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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

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

were investigated for RBC indices by automated hematology analyzer.

A nemia is considered a public health problem in developing countries, and it has been estimated that 2 billion people suffer from anemia worldwide. About 75% of one million deaths a year in Africa and South Asia is caused by anemia. The underlying causes of anemia are many, varied and preventable, such as nutritional deficiencies, infections, and hemoglobin (Hb) disorder.^[1]

Anemia may be defined as a reduction in the concentration of Hb which leads to reduced oxygen carriage and delivery that leads to symptoms such as shortness of breath, exertion, tiredness, headache, or angina if anemia is severe of rapid onset and in elderly.^[2,3]

The anemia of iron deficiency (IDA) may occur as a result of an iron-deficient diet, intestinal iron malabsorption, and chronic blood loss due to

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many factors like hemorrhage or hemoglobinuria because of intravascular hemolysis.^[4,5]

Thalassemias are defined as a heterogeneous group of genetic disorders of Hb synthesis due to the reduction of one or more of the globin chains production.^[6] Thalassemia is a growing global public health problem as it was expected that about 900,000 births of clinically significant thalassemia disorders will occur in the next 20 years.^[7] It is estimated that about 1.5% of world population are carriers of the associated genetic mutation.^[8,9]

It is so important to differentiate between IDA and β -thalassemia trait (β -TT) avoid unnecessary iron therapy as an iron treatment is indicated in IDA and contraindicated β -TT.^[10] A definitive differential diagnosis between β -TT and IDA is based on the result of HbA2 percentage, serum

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

iron, and ferritin concentration. Electronic cell counters have been used to determine red cells indices as the first indicator of β -TT. The use of indices to detect subjects who have a high probability to reduce unnecessary investigating costs.^[11]

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.^[12]

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.^[13] In the last four decades, many formulas based on results of indices have been proposed to differentiate between β -TT and IDA.^[14] 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 β -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 β -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 β -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 \pm 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 β -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 β -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 β -TT subjects

as shown in Table 6 whereas Table 7 shows that MCV in iron deficient subjects less than that in β -TT subjects.

Discussion

As shown in Table 1, the age in both IDA subjects and β -TT was limited to the adult population. This limitation was done as studies revealed that indices generally performed better in adults than in children.^[15]

Regarding female predominance in IDA, subjects are shown in Table 1. Studies documented that IDA is more common in adult female.^[16] 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 β -TT [Table 4]. The RBC count is often in the high to normal range with β -TT.^[19-22] 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 β -TT subjects. Same results were reported.^[21,22] Most patients with β -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 β-TT subjects [Table 6] and that agrees with other studies.^[21-24] In β-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 β -thalassemia trait subjects

Group	Total number	Age range	Sex	
			Male	Female
Iron deficiency anemia	50	18-65	12	38
β-thalassemia trait	50	17-66	20	30

Table 2:	Serum ir	on and to	otal iron	binding	capacity
levels in	subjects	with iro	n deficie	ncy aner	nia

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 c	apacity, SD = Stan	dard deviation	

Table 3: Hemoglobin A2 level in subjects with $\beta\text{-thalassemia trait}$

Group	Total number	HbA2%, mean±SD
β-thalassemia trait	50	5.09±0.53
SD - Standard doviation	HhA2 - Homoglobin A2	

SD = Standard deviation, HbA2 = Hemoglobin A2

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

Group	Total number	Mean±SD			
		RBC count ×10 ¹² /L	Hb (g/dl)	Hct %	
Iron deficiency anemia	50	4.11±0.49	7.92±1.38	26.07±3.69	
β -thalassemia trait	50	5.6±0.72	11.38±1.5	35.95±4.75	
<u>P</u>		<0.001*	<0.001*	<0.001*	

Table 4: Red blood cell count, hemoglobin level and hematocrit in iron deficient subjects and in β -thalassemia trait subjects

*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	Mean±SD	
		MCH (pg)	MCHC (g/dl)
Iron deficiency anemia	50	19.25±2.37	30.22±1.33
β-thalassemia trait	50	20.56±2.28	31.62±0.72
P		0.01*	0.001*

*Significant using Student's *t*-test for two independent means at 0.05 level of significance. SD = Standard deviation, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration

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
Р		<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.^[6]

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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 $_{\rm V}$ between IDA subjects and β -11 subjects.

Conflicts of interest

There are no conflicts of interest.

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

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