Bangabandhu Sheikh Mujib Medical University Journal 2024;17(3):e74482 bsmmuj.org
ISSN 2074-2908 | eISSN 2224-7750 | © CC-BY-NC-4.0 | Open access doi: 10.3329/bsmmuj.v17i3.74482

RESEARCH ARTICLE

Reticulocyte haemoglobin content in the differential diagnosis of iron deficiency anaemia and thalassemia traits in pregnancy

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ABSTRACT

Background: Iron deficiency anaemia (IDA) and thalassemia traits are the common conditions of microcytic hypochromic anaemia in pregnant women. However, the laboratory tests to differentiate them are expensive. The reticulocyte haemoglobin (Ret-Hb) test is relatively cheap. This study aimed to assess whether Ret-Hb can differentiate IDA from thalassemia traits in pregnant patients.

Method: This cross-sectional study was conducted in the Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2023 to February 2024. We recruited pregnant women aged 18 to 40 through antenatal visits in the Obstetrics and Gynecology Department. Microcytic hypochromic anaemia was diagnosed by complete blood counts, IDA by iron profile and thalassemia traits by haemoglobin electrophoresis. Ret-Hb was measured using the flow cytometric method. Ninety pregnant women—30 each with IDA, thalassemia traits and healthy individuals—were enrolled.

Result: The mean age was 27 years. The IDA patients had significantly (P<0.001) lower levels of Ret-Hb (mean 18.1, standard deviation 3.2 pg) compared to thalassemia traits (20.8, 2.2 pg) and healthy pregnant women (29.2, 1.9 pg). Using a Ret-Hb cut-off point of 19 pg, the test had 86.7% sensitivity and 53.3% specificity to differentiate thalassemia traits from IDA.

Conclusion: Ret-Hb could be considered a diagnostic test to differentiate thalassemia traits from IDA in clinical settings before expensive confirmatory tests are performed.

Keywords: *microcytic hypochromic anaemia, iron deficiency anaemia, thalassemia traits, reticulocyte haemoglobin content*

INTRODUCTION

Anaemia is a condition in which the number of red blood cells or haemoglobin concentration is lower than normal according to age and sex.[1] It is an important global public health problem that mainly affects young children, menstruating adolescent girls, and pregnant and postpartum women[2] mainly in low-resource populations. Anaemia occurs in pregnancy due to inadequate iron intake, greater fetal demand and hemodilution, and physiological anaemia.[2, 3] Iron deficiency anaemia is associated with several complications, such as early labour, low birth weight, preeclampsia and a higher risk of miscarriage.[4] The World Health Organization estimated, in 2017, that 40% of children aged 6-59 months, 37% of pregnant women, and 30% of women aged 15-49 years worldwide are anaemic.[5] Anaemia affects about 40% of pregnant women in Bangladesh.[6, 7]

The other important cause of anaemia in pregnancy is thalassemia. It must be differentiated from IDA before initiating treatment. The prevalence of thalassemia traits is up to 16% in the Cypriot population, 3-14% in Thai populations, and 3-8% in Indian, Pakistani, Bangladeshi and Chinese populations. However, it's relatively rare in the Black Caribbean and African

Received: 26 June 2024 | **Revised version received:** 19 Aug 2024 | **Accepted:** 10 Sep 2024 | **Published online:** 17 Sep 2024
Handiling Editor: Mohammed Saiful Islam Bhuiyan | **Reviewer C:** Anonymous; **E:** Najnin Akhter
This article encompasses MD thesis of Dr. Nayla Islam

HIGHLIGHTS

1. Few studies have been done on pregnant anaemic patients to differentiate thalassemia traits from iron deficiency anaemia using reticulocyte haemoglobin content in Bangladesh.
2. Reticulocyte haemoglobin level of < 19 pg showed high sensitivity but moderate specificity.
3. Reticulocyte haemoglobin content can differentiate thalassemia traits, but false negativity has to be kept in mind.

