Usefulness of red blood cell flags in diagnosing and differentiating thalassemia trait from iron-deficiency anemia

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Background: Thalassemia trait (THA) is an important differential diagnosis of iron deficiency anemia (IDA). The red cell distribution width (RDW) is usually elevated in IDA, but often is normal in THA.

Objective: This study was conducted to determine the usefulness of red cell flags in differentiating iron deficiency anemia and thalassemia trait.

Methods: Peripheral blood samples from 50 patients suffering iron deficiency anemia and 64 patients suffering thalassemia trait were used to determine red cell flags (RCF) along with complete blood count, red cell distribution width, serum iron and total iron binding capacity, ferritin and hemoglobin electrophoresis. According to the data collected in this study, the first digit of red cell flags (RCF1)=0 was almost three times higher in thalassemic patients compared to those of iron deficiency anemia. Another reverse significant difference was observed in RCF1=2 (63% versus 42% for thalassemia and iron deficiency anemia respectively).

Conclusion: We conclude that RCF findings are sensitive and specific enough to be used as an approach in differentiating iron deficiency anemia from beta-thalassemia trait.

Keywords: Iron deficiency, thalassemia, anemia

Introduction

In iron deficiency (ID), a normocytic normochromic anemia along with anisocytosis precedes the development of anisochromia, hypochromia and microcytosis. Using the most automated full blood counters, the earliest evidence of iron deficiency is an increase in the red cell distribution width (RDW). This is an indicative of anisocytosis which precedes anemia.¹ Thalassemia trait (THA) is considered to be an important differential diagnosis of iron deficiency anemia (IDA). The RDW is usually elevated in ID, but often is normal in THA.²

Red cell distribution width could be measured automatically by BAYER H3 instrument. Red cell flags (RCF) is another parameter which may be observed in the BAYER H3 reports. It consists of four digits corresponding with the range of RDW, microcytosis, hematocrit distribution width (HDW) and hypochromia respectively. The first digit of RCF may simply to be taken as an indicative of RDW. The purpose of this study was to determine the usefulness of RCF in differentiating between IDA and THA.

Methods and patients

Fifty patients with IDA and 64 patients with THA were enrolled in this study during 2004–2006. The complete blood count (CBC) was measured by Bayer H3 in the first hour of blood sampling. Another
sample was taken for electrophoresis of hemoglobin, and determining iron, total iron binding capacity and serum ferritin level. The levels of hemoglobin (Hb) mean cell volume (MCV), RDW, percentage of microcytes, hypochromic cells and RCF was also measured in all subjects. Measurement of serum iron was performed using RA1000 autoanalyser (Random Access, Technicon, USA) Pars Azemoon kit (Iran) and 60 μl serum taken from 3 ml of fasting blood. Iron was dialyzed and the reduced iron reacted with a chromogen to produce an iron–chromogen complex resulting in a peak of optical density at 595 nm. Using magnesium carbonate precipitating method, total iron binding capacity (TIBC) was measured by RA1000 autoanalyser and Darman Kaveh kit (Iran) and serum taken from fasting blood. Iron was added to the serum to saturation followed with removal of unbound iron through iron absorbent (magnesium carbonate). Total transferrin bound iron was measured by ‘TIBC*mg/dl=iron (mg/dl)*3’.

Ferritin was measured through chemiluminescence’s assay using autoanalyser, Liaison kit (Italy) and 10 μl of serum taken from fasting blood. This method is a two-site immunoluminometric assay (sandwich principle) in which monoclonal antibodies are used for coating both the solid phase (magnetic particles) and the tracer. Applying low pressure cation exchange chromatography along with gradient elution to separate human hemoglobin subtypes and variants from haemolysed whole blood, electrophoresis of hemoglobin was conducted by HbGold Analyzer (Drew Company, England) using 10 μl of whole blood in one ml of distilled water. Patients with serum ferritin level less than 15 ng/ml or transferrin saturation less than 15% were diagnosed as IDA and patients who had hemoglobin A2 more than 3.5% on hemoglobin electrophoreses were diagnosed as THA. The first digit of RCF (RCF1) was considered as an indicator of RDW in this study. Two groups were compared in terms of RCF using 95% confidence intervals. No patient in this study had THA and IDA simultaneously. In the patients with IDA, α-thalassemia could not be excluded, however all IDA patients had rise in hemoglobin level and had normal or large sized new red cells.

Results
In this study, 64 patients having diagnosed with thalassemia were compared to 50 patients suffering iron deficiency anemia. The mean age (standard deviation) was 29.7 years (16.1) and 37.5 years (15.3) for thalassemic and IDA patients respectively. Fifty-five per cent of thalassemic subjects were male while the same figure for IDA patients was 30%. This study detects the significance of first digit of RCF (RCF1) in differentiating IDA and THA.

