

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

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Abstract

Background: There are various causes of hypochromic microcytic anaemia. Among these β -thalassemia trait and iron deficiency anaemia are much more common in developing country like India. Various parameters obtained on automated haematology analyser by calculating discriminant functions to differentiate iron deficiency anaemia and β -thalassemia trait becomes easy and cost effective.

Objective: To differentiate iron deficiency anaemia and β -thalassemia trait by blood indices, red cell distribution width and various discriminant functions.

Materials and Methods: This study was carried out in the pathology department of AMC MET medical college, L.G.Hospital, Maninagar, Ahmedabad. 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 2019 to December 2019). 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 MCV, RBC count, RDW, RDWI, SI, GI and MI.

Results: Out of 100 cases seventy five were diagnosed having IDA, whereas twenty five were having β -TT. The RBC count was higher, and MCV was much lower in β TT as compared to IDA. 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 IDA and β -TT.

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

Introduction

Anemia is defined as a qualitative or quantitative deficiency of hemoglobin, a molecule found inside red blood cells (RBCs). 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.

Microcytosis and hypochromia are the common presentations of both the disease processes of iron deficiency anemia (IDA) and β -thalassemia trait (β -TT). 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 battery of tests including serum ferritin and HbA2 level estimation.¹ Ideally, one needs a battery of tests including 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.² 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.^{3,4,5} RDWI is more advantageous as all the discriminating factors including RBC count, MCV and RDW are incorporated in its formula.⁶ 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. RBC morphology resembles strongly to iron deficiency anemia with few differences i.e. rarely seen nucleated

RBCs, normal electrophoretic mobility and alkali resistance in Hb.¹ Fetal Hb is not increased & the characteristic, and diagnostic rise in HbA2 are often not seen in coexisting iron deficiency.⁷

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.⁸⁻¹⁰ A rather improvised index, RDWI has proven to be a reliable discrimination index in the differentiation of β TT and IDA.⁶ RDWI can be easily calculated as (MCV x RDW /RBC).

Aims & Objective:

1. To study various causes of hypochromic microcytic anemia

2. To differentiate Iron deficiency anemia and β -thalassemia trait by blood indices, red cell distribution width and various discriminant functions.
3. Differentiation of hypochromic microcytic anemia by Hb-electrophoresis and biochemical parameters.
4. Study of RDW as a parameter for differentiation between IDA and β -TT

MATERIAL & METHODS: This prospective cross-sectional study was conducted from January 2019 to December 2019 at the pathology department of AMC MET medical college, L.G.Hospital, Maninagar, Ahmedabad. Venous blood was taken into an EDTA tube, the CBC and RBC indices were measured by automated cell counter (XT 1800i sysmex- 5part) on the same day of collection. The Hb electrophoresis was done on cellulose acetate. Two additional tests of serum iron and ferritin were carried out in individuals having a hypochromic microcytic picture (hemoglobin < 8 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.¹² 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. Non cooperative patients
2. Other than iron deficiency anemia and B-thalassemia trait
3. Hemoglobin level more than 11 gm/dl.

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. Discriminant functions were calculated from these indices.

The Hb characteristics of both the groups i.e. IDA and β TT are shown in (Table-I). 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 ± 4.8 fL, the corresponding values for β -TT were 58.6 ± 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 ± 0.2 ng/ml whereas its levels were on the higher side in β TT patients with mean value of 32.68 ± 0 ng/ml. Though in some the patients having hemoglobin A2 above the cut-off limits, the serum ferritin levels were below the cutoff limit, indicating the coexistence of IDA and β -TT. Findings of our study were comparable with study by Trivedi Dhara P 67 in 2010 at

Jamnagar, Gujarat showed RBC count, RDW and other discriminant functions were helpful for distinguishing between IDA and β -TT.

Concerning the hematologic parameters, MCH is very sensitive (96%) and RBC is more specific (90%) resulting in a good efficiency of discrimination between B-TT and IDA.

