
Median (IQR)	30.22 (3.80)	29.00 (3.52)	32.10 (3.10)	30.60 (3.80)	32.80 (4.15)
Ferritin					
Mean (SD)	39.14 (88.09)	4.69 (2.74)	5.90 (3.55)	66.59 (87.85)	134.16 (77.32)
Median (IQR)	10.30 (24.63)	3.98 (4.20)	6.10 (7.40)	28.00 (62.90)	55.00 (79.25)
Ret-He					
Mean (SD)	24.96 (7.45)	19.82 (5.27)	26.84 (2.05)	30.04 (5.56)	29.80 (9.14)
Median (IQR)	25.10 (9.10)	20.00 (7.80)	26.40 (1.50)	29.00 (7.65)	31.80 (9.62)

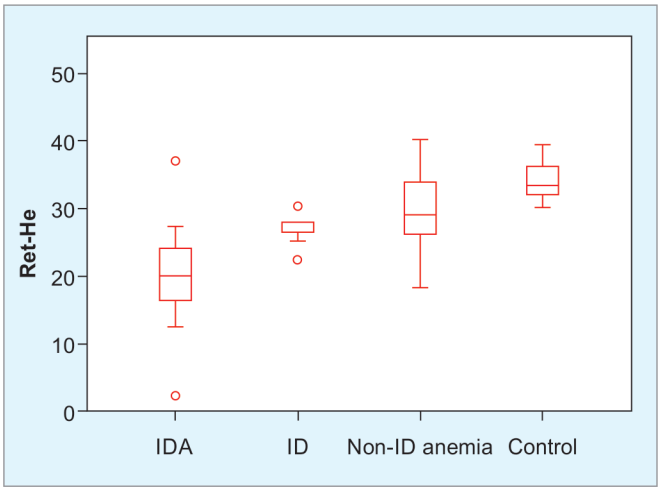


Fig. 1: Reticulocyte hemoglobin equivalent (Ret-He) readings according to iron levels in categories of patients

for four patient groups as shown in Figure 1. According to Table 2, the median (IQR) for the IDA, ID, non-ID with anemia, and control groups, respectively, were 20.00 (7.80), 26.40 (1.50), 29.00 (7.65), and 31.80 (9.62). IDA patients showed considerably lower Ret-He than the control group, which did not have either anemia or ID *p*-value (0.001). Reticulocyte hemoglobin equivalent levels also decreased in accordance with the degree of iron insufficiency (IDA vs ID, *p*-value 0.001; ID vs control, *p*-value 0.001). Reticulocyte hemoglobin equivalent levels in the patients in the IDA and ID groups were considerably lower than those in the non-ID with anemia group *p*-value (0.001) (Fig. 1).

Correlation of Ret-He with Parameter of Iron Deficiency

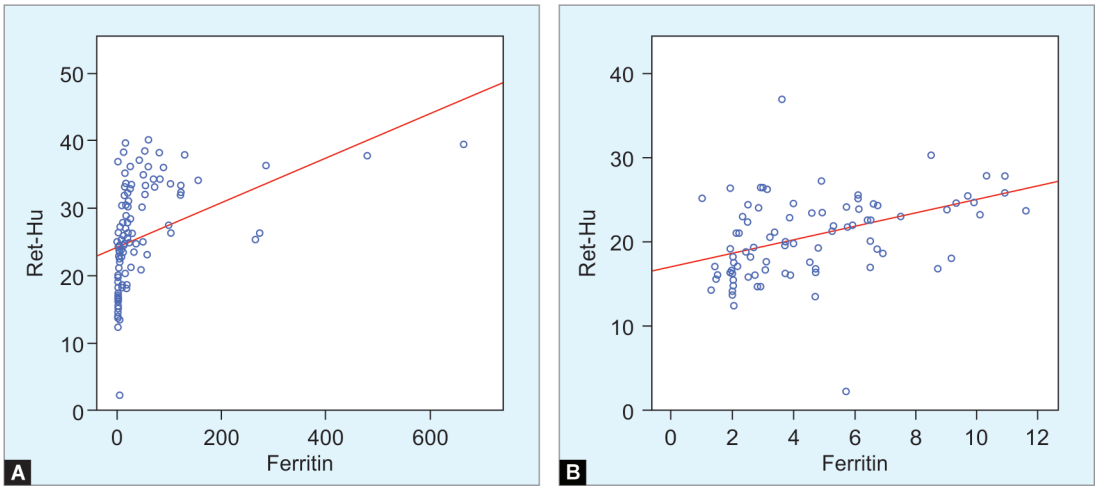
Figure 2 shows the relationship between the amount of ferritin in the blood and Ret-He. Reticulocyte hemoglobin equivalent and ferritin concentration in the blood were shown to have a positively correlated relationship when every participant in the study was compared ($r = 0.351$, *p*-value 0.001). This positive correlation increased when just the patient group with ID was examined ($r = 0.496$, *p*-value 0.001).