Table 1 and Fig. 1 present the frequency distribution of RCF in thalassemia compared to iron-deficiency anemia patients. The RCF1=0 was almost three times higher in thalassemia patients (64.1%, CI 95%: 52.1–75.8) compared to iron deficiency anemia (20%, CI 95%: 8.9–31.1). Another reverse significant difference was also observed in RCF1=2 (6.3%, CI

### Table 1: Frequency distribution of red blood cell flags in thalassemia and iron-deficiency anaemia

| Red blood cell flags | Thalassemia | | | Iron-deficiency anaemia | | |
|----------------------|-------------|-------------------------------------------------|-----------------|-------------------------|-------------------------------------------------|
|                      | (#64)       | % 95% CI                                        | (#50)          | % 95% CI                | Significance*                                    |
| First digit          |             |                                                 |                |                         |                                                 |
| 0                    | 41          | 64.1 (52.1–75.8)                                | 10             | 20 (8.9–31.1)           | s.                                               |
| 1                    | 17          | 26 (15.7–37.4)                                  | 16             | 32 (19.1–44.9)          | Ns.                                              |
| 2                    | 4           | 6 (0.3–12.2)                                    | 21             | 42 (28.3–55.7)          | s.                                               |
| 3                    | 2           | 3 (–1.1–7.4)                                    | 3              | 6 (–0.5, 12.6)          | Ns.                                              |
| Second digit         |             |                                                 |                |                         |                                                 |
| 0                    | 0           | 0                                              | 1              | 2 (–1.5–9)              | Ns.                                              |
| 3                    | 1           | 1.5 (–1.4–6)                                    | 5              | 10 (1.7–18.3)           | Ns.                                              |
| 6                    | 63          | 98.4 (95.3–99.9)                                | 44             | 88 (78.9–97)            | Ns.                                              |
| Third digit          |             |                                                 |                |                         |                                                 |
| 0                    | 36          | 56.3 (44.6–68.4)                                | 34             | 68 (55.1–80.9)          | Ns.                                              |
| 1                    | 25          | 39.1 (27.1–51)                                  | 13             | 26 (13.8–38.2)          | Ns.                                              |
| 2                    | 1           | 1.5 (–1.4)                                      | 3              | 6 (0.125)               | Ns.                                              |
| 3                    | 2           | 3 (–1–7.4)                                      | 0              | 0                      | Ns.                                              |
| Fourth digit         |             |                                                 |                |                         |                                                 |
| 3                    | 8           | 12.5 (4.4–20.6)                                 | 2              | 4 (–1.4–9.4)            | Ns.                                              |
| 6                    | 56          | 87.5 (79.4–95.6)                                | 48             | 96 (90.5–99.9)          | Ns.                                              |

*s.: significant; Ns.: not significant.
95%: 0.3–12.2 versus 42%, CI 95%: 28.3–55.7 for thalassemia and iron deficiency anemia respectively. The difference between two groups of patients in terms of RCF1 = 1 was not significant.

Discussion
This study demonstrates relation of RDW and RCF findings with IDA and THA. Several studies have been conducted to differentiate IDA and THA using automated cell counters1–10 and some formula has been developed for this purpose.11–15 Bayer Technicon instrument has been utilized in several studies.16–21 Other works have been focused on using red cell indices and RDW.22–25 Red cell flag is a number consisting of four digits and is routinely measured by H3 instrument. Normal findings are represented by ‘(0000)’, where the first digit denotes RDW ranging from 0 to 3 while 0 stands for RDW < 16.0, 1 for 16.0 < RDW < 18.0, 2 for 18.0 < RDW < 22 and 3 for 22.0 < RDW. Second digit denotes percentages of microcytes and macrocytes ranging between 0 and 8. The third digit denotes HDW ranging from 0 to 3 and the forth digit denotes percentages of hypochromia and hyperchromia of red cells also ranging between 0 and 8. (H3 guideline) Therefore, we can use the first digit of RCF (RCF1) as RDW. Even though, the analysis of red blood cell volume distribution, not accurate enough to be confident in diagnosis, it appears to be a useful method to initially screen the patients with microcytosis and to determine the additional test.23 We used RCF1 as a tool for this purpose. Red cell distribution width has also been used for differentiation of hypochromic microcytic anemia. According to Romero26 and coworkers, RDW is a more sensitive indicator than MCV in establishing the possible origin of microcytic hypochromic anemia and both should be used along in early diagnosis. We used RCF for differentiation of two microcytic states. It is determined readily and is significant in THA diagnosis. Red cell distribution width is elevated in some anemic THA patients, and we have observed cases with high levels of RDW (RCF1 = 1 or 2), while it is usually normal and RCF1 = 0. In contrast to THA, IDA has high RDW level (high RCF1). We have also observed many IDA cases with RCF1 = 2 which are significant in IDA diagnosis.

Red cell distribution width index, obtained by Coulter Counter S Plus IV, in normal subjects and in patients with beta-thalassaemia trait and iron deficiency anemia in order to make a differential diagnosis between beta-thalassaemia trait and iron deficiency anemia. The correct beta-thalassaemia trait diagnoses were 94.4%, while the same figure was 86.2% for iron deficiency anemia using red cell distribution index.27

There are some laboratory tests proposed and reported for the differentiation of beta thalassemia from iron deficiency, including decision functions based on red blood cells indices generated by electronic cell counters. These screening methods appeared to be useful techniques in the initial screening of patients with microcytosis.28 Youden’s index is the most reliable method to measure the validity of a particular technique, because it takes into accounts both the sensitivity and specificity.29 In this report, eight discrimination indices including Mentzer Index, England and Fraser Index, Srivastava Index, Green and King Index, Shine and Lal Index, red blood cell (RBC) count, red blood cell distribution width and red blood cell blood distribution width index (RDWI) were determined. None of the discrimination indices showed a sensitivity and specificity of 100%. Youden’s indices of RBC count
and RDWI were the highest with the value of 82 and 80% respectively. Ninety per cent and 92% of the patients were correctly identified with RBC and RDWI, respectively. Red cell flags findings are suitable tool for differentiating IDA from THA, another capability achieved by the H3 instruments.

**Conclusion**

We conclude that RCF findings are sensitive and specific enough to be used in differentiating iron deficiency anaemia from beta-thalassemia trait. Further studies should be conducted for detecting its role in screening microcytic anaemias.

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**References**


