Comparative behavior of red blood cells indices in iron deficiency anemia and β-thalassemia trait

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Abstract:
BACKGROUND: β-thalassemia trait (β-TT) is an important differential diagnosis of iron deficiency anemia (IDA). It is important to distinguish between the above conditions to avoid unnecessary iron therapy. IDA and β-TT are the two most common causes of microcytic hypochromic anemia. Red blood cells (RBCs) indices are a simple, easy, and cost-effective method to get a primary and valuable information regarding the diagnosis of IDA and β-TT.

OBJECTIVES: This study was focused on the comparison of RBC indices behavior: hemoglobin (Hb), hematocrit (Hct), RBC count, mean cell volume (MCV), mean cell hemoglobin (MCH), MCH concentration (MCHC), and red cell distribution width (RDW) in IDA and β-TT.

PATIENTS AND METHODS: Fifty subjects with IDA (12 males and 38 females, age range: 18–65 years) and fifty subjects with β-TT (twenty male and thirty females with age range: 17–66 years) were chosen. Both groups were investigated for RBC indices by automated hematology analyzer.

RESULTS: RBC count, Hb, and Hct were significantly lower with \( P < 0.001 \) in IDA subjects than in β-TT subjects. MCH and MCHC were significantly lower with \( P = 0.01 \) and 0.001, respectively) in IDA subjects than in β-TT subjects. RDW was significantly higher with \( P < 0.001 \) in IDA subjects than in β-TT subjects. There is no significant difference with \( P = 0.2 \) regarding MCV between IDA subjects and β-TT subjects.

CONCLUSION: The study showed that RBC count, Hb, Hct, MCH, and MCHC were significantly lower in IDA subjects than in β-TT subjects, whereas RDW was significantly higher in IDA subjects than in β-TT subjects. There was no significant difference regarding MCV between IDA subjects and β-TT subjects.

Key words:
Iron deficiency, red blood cell indices, β-thalassemia
iron, and ferritin concentration. Electronic cell counters have been used to determine red cells indices as the first indicator of \( \beta \)-TT. The use of indices to detect subjects who have a high probability to reduce unnecessary investigating costs.[10]

The modern hematology laboratory uses the automated blood cells analysis as a rapid, cost-effective and accurate analysis of red cell indices which have an important diagnostic utility. Most of these analyzers measure the red blood cell (RBC) count, the mean cell volume (MCV), and Hb concentration. The other indices such as the hematocrit (Hct), mean cell hemoglobin (MCH), and MCH concentration (MCHC) are derived from the primary measurements.[11]

The MCV is either directly measured by the instrument, or it is calculated by certain formula. The red cell distribution width (RDW) is calculated as standard deviation (SD) of RBC or as a coefficient of variation.[12] In the last four decades, many formulas based on results of indices have been proposed to differentiate between \( \beta \)-TT and IDA.[13] These include Shine and Lab Index, Ehans formula, Sirdah formula.[8]

This study will not focus on the above formula as it is designed to compare the RBC indices of IDA subjects with those of \( \beta \)-TT subjects to have detailed information about the behavior of the RBC indices in the above two different conditions.

**Patient and Methods**

This is a retrospective study which carried out at Hematology Department, Central Public Health Laboratory. From referral cases to Hematology Laboratory for evaluation of anemia and for checkup, fifty subjects who diagnosed as IDA (12 males and 38 females with age range: 18–65 years) and fifty subjects (twenty male and thirty females with age range: 17–66 years) who diagnosed as \( \beta \)-TT were selected.

Both two groups were investigated by automated hematology analyzer (model: MEK-6410K, Nihon series – Japan). The IDA was diagnosed by measuring the serum iron and serum total iron binding capacity concentrations using colorimetric method (Randox – UK). The \( \beta \)-TT was diagnosed by a high performance liquid chromatography technique using Hb Testing System (Variant I, BioRad - USA).

**Statistical analysis**

The Student’s \( t \)-test was used for statistical tests. Results were expressed as the mean ± SD and considered statistically significant when the value of \( P \) < 0.05.

**Results**

As shown in Table 1 the age in both IDA subjects and \( \beta \)-TT was limited to adult population. Tables 2 and 3 show the mean and SD for both IDA and \( \beta \)-thalassemia minor, respectively. Table 4 shows a comparison between IDA and \( \beta \)-thalassemia in regards to RBC count, Hb level, and Hct which is more decrease on the side of iron deficiency. Table 5 compares between MCH and MCHC level in iron deficient subjects and in \( \beta \)-TT subjects in which both indices were slightly higher in \( \beta \)-thalassemia that IDA with a significant \( P \) value. RDW was significantly higher in IDA subjects than in \( \beta \)-TT subjects as shown in Table 6 whereas Table 7 shows that MCV in iron deficient subjects less than that in \( \beta \)-TT subjects.

