



ORIGINAL RESEARCH PAPER

Pathology

UTILITY OF BLOOD INDICES AND PARAMETERS FOR DIFFERENTIATING IRON DEFICIENCY ANAEMIA AND β -THALASSEMIA TRAIT BY CALCULATING VARIOUS DISCRIMINANT FUNCTIONS

KEY WORDS: β -Thalassemia trait, Iron deficiency anaemia, Red Cell Distribution Width Index (RDWI), Mentzer Index, Srivasta index, Green and king Index.

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ABSTRACT

The most commonly encountered disorders with microcytic anemia are iron deficiency anemia (IDA) and β -thalassemia trait (BTT). It is important to distinguish between IDA and BTT to avoid unnecessary iron therapy and the development of hemosiderosis. Various parameters obtained on automated haematology analyser by calculating discriminant functions have been helpful to differentiate iron deficiency anemia and β -thalassemia trait. **Materials and Methods:** This study was carried out in the pathology department of Gujarat Adani Institute Of Medical Science. We had studied 100 cases of differentiating Iron deficiency anaemia and β -Thalassaemia trait from patient's case records and laboratory reports irrespective of age and sex over a period of one year (January 2020 to December 2020). Tests for serum iron and ferritin were carried out in individuals showing low hemoglobin (Hb). All the selected subjects' samples were subjected to blood morphology, comparison of mean cell volume (MCV), RBC count, red cell distribution width (RDW), Red cell distribution width Index (RDWI), Srivastav Index (SI), Green and king Index (GI) and Mentzer's Index (MI). **Results:** Out of 100 cases seventy five were diagnosed having iron deficiency anaemia, whereas twenty five were having β -thalassemia trait. The RBC count was higher and MCV was much lower in β -thalassemia trait as compared to iron deficiency anaemia. Both groups were subjected to RDW, RDWI, SI, MI and GI. **Conclusion:** Among various discriminant function calculated from different haematological parameters and blood indices, Mentzer index and Srivasta index are more sensitive and specific for differentiating iron deficiency anaemia and β -thalassemia trait.

Introduction

Anemia is defined as a qualitative or quantitative deficiency of hemoglobin, a molecule found inside red blood cells (RBCs). Microcytic anemia is commonly the consequence of iron deficiency anemia (IDA), or thalassemia trait or a combination of these. IDA is a very frequent finding, not only in developing countries due to deficient nutritional status, but also in the western world, where women of childbearing age are often diagnosed with IDA due to intermittent blood loss in combination with insufficient iron intake.¹ Beta thalassemia is one of the most prevalent hemoglobinopathies worldwide particularly in the Mediterranean countries, the middle east and Asia.^{1,2} The differentiation between IDA and β -TT is important because of two main reasons, first because Hb won't improve in β -TT if it is misdiagnosed as IDA and unnecessary iron being prescribed by the attending physician. The second grave reason is that misdiagnosed β -TT as IDA may get married to a β -TT, resulting in homozygous or thalassemia major in the offspring.

Aims & Objective:

1. To differentiate Iron deficiency anemia and β -thalassemia trait by blood indices, red cell distribution width and various discriminant functions.
2. Differentiation of hypochromic microcytic anemia by Hb electrophoresis and biochemical parameters.

MATERIAL & METHODS:

The study was conducted from January 2020 to December 2020 at the pathology department of Gujarat Adani Institute of Medical Science, Bhuj. Venous blood was taken, the CBC and RBC indices were measured by automated cell counter on. The Hb electrophoresis was done. Two additional tests of serum iron and ferritin were carried out in individuals having a hypochromic microcytic picture (hemoglobin < 11 gram/dl and MCV < 80fl). All the selected samples were subjected to blood morphology, comparison of MCV, RBC count, RDW and

RDWI. Cases were diagnosed as IDA and β TT on the basis of two standard tests, Hb electrophoresis and serum iron plus ferritin estimation. Patients with HbA2 more than 3.2% were identified as β TT cases and patients with serum ferritin less than 12 ng/ml were identified as IDA cases.³ The cutoff values of RDW & RDWI for differentiation are shown in (Table-.I)

Inclusion criteria: All indoor and outdoor patients diagnosed as hypochromic microcytic anemia and suspected for B-thalassemia trait and iron deficiency anemia.