populations (0.9%), and northern Europeans (0.1%).[8] In Bangladesh, total carriers of ß-globin gene mutations are 11.9%. Among them, 8.7% are HbE traits, and 2.2% are beta-thalassemia traits.[9] During pregnancy, women with these traits usually present with more severe anaemia, which requires close maternal and fetal surveillance.[10]

The complete blood count can detect anaemia. IDA is confirmed by evaluating the iron profile. Thalassemia traits is diagnosed by Hb electrophoresis.[11] Differential diagnosis of hypochromia is important clinically because treatment and prognosis depend on it. IDA is managed with iron supplementation. Iron overload can occur in the case of thalassemia traits.[4] The confirmatory diagnostic assays for IDA and thalassemia traits are expensive, and the process is complex, which is sometimes difficult for a country with low resources.[12] More cost-effective and reliable screening tests are required.

Reticulocyte haemoglobin (Ret-Hb) content is a relatively new index that can help us understand marrow erythropoietic activity. Therefore, it is useful in evaluating microcytosis and hypochromia.[13] Moreover, it is effective in evaluating a large number of samples.[14] This study aimed to assess whether Ret-Hb can differentiate IDA and thalassemia traits in pregnant women.

METHODS

Study design

This cross-sectional study was conducted from March 2023 to February 2024 in the Department of Laboratory Medicine in collaboration with the Department of Hematology and, Obstetrics and Gynecology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

Sample and sampling of subjects

We estimated the sample size of 30 for each group using the following formula[15]

$$n = \frac{(u+v)^2(\sigma_1^2+\sigma_0^2)}{(\mu_1-\mu_0)^2}$$

Where, μ1 (Mean in group I)=13.8, μ0 (Mean in group II)=11.9[4], σ1 (Standard deviation in group 1)= 2.5, σ0 (Standard deviation in group II)=2.5, u= 1.96 for 1% level of significance, v=0.84 (from Z table) at 80% power of the test. Therefore, we recruited 90 pregnant women (30 for each group).

We examined a total of 100 pregnant women consecutively. We included pregnant patients aged 18 to 40 through antenatal visits to the in- and out-patients. Microcytic hypochromic anaemia was diagnosed by CBC where MCV <80 fL, MCH <27 pg, and Hb <11.5 g/dl. IDA was confirmed by doing an iron profile (iron, ferritin, transferrin saturation, and total iron binding capacity). The thalassemia traits were confirmed by haemoglobin electrophoresis. Patients with IDA comprised 30 in number, and 30 patients with thalassemia traits were included.

Patients taking iron therapy, anaemia from chronic disorders, macrocytic anaemia, hereditary hemolytic anaemia other than thalassemia traits, history of blood transfusion within 30 days, and known cases of haematological malignancy were excluded from the study. Thirty healthy pregnant women were also recruited from the same setting.

Ethical standards of the Helsinki Declaration were maintained. All the participants were informed about the study objective, possible discomfort during venipuncture, and the study's benefits. Finally, written consent was obtained from all of them.

Measurement of Ret-Hb

Six mL blood was collected from the antecubital vein with aseptic precaution and separated into three aliquots: (a). 2 mL was taken into two ethylene diamine tetra-acetic acid tubes for analysis of Hb electrophoresis and CBC, and Ret-Hb, and (b) 2 mL into a plain tube for the iron profile. The tubes were labelled with the participant's identification number. All the laboratory tests were done on the day of blood collection.

The reticulocyte indices were measured by Automated Full Digital Cell Counter (Abbott Alinity HQ, Abbott Laboratories, USA) using flow cytometric methods in the Department of Laboratory Medicine, BSMMU. Complete blood count included estimation of Hb, mean corpuscular volume, mean corpuscular Hb, and red cell distribution width coefficient of variation. The flow cytometry methods used fluorescent staining of reticulocytes. The intensity of fluorescence was proportional to the RNA content of reticulocytes. Young, immature or stress reticulocytes had higher fluorescence than mature reticulocytes. The reticulocyte indices were derived from the reticulocyte scattergram generated by the forward light signals. Ret-Hb was the product of the reticulocyte volume and haemoglobin concentration determined by measuring individual reticulocytes' light scatter.

