

# Building a Score to Discriminate Between Iron Deficiency Anemia and Beta Thalassemia Trait

## Fatimah Suliman Mohammad Aljebaly<sup>1</sup>

<sup>1</sup>Department of Pathology, College of Medicine, Qassim University, Qassim, Saudi Arabia.

#### **KEYWORDS**

#### **ABSTRACT**

Anemia, Beta, Deficiency, Iron, and Thalassemia Trait Objective: Iron deficiency anemia (IDA) and thalassemia are the most common causes of anemia. Differentiating Beta thalassemia trait ( $\beta$ TT) from IDA can be challenging and often requires sophisticated procedures such as hemoglobin electrophoresis, High Performance Liquid Chromatography (HPLC), genetic and molecular studies, which are time and money consuming. However, many equations are made using the hemoglobin, MCV, RDW, MCH and R.B.Cs count to discriminate between IDA and  $\beta$ TT. An example of these equations are Metzer, Sirdah, Green and king, Shine and Lal, and Ehsani also many other equations are present, but no one equation is currently superior to the others. In our paper, we are aiming to build a scoring system to differentiate between IDA and  $\beta$ TT with higher sensitivity and specificity. Methodology: We used the five equations with high sensitivity and specificity and gave a point of either 1 or zero for each result, either IDA or  $\beta$ TT. If the final score is more than 3, it is most properly IDA, and if less than 3, it could be  $\beta$ TT. Results: We applied this method to 50 patients diagnosed with IDA or  $\beta$ TT and obtained a result with a good confidence interval (9.9 to 21) and high precision. Conclusion: We recommend utilizing this scoring system with the help of Artificial Intelligence (AI) as an easy, cheap, faster, more specific, and more sensitive tool to discriminate between IDA and  $\beta$ TT.

#### 1. Introduction

Iron deficiency anemia (IDA) is the most common cause of anemia (1). Also, Thalassemia is not a rare type of anemia in the Middle East (2). One type of  $\beta$  Thalassemia, known as  $\beta$  Thalassemia trait ( $\beta$ TT), has a blood picture similar in many aspects to Iron deficiency anemia (3). In both types of anemia, we get low hemoglobin levels (Hb), Low volume of Mean Corpuscular Volume (MCV), an increased degree of variation in Red Cell Distribution Width (RDW), and low Mean Corpuscular Hemoglobin (MCH). This makes the differentiation between them a complicated process (4, 5).

Although there is a similarity in the blood picture, the treatment is different, and the prescription of iron for  $\beta$  Thalassemia trait may not be useful or even harmful (6, 7). To differentiate between them, hemoglobin electrophoresis, High-Performance Liquid Chromatography (HPLC), genetic and molecular techniques are used. However, these methods are expensive and time consuming (8, 9, 10). In order to get rapid and easy methods to differentiate between them, many equations and formulae are represented using the R.B.Cs, MCV, MCH, and RDW to discriminate between  $\beta$  Thalassemia trait and Iron deficiency anemia.

The most widely used methods are listed in Table 1:

Table 1:

Name	Equation
Green and king	MCV*MCVX RDW/Hb*100
Shine and Lal	MCV*MCV*MCH/100
Mentzer index	MCV/R.B.Cs
Ehsani	MCV –(10*R.B.Cs)
Sirdah	MCV– R.B.Cs –(3*Hb)
Ricerca	RDW/R.B.Cs
MDHL	(MCH/MCV)*R.B.Cs
England and Fraser	MCV-(5*Hb)-RBCs-3.4
RDWI	MCV*RDW/R.B.Cs



No method has a sensitivity and specificity of 100%, but variable degrees of sensitivity and specificity are present between these methods (5). Thus, this study aims to develop a scoring system, which is expected to have higher sensitivity and specificity.

This score system depends on the sum of the above methods and uses this score to discriminate between  $\beta$ TT and IDA. We are going to use the higher equations for sensitivity and specificity as the base of the scoring system; this score will be applied to 50 cases of IDA and BTT and will be tested to see if the score can be used as a method to discriminate between iron deficiency anemia and beta thalassemia trait.

We are going to use the following equations to build the score:

MCV/R.B.Cs

If > 13 equals 1 in the score system

If< 13 equals 0 in the score system

2. MCV-(5\*Hb) - R.B.Cs - 3.4

If> 0 equals 1 in the score system

If < 0 equals 0 in the score system

3. MCV - (10\*R.B.Cs)

If> 15 equal 1 in the score system

If< 15 equal 0 in the score system

4. MCV - R.B.Cs - (3XHb)

If > 27 equal 1 in the score system

If < 27 equal 0 in the score system

5. MCV\*RDW/R.B.Cs

If> 220 equals 1 in the score system

If < 220 equals 0 in the score system

In the end, if the total score Number > 3, the diagnosis will be IDA, and if the total score < 3, it will be  $\beta$ TT. So, we are going to apply this hypothesis to 30 patients who are diagnosed with IDA and 20 patients who are diagnosed with  $\beta$ TT and see if it is true or not.