Exclusion criteria:

1. Other than iron deficiency anemia and B-thalassemia trait
2. Hemoglobin level more than 11 gm/dl.

Discussion:

Anaemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. Microcytosis and hypochromia are the common presentations of both the disease processes of iron deficiency anemia (IDA) and β -thalassemia trait (β -TT). A correct diagnosis in patients with microcytic anemia is important: it can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes by premarital counseling in high-prevalence areas.^{1,2} The morphological findings in both the IDA and β -TT are at times so close that it is really difficult to differentiate one from the other. Differentiation between β -TT and IDA can be carried out effectively by involving the other tests including serum ferritin and HbA2 level estimation.³ Ideally, one needs detailed peripheral blood picture, Hb A2 estimation, serum iron, Total Iron Binding Capacity (TIBC), serum ferritin and transferrin saturation to differentiate IDA from β TT clearly.⁴ But all these tests are either not available in all clinical set ups, or these are relatively time-consuming and expensive

techniques. A number of studies have revealed that derived red cell indices including Red Cell Distribution Width (RDW) can be very helpful in differentiation of anisocytosis caused by IDA or β TT and a recently added Red Cell Distribution Width Index (RDWI) provide valuable help to the attending physician.^{5,6} RDWI is more advantageous as all the discriminating factors including RBC count, MCV and RDW are incorporated in its formula.⁷ We focused on individuals who were found to be anaemic but were either having features of IDA or β -TT. Thalassemia minor or trait is essentially asymptomatic, but the only finding is mild anaemia unresponsive to medicine. Derived indices like RDW Index, Srivasta Index, Green and King Index and Mentzer Index can be calculated using the automated blood cell counters for differentiation between IDA and β -TT. Many studies have revealed that RDW is the first index to become abnormal in iron deficiency.^{8,9,10} A rather improvised index, RDWI has proven to be a reliable discrimination index in the differentiation of β TT and IDA.⁶

Observations & Results:

Out of 100 cases screened initially by haematology analyser, 75 were of Iron deficiency anaemia and 25 cases were B-thalassemia trait. This distinction into two groups was made on the basis of serum ferritin estimation and HbA2 estimation by HPLC. On comparison of mean Hb, RBC, MCH, MCV and RDW between the BTT and IDA group [Table- I], we found a significant difference between this two groups in all the red cell indices and discriminant functions were calculated from these indices.

The RBC count was found to be higher in patient of β -TT with the mean of $4.85 \times 10^{12} \pm 0.15/L$ as compared to IDA patients in which mean is $3.18 \times 10^{12} \pm 0.02/L$. Mean MCV in IDA was $67.1 \pm 4.8fL$, the corresponding values for β -TT were $58.6 \pm 0.1 fl$. MCH values did not show much difference among both the groups. RDW has been known as a valuable differentiation index against β TT and IDA but in our patients, its results were not conclusive. The mean values of RDW, found in IDA and β TT were $23.37 \pm 0.25\%$ and $21.0 \pm 2.1\%$ respectively. Serum ferritin was remarkably low in patients diagnosed as IDA with mean value of $6.10 \pm 0.2 ng/ml$ whereas its levels were on the higher side in β TT patients with mean value of $32.68 \pm 0 ng/ml$. Findings of our study were comparable with study by TrivediDhara P 67 in 2010 at Jamnagar, Gujarat showed RBC count, RDW and other discriminant functions were helpful for distinguishing between IDA and β -TT.