ROC Analysis

Receiver operating characteristic analysis was used to test the efficacy of Ret-He in detecting ID (serum hemoglobin 12 ng/mL), as seen in Figure 3. The ROC analysis establishes the test's usefulness by calculating its degree of sensitivity and specificity. When the sensitivity values were checked against the value of 1-specificity, the analysis's findings can be seen as a curve. The test's accuracy is considered acceptable in the event that the AUC, or area under the ROC curve, is almost 1. Reticulocyte hemoglobin equivalent had an AUC of 0.906 for iron insufficiency. We determined Ret-He's sensitivity to diagnose ID with a specificity greater than 96% using a ROC analysis result. The Ret-He threshold value and sensitivity in that calculation were 28.2 pg and 67%, respectively. In order to diagnose ID at the point of diagnosis with both high susceptibilities along with elevated preciseness, we calculated the Ret-He cut-off and found that the cut-off value should be 25.25 pg. This figure would result in 88% of responsiveness and 80% of selectivity.

Discussion

The knowledge about the use of Ret-He as an ID and IDA diagnostic tool by Bangladeshi people is the main goal of this study. Currently, laboratory indicators, such as serum ferritin, transferrin saturation, total iron-binding capacity, and serum iron concentration are used to diagnose IDA. But in places with limited resources, like Bangladesh, these tests need extra equipment, which is neither always possible nor affordable. Reticulocyte hemoglobin equivalent has been apparent as a viable substitute for diagnosing IDA in certain conditions. This test provides a number of benefits, including automated processing, a full blood count without the need for extra reagents, and a quick measurement that takes less than two minutes.[16] Reticulocyte hemoglobin equivalent showed



Figs 2A and B: Correlation between Ret-He and serum ferritin. (A) All patients; (B) Patients with ID

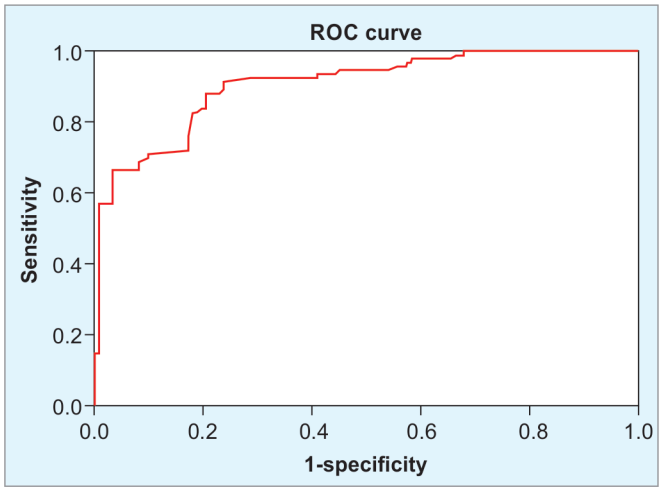


Fig. 3: Receiver operating characteristic (ROC) analysis of Ret-He to diagnose ID (serum ferritin <12 ng/mL)

promising findings as a useful marker of ID for patients with certain conditions (i.e., iron-deficient neonates and children, adult blood donors, elderly patients, pregnant women, and patients undergoing hemodialysis with chronic kidney disease) as well as for the general population.[6,8–13] Additionally, as a significant portion of patients with liver problems also experience anemia, this may be of interest to them.[14] Even though this metric might be used to quantify IDA, very few studies have evaluated it for the population of Bangladesh. Therefore, our goal was to assess Ret-He's utility in Bangladeshi patient diagnosis of IDA.