Statistical analysis

Data were analysed using SPSS version 26. The mean (standard deviation) values were calculated for continuous variables, and frequency (per cent) was presented for the quantitative variables. The ANOVA (or Kruskal–Wallis) test, followed by the Tukey test, was used to compare continuous variables between IDA and thalassemia traits. The chi-square test was used to compare categorical data. Sensitivity and specificity were calculated to examine the performance of Ret-Hb<19 pg to differentiate IDA from thalassemia traits. A receiver operating characteristic curve analysis was performed, plotting sensitivity and 1-specificity values, to determine the area under the curve. P<0.05 was considered statistically significant.

RESULTS

The mean (standard deviation) age of the participants was 27 (4) years, with negligible difference between groups (P=0.89). As shown in **TABLE 1**, Hb, Hb, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, and red cell distribution width coefficient of variation values in patients with IDA and beta traits were significantly different (P<0.05) from the healthy pregnant women. Mean corpuscular Hb concentration (MCHC) was significantly lower in patients with IDA compared to the healthy group (P<0.05). Ret-Hb was significantly (P<0.001) lower in

TABLE 1 **Mean (standard deviation) of haematological indices including reticulocyte haemoglobin in pregnant women with iron deficiency anaemia, thalassemia traits and healthy individuals (n=90)**

Haematological indices	iron deficiency anaemia (n=30)	Thalassemia traits (n=30)	Healthy (n=30)	*P*
Haemoglobin%, gm/dL	9.7 (0.9)[a*]	9.6 (0.7)[b]	11.8 (0.5)	0.000
Mean corpuscular volume, fL	74.1 (6.5)[a]	71.8 (4.9)[b]	87.4 (5.7)	0.000
Mean corpuscular haemoglobin, pg	24.4 (2.5)[a]	23.4 (1.7)[b]	29.4 (2.2)	0.000
Mean corpuscular haemoglobin concentration, gm/dL	32.7 (0.5)[c]	33.1(0.5)	33.3 (1.0)	0.007
Red cell distribution width coefficient of variation, %	16.3 (1.5)[c]	15.6 (4.9)	13.6 (0.7)	0.003
Reticulocyte haemoglobin, pg	18.1 (3.2)[a, d]	20.8 (2.2)[b]	29.2 (1.9)	0.000
Serum iron, µmol/L	8.8 (8.4)[a,d]	16.4 (5.8)	23.7 (2.5)	<0.001
Serum ferritin, ng/mL	13.4 (6.5)[a,d]	237.0 (182.7)	108.2 (35.0)	<0.001
Transferrin, saturation, %	12.6 (17.3)[a,d]	43.9 (83.0)	44.1 (3.3)	0.020
Total iron binding capacity, µmol/L	90.8 (30.5)[a,d]	56.8 (16.5)	54.0 (7.7)	<0.001

A statistically significant difference between a: IDA and healthy; b: thalassemia traits and healthy women; c: IDA and healthy women; d: IDA and thalassemia traits.

TABLE 2 **Sensitivity and specificity of reticulocyte haemoglobin concentration in differentiating thalassemia traits from iron deficiency anaemia (n=60)**

Reticulocyte haemoglobin, pg	Thalassemia traits (n=30)	Iron deficiency anaemia (n=30)	*P*
≥19.0	26 (86.7%) (Sensitivity[a])	14 (46.7%) (False positive)	0.001
<19.0	4 (13.3%) (False negative)	16 (53.3%) (Specificity[b])	

a: true positives, b: true negatives

patients of IDA than those of thalassemia traits.