Table-I : Hematological parameters and biochemical data

HEMATOLOGICAL DATA	NORMAL	IDA (MEANVALUE \pm SD)	BTT (MEANVALUE \pm SD)	Trivedidhara P 2010 ¹³	
				IDA	β -TT
Hemoglobin (Hb)	14-18g/dl	6.18 ± 0.30	8.34 ± 0	9.3	10.4
Red cell count (RBC)	4.7-6.1x10 ¹² /L	3.18 ± 0.02	4.85 ± 0.15	4.3	5.6
Mean corpuscular volume (MCV)	81-99fl	67.1 ± 4.8	58.6 ± 0.1	70.6	63.1
Mean corpuscular hemoglobin (MCH)	27-31pg/cell	15.69 ± 0.25	17.24 ± 0	21.5	18.8
Red cell distribution width (RDW)	11-14%	23.37 ± 0.25	21.0 ± 2.1	17.9	17.1
Serum Iron	35-45gm/dl	33.58 ± 1	83.4 ± 0	30	75

Serum Ferritin	12-300 ng/ml	6.10 ± 0.2	32.68 ± 0	8	40
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Table- II :Discriminate functions obtain from above parameters

Blood Indices	Formulae	IDA (MEANVALUE \pm SD)	BTT (MEANVALUE \pm SD)
Red cell distribution width Index (RDWI)	MCV x RDW/RBC	$639.6 \pm 3.77 (<220)$	$239.82 \pm 70 (>220)$
Mentzer's Index	MCV/RBC	$26.10 \pm 0.32 (<13)$	$11.52 \pm 2.3 (>13)$
Srivastav Index	MCH/RBC	$5.6 \pm 0.04 (<3.8)$	$3.41 \pm 4.3 (>3.8)$
Green and King Index	MCV x MCV x RDW/Hb x 100	213.62 ± 0.18	86.23 ± 41.07

Mentzer Index remained powerful since it had the best youden's index (YI) (66%) with high Sensitivity(76%) and specificity(90%).

Srivasta index is second most reliable after Mentzerindex having sensitivity (72%) and specificity (78.7%) with YI (51%).

Findings of our study were comparable with study by ChaimaAbdelhafidhSahli 2013showed Mentzer Index and Shrivasta index is reliable index for distinguishing between IDA and β -TT.¹⁴

Table-III : Predictive value of 8 evaluated indices at published cut-off value for the differential diagnosis of β -TT and IDA

Indices	Cut-off value	B-TT (n=25)	IDA (n=75)	Se, %	Sp, %	PPV %	NPV %	YI	ChaimaAbdelhafidhSahli 2013 ¹⁴		
									Se%	Sp%	YI
RBC count											
B-TT	>5	14 (tp)	07 (fn)	56%	90%	66%	86%	46%	91%	81%	72%
IDA	<5	11 (fn)	68 (tp)								
MCV											
B-TT	<75	22	55	88%	27%	29%	87%	15%	84%	66%	51%
IDA	>75	03	20								
MCH											
B-TT	<23	24	30	96%	60%	45%	100%	60%	95%	85%	83%
IDA	>23	01	45								
RDW											
B-TT	<14	23	72	92%	04%	24%	60%	4%	97%	35%	32%
IDA	>14	02	03								
RDWI											
B-TT	<220	10	05	70%	93%	7%	82%	33%	70%	87%	57%
IDA	>220	15	69								
MI											
B-TT	<13	19	07	76%	90%	73%	92%	66%	83%	89%	72%
IDA	>13	06	68								
GK											
B-TT	<65	03	02	12%	97.3%	60%	77%	9%	30%	96%	16%
IDA	>65	22	73								
SI											
B-TT	<3.8	18	16	72%	78.7%	53%	89%	51%	88%	100%	88%
IDA	>3.8	07	59								

Summary and Conclusion:

1. In present study, Meanvalue of MCH were decreased in both IDA and β -TT. Mean RBC count in IDA and β -TT were 3.18 ± 0.02 and 4.85 ± 0.15 respectively. So RBC count was higher in β -TT compare to IDA.

2. In our study, Mentzer Index was found to be the most reliable index in differentiating between B -TT and IDA.

3. Serum Ferritin was remarkably low in patients diagnosed as IDA with the mean of 5.02 whereas its levels were on the higher side in β -TT patients.

4. RDW denotes anisocytosis. Its value is increased in IDA, and it is near normal or mildly increased in β -TT. Although RDW is a valuediscrimination index for differentiating β -TT and IDA,¹¹ our results found that RDW is almost equally elevated in both β TT and IDA and YI was found, which would not be a good discriminator of β TT and IDA.^{10,12}

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