**Discussion**

As shown in Table 1, the age in both IDA subjects and \( \beta \)-TT was limited to the adult population. This limitation was done as studies revealed that indices generally performed better in adults than in children.[13]

Regarding female predominance in IDA, subjects are shown in Table 1. Studies documented that IDA is more common in adult female.[14] Blood loss is the most common cause of IDA in adults, and the loss is usually from the genital tract in women[17] also it was reported that women are significantly more exposed to IDA than men and IDA has been observed in girls ten times more than boys.[18]

Highest RBC count was observed in \( \beta \)-TT [Table 4]. The RBC count is often in the high to normal range with \( \beta \)-TT.[19,20] This increment is related to the disease pathophysiology as excess globin chain that precipitate in erythroid precursors and circulating RBC leads to a discrete inefficacious erythropoiesis, resulting in increased RBC production trying to compensate for anemia.[20]

Hb and Hct as shown in Table 4 were higher in \( \beta \)-TT subjects. Same results were reported.[21,22] Most patients with \( \beta \)-TT have mild anemia (Hb level is rarely >9.3 g/dl). This may be explained by the mechanism associated with thalassemia minor.[20]

RDW was significantly higher in IDA subjects than in \( \beta \)-TT subjects [Table 6] and that agrees with other studies.[21-24] In \( \beta \)-TT, almost all RBC are microcytic because deficient synthesis of globin chains resulting from thalassemia mutations expresses itself in all of the RBC precursors. Consequently, RDW values are relatively constant.[24] IDA is progressive rather than stable and if the patient suffers from chronic blood loss and have MCV of 75 fl, later on with continuous loss may have MCV of 65 fl. Furthermore, IDA per se leads to abnormal erythropoiesis those

**Table 1: Age and sex distribution in iron deficient subjects and in \( \beta \)-thalassemia trait subjects**

| Group                  | Total number | Age range | Sex |
|------------------------|--------------|-----------|-----|
| Iron deficiency anemia | 50           | 18-65     | 12  | 38 |
| \( \beta \)-thalassemia trait | 50       | 17-66     | 20  | 30 |

**Table 2: Serum iron and total iron binding capacity levels in subjects with iron deficiency anemia**

| Group                  | Total number | Mean±SD (mm/L) | Serum iron | Serum TIBC |
|------------------------|--------------|----------------|------------|------------|
| Iron deficiency anemia | 50           | 3.66±1.53      | 69.06±3.85 |

TIBC = Total iron binding capacity, SD = Standard deviation

**Table 3: Hemoglobin A2 level in subjects with \( \beta \)-thalassemia trait**

| Group                  | Total number | HbA2%, mean±SD |
|------------------------|--------------|-----------------|
| \( \beta \)-thalassemia trait | 50           | 5.09±0.53       |

SD = Standard deviation, HbA2 = Hemoglobin A2
Table 4: Red blood cell count, hemoglobin level and hematocrit in iron deficient subjects and in β-thalassemia trait subjects

| Group                      | Total number | RBC count ×10^12/L | Mean±SD       |
|----------------------------|--------------|-------------------|---------------|
| Iron deficiency anemia     | 50           | 4.11±0.49         | 7.92±1.38     |
| β-thalassemia trait        | 50           | 5.6±0.72          | 11.38±1.5     |
| *P                         |              | <0.001*           | <0.001*       |

*Significant using Student’s t-test for two independent means at 0.05 level of significance. SD = Standard deviation, Hb = Hemoglobin, Hct = Hematocrit, RBC = Red blood cell

Table 5: Mean cell hemoglobin and mean cell hemoglobin concentration level in iron deficient subjects and in β-thalassemia trait subjects

| Group                      | Total number | MCH (pg) | MCHC (g/dl) | Mean±SD       |
|----------------------------|--------------|----------|-------------|---------------|
| Iron deficiency anemia     | 50           | 19.25±2.37 | 30.22±1.33  | 7.92±1.38     |
| β-thalassemia trait        | 50           | 20.56±2.28 | 31.62±0.72  | 5.6±0.72      |
| *P                         |              | 0.01*     | 0.001*      | <0.001*       |

*Significant using Student’s t-test for two independent means at 0.05 level of significance. SD = Standard deviation, RDW = Red cell distribution width

Table 6: Red cell distribution width in iron deficient subjects and in β-thalassemia trait subjects

| Group                      | Total number | RDW %, mean±SD |
|----------------------------|--------------|----------------|
| Iron deficiency anemia     | 50           | 16.74±1.42     |
| β-thalassemia trait        | 50           | 13.6±2.69      |
| *P                         |              | <0.001*        |

*Significant using Student’s t-test for two independent means at 0.05 level of significance. SD = Standard deviation, RDW = Red cell distribution width

Table 7: Mean cell volume in iron deficient subjects and in β-thalassemia trait subjects

| Group                      | Total number | MCV fl, mean±SD |
|----------------------------|--------------|-----------------|
| Iron deficiency anemia     | 50           | 62.36±9.63      |
| β-thalassemia trait        | 50           | 64.26±4.59      |
| *P                         |              | 0.2*            |

*Not significant using Student’s t-test for two independent means at 0.05 level of significance. SD = Standard deviation, MCV = Mean cell volume

Results in increased variation in shape and size: Poikilocytosis and anisocytosis.[23]

MCH and MCHC show conflict results with other studies between agreement[22] and disagreement.[21] The same conflict was found for MCV results between agree[24] and disagree.[21,22] This can be due to the fact that β-thalassemia shows remarkable phenotypic variability and the molecular basis for this diversity is partly understood.[24]

Conclusion

The study showed that RBC count, Hb, Hct, MCH, and MCHC were significantly lower in IDA subjects than in β-TT subjects, whereas RDW was significantly higher in IDA subjects than in β-TT subjects. There was no significant difference regarding MCV between IDA subjects and β-TT subjects.

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Conflicts of interest
There are no conflicts of interest.

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