The mean values of serum iron, serum ferritin and transferrin saturation were significantly lower in patients with IDA. The total iron binding capacity on the other hand, was significantly higher compared to those with thalassemia and healthy group (P<0.05).

The distribution of low Ret-Hb (<19 pg) significantly (P<0.001) varied between IDA and thalassemia traits **(TABLE 2)**. Ret-Hb had high (86.7%) sensitivity but moderate (53.3%) specificity for this cut-off point. A receiver operating characteristic curve analysis of all subjects combined provided a moderate area under the curve (0.742), although with degrees of instability for a few cut-off points.

DISCUSSION

Anaemia in pregnancy can have adverse maternal and fetal outcomes.[16] Its early detection and differential diagnosis can prevent complications from anaemia and the treatment itself. We report that the Ret-Hb levels can differentiate IDA from thalassemia traits and healthy pregnant women with reasonable sensitivity and moderate specificity. Reticulocytes are immature, non-nucleated erythrocytes that contain ribosomal RNA residues. They mature three days after being produced in the bone marrow, while in circulation, maturity occurs after one day of release.[17]

Thalassemia includes α and β thalassemia traits caused by the defective synthesis of α and β chains in haemoglobin, respectively.[18] Thalassemia is classified as major, intermedia, minor, or traits. The classification is done according to the impaired globin chain synthesis, resulting in variable phenotypes ranging from severe anaemia to clinically asymptomatic individuals.[19]

Our findings are consistent with those of Düzenli Kar *et al.*,[9] who found that Ret-Hb was significantly lower in patients with IDA than those with thalassemia traits. The Ret-Hb level is a direct assessment of the incorporation of iron in Hb. In patients with IDA, the reason for low Ret-Hb is reduced iron storage. On the contrary, the iron storage in thalassemia traits remains unaffected.[20]

Lian *et al.*[21] performed a receiver operating characteristics curve analysis for the differential diagnosis of IDA and beta thalassemia traits. They mentioned that when 19.1 pg was considered the Ret-Hb cutoff value, the area under the curve was 0.714 with a specificity of 84.1% and a sensitivity of 68.4%. This is similar to our findings. However, there is a lack of consensus about the cut-off point of Ret-Hb. Many authors used different cut-off points. For instance, Düzenli Kar[4] used a cut-off value <18.2 pg. Their sensitivity (65.3%) and specificity (96.9%) were a little different, but the area under the curve was almost similar (0.765).[4] Padang *et al.*[8] determined the cut-off value for Ret-Hb in differentiating the cases of iron deficiency anaemia with thalassemia traits of 27.8 pg with a sensitivity of 90.5% and a specificity of 71.4%.

Conclusion

We acknowledge the limitation of the findings based on purposively selected subjects. The reticulocyte indices can be used as a preliminary diagnostic tool to diagnose and differentiate IDA from thalassemia traits in Bangladeshi pregnant women attending tertiary care hospitals before expensive tests are considered.

Acknowledgments

We thank the authority of the Department of Laboratory Medicine, Department of Hematology, and Department of Obstetrics and Gynecology of BSMMU for cooperating during blood collection and testing. We are also thankful to the study subjects for their cooperation.

Author contributions

Conception and design: NI, DP, RKK. *Acquisition, analysis, and interpretation of data:* DP. *Manuscript drafting and revising it critically:* NI, DP, RKK, TRA, SR. *Approval of the final version of the manuscript:* NI, DP. *Guarantor of accuracy and integrity of the work:* DP.

Funding

We did not receive any external funds for this study

Conflict of interest

We do not have any conflict of interest.

Ethical approval

Before commencing the study, the Institutional Review Board of BSMMU approved this project proposal (memo no. BSMMU/2023/10206) on 31/07/2023.

Data availability statement

We confirm that the data supporting the findings of this study will be shared upon reasonable request.

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