Table-I : Hematological parameters and biochemical data

HEMATOLOGICAL DATA	NORMAL	IRON DEFICIENCY ANEMIA (MEANVALUE \pm SD)	BETA THALASSEMIA TRAIT (MEANVALUE \pm SD)	Trivedi dhara P 2010 ⁶⁷	
				IDA	β -TT
Hemoglobin (Hb)	14-18g/dl	6.18 \pm 0.30	8.34 \pm 0	9.3	10.4
Red cell count (RBC)	4.7-6.1x10 ¹² /L	3.18 \pm 0.02	4.85 \pm 0.15	4.3	5.6
Mean corpuscular volume (MCV)	81-99fl	67.1 \pm 4.8	58.6 \pm 0.1	70.6	63.1
Mean corpuscular hemoglobin (MCH)	27-31pg/cell	15.69 \pm 0.25	17.24 \pm 0	21.5	18.8
Red cell distribution width (RDW)	11-14%	23.37 \pm 0.25	21.0 \pm 2.1	17.9	17.1
Serum Iron	35-45gm/dl	33.58 \pm 1	83.4 \pm 0	30	75
Serum Ferritin	12-300 ng/ml	6.10 \pm 0.2	32.68 \pm 0	8	40

Table- II : Discriminate functions obtain from above parameters

Blood Indices	Formulae	IRON DEFICIENCY ANEMIA (MEANVALUE \pm SD)	BETA THALASSEMIA TRAIT (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 ± 0.32 (<13)	11.52 ± 2.3 (>13)
Srivastav Index	MCH/RBC	5.6 ± 0.04 (<3.8)	3.41 ± 4.3 (>3.8)
Green and King Index	MCV x MCV x RDW/Hb x 100	213.62 ± 0.18	86.23 ± 41.07

In the present study, the mean RDW was not statistically significant ($p=0.269$) whereas the derived index i.e. RDWI showed better discriminative effect between β TT and IDA. Sensitivity and specificity of RDWI for detection of β TT was found 70% and 93%. Again for IDA, sensitivity and specificity were found 88.0% and 86.0% respectively. Youden's index (YI) takes into account both sensitivity and specificity and gives an appropriate measure of the validity of a particular technique. (Table-II-III)

Mentzer Index remained powerful since it had the best YI(66%) with high Se(76%) and sp(90%). Our results match

those of an Iranian study where MI at the same cut-off (13) was also the best discriminating indice with YI = 90.1. (Table-IV)

Green and King Index has high specificity (97.3%) and low sensitivity (12%).

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

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

Table-III: Cutoff values of RDW& RDWI for iron deficiency anemia (IDA) and beta-thalassemia trait (β TT).

Red cell indices	β -TT	IDA
RDW (%)	<14	>14
RDWI	<220	>220

RDW=Red Cell Distribution Width,
RDWI=Red Cell Distribution Width Index.

Table-IV : 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	Chaima Abdelhafidh Sahli 2013		
									Se%	Sp%	YI
RBC count B-TT IDA	>5 <5	14 (tp) 11 (fn)	07(fn) 68 (tP)	56%	90%	66%	86%	46%	91%	81%	72%
MCV B-TT IDA	<75 >75	22 03	55 20	88%	27%	29%	87%	15%	84%	66%	51%
MCH B-TT IDA	<23 >23	24 01	30 45	96%	60%	45%	100%	60%	95%	85%	83%
RDW B-TT IDA	<14 >14	23 02	72 03	92%	04%	24%	60%	4%	97%	35%	32%
RDWI B-TT IDA	<220 >220	10 15	05 69	70%	93%	7%	82%	33%	70%	87%	57%
MI B-	<13	19	07	76%	90%	73%	92%	66%	83%	89%	72%

TT IDA	>13	06	68								
GK B- TT IDA	<65 >65	03 22	02 73	12%	97.3%	60%	77%	9%	30%	96%	16%
SI B- TT IDA	<3.8 >3.8	18 07	16 59	72%	78.7%	53%	89%	51%	88%	100%	88%

Summary and Conclusion:

1. In present study, Mean value 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 with high Se(76%), Sp (90%) and YI (66%) in differentiating between B -TT and IDA. Srivasta index was also proved to be among the most reliable indices with high Se (72%), Sp(78.7%) and YI (51%) in differentiating between B -TT and IDA. RDW is among the lowest indices with high Se (92%) and a very low Sp (4%) resulting in a low YI (4%). RDW alone is not sufficient enough to differentiate

between B -TT and IDA and that this index had one of the lowest YI values. SI and MI are the most reliable indices with their new cut-offs, they are < 3.7 , and <12.5 respectively, in B -TT and > 3.7 and >12.5 respectively, in IDA.

3. Serum Ferritin was remarkably low in patients diagnosed as IDA (2.6-9.7 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 valued discrimination 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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