Serum ferritin is now considered to be the gold standard test for IDA[17] diagnosis. A very precise diagnostic of iron insufficiency was found to have a serum ferritin cut-off value of 12 ng/mL, according to research that rigorously characterized iron deficit based on bone marrow iron reserves.[15] Additionally, this cut-off value[18] was a useful tool in evaluating the sensitivity and specificity of Ret-He to diagnose ID in the Asian population. As a result, 12 ng/mL was established as the serum ferritin cut-off level for the diagnosis of ID in the present experiment.

The results showed that Ret-He was significantly lower in IDA patients than in the control group (*p*-value 0.001), which did not have anemia or ID. The degree of ID was likewise correlated with a reduction in Ret-He levels (*p*-value 0.001 for IDA vs ID and *p*-value 0.001 for ID vs control). Furthermore, compared with the non-ID with anemia group, the patients in the IDA and ID groups had substantially lower Ret-He levels (*p*-value 0.001). These results suggest that Ret-He, which represents patients' iron status, might be a useful diagnostic marker for IDA. But there was a notable variation in Ret-He in the group of individuals without ID and anemia. Numerous anemias, such as myelodysplastic anemia, aplastic anemia, renal insufficiency, and chronic disease anemia, may be present in these people. Reticulocyte hemoglobin equivalent levels may range significantly since anemia can be caused by various illnesses in a multitude of ways. For example, Ret-He stays somewhat normal in aplastic anemia but drastically decreases in anemia brought on by chronic diseases. For the non-ID with anemic group, further study is advised in order to identify the usefulness of Ret-He.

Reticulocyte hemoglobin equivalent is a marker that is often used to evaluate the degree of ID in patients. It was discovered to be connected with many characteristics that were discussed throughout our conversation. Even though we were unable to measure all of these parameters, our results showed that Ret-He level was positively related with serum ferritin level, which is considered the gold standard.

Reticulocyte hemoglobin equivalent has an excellent capacity to identify ID, according to the ROC analysis, with an AUC of 0.906 for ID diagnosis. This outcome is in line with previous studies. In this study population, Ret-He had a cut-off value of 28.2 pg with a 96% specificity and a 67% sensitivity for the diagnosis of ID. This finding is also in line with another research that was done on Japanese individuals, where the cut-off value (28.4 pg) had a specificity of 91% and a sensitivity of 68%. This indicates that the accuracy of the ID diagnosis was quite similar to other iron metabolism indicators.

There are some limits to the research. First and foremost, we included people from hospital settings, which may limit our ability to make population-wide generalizations. In addition, since serum ferritin has been the primary factor used to diagnose IDA, it has not been feasible to compare Ret-He to other iron

metabolism parameters such as iron concentration, total iron-binding capacity, unsaturated iron-binding capacity, transferrin saturation, and soluble transferrin receptor level. Moreover, the relationship between the Ret-He and parameters of concurrent diseases, such as infection indicators (e.g., CRP), could not be taken into consideration.

Conclusion

In the present research, the efficacy of Ret-He as a stand-in biomarker for confirming IDA in patients from Bangladesh was evaluated. As a result of our findings, it was possible to employ this test to identify ID in Bangladeshi patients as a therapeutically meaningful marker. The measurement of Ret-He in anemic patients before other measures like serum iron or ferritin level may therefore be helpful because it is quick, automated, and only needs peripheral blood samples. Also, there should more studies if this marker bear clinical implications in different forms of anemia and in patients with chronic diseases, such chronic liver diseases and their sequela.

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