## 2. Methodology

This retrospective study was approved by ethical approval from the Qassim University Research Ethics Committee. Given the nature of the study, patient consent was not required, as all data were anonymized and obtained from the electronic medical records from the archive of the hematology lab at King Khalid University Hospital, Riyadh, Saudi Arabia, in a time period between June 1, 2024, to September 1, 2024. We collected and analyzed Data of 50 patients with microcytic anemia (mean age: 11- 41 years), and with no clinical symptoms of acute or chronic inflammation or infectious disease. We selected patients who were definitively diagnosed with either Iron Deficiency Anemia (IDA) or Beta Thalassemia Trait ( $\beta$ TT) based on clinical and laboratory results, including Complete Blood Count (CBC), Serum Iron, Total Iron Binding Capacity (TIBC), and Ferritin levels. Hemoglobin electrophoresis was also performed for the  $\beta$ TT group to confirm the diagnosis.

30 patients of them were diagnosed with IDA. The diagnosis was made after clinical and laboratory tests, including CBC, Serum Iron, TIBC, and Ferritin. Table 2 shows various hematological parameters for Iron Deficiency Anemia (IDA) group. While the criteria of iron deficiency anemia are shown in table 3.



 Table 2: IDA group

	Hb	R.B.Cs	MCV	МСН	RDW
1	6.8	3.65	58	17.2	16.9
2	7.5	3.82	60	19.6	19.3
3	8.8	4.01	64	21.1	18.2
4	7.2	3.71	56	19.4	17.2
5	10.6	4.10	73	25.8	19.4
6	8.7	3.02	63	28.8	18.3
7	7.0	3.78	60	18.5	16.7
8	9.1	3.51	64	25.9	17.0
9	7.7	3.92	59	19.6	18.8
10	9.3	3.92	66	23.7	17.7
11	10.9	4.10	72	26.5	16.6
12	8.9	3.12	67	28.5	19.6
13	7.3	3.45	55	21.1	19.5
14	9.5	3.56	68	26.6	18.1
15	10.0	4.20	71	23.8	17.1
16	7.6	3.32	54	22.8	18.7
17	10.2	4.00	72	25.5	17.3
18	10.7	4.52	74	25.1	17.0
19	9.0	3.32	62	27.1	17.4
20	7.9	3.12	59	25.3	19.1
21	8.7	3.91	63	22.2	17.9
22	9.6	3.78	67	25.3	18.4



23	9.7	3.99	66	24.3	19.1
24	7.1	3.01	57	23.5	19.0
25	10.5	4.32	75	24.3	16.8
26	9.3	3.78	67	24.6	17.9
27	7.4	3.21	58	23.0	19.4
28	10.7	4.12	73	25.9	16.9
29	11.2	4.71	77	23.7	16.0
30	11.3	4.61	76	24.5	14.0

Note: Hb: Hemoglobin (g/dL), R.B.Cs: Red Blood Cell Count (million/µL), MCV: Mean Corpuscular Volume (fL), MCH: Mean Corpuscular Hemoglobin (pg), RDW: Red Cell Distribution Width (%)

Table 3: The criteria of iron deficiency anemia

Test	Normal Range for Men	Normal Range for Women
Serum Iron	75–150 mcg/dL	60–140 mcg/dL
<b>Total Iron-Binding Capacity (TIBC)</b>	250–450 mcg/dL	250–450 mcg/dL
Ferritin	30–300 ng/mL	30–300 ng/mL
Transferrin Saturation	20–50%	20–50%

**Note:** TIBC – Total Iron-Binding Capacity, Serum Iron – Amount of circulating iron in the blood, Ferritin – Protein that stores iron, Transferrin Saturation – Percentage of transferrin bound to iron.

The above table 3 provides normal reference ranges for various iron-related blood tests. These values are used to assess an individual's iron status and help diagnose iron-deficiency anemia. Serum iron: This measures the amount of iron circulating in the blood. (Men=75-150 mcg/dL, Women= 60-140 mcg/dL). Total iron-binding capacity (TIBC): This measures the maximum amount of iron that transferrin, a protein in the blood, can bind to (Normal range: 250-450 mcg/dL). Ferritin: This protein stores iron in the body (Normal range: 30-300 ng/mL). Transferrin saturation: This is the percentage of transferrin that is bound to iron (Normal range: 20-50%).

The other 20 patients were diagnosed with  $\beta$ TT by doing CBC, Iron Profile and Hemoglobin electrophoresis.

The normal value of HBA2 is less than 3.2%, while 3.2% to 3.6% is considered borderline, which warrants further investigations. Values between 3.6% to 7% are considered beta thalassemia carriers (5). Table 4 shows results of the  $\beta$ TT group.

Table 4: *βTT Group* 

Hb R.B		МСН	RDW
--------	--	-----	-----



1	7.0	4.34	52	16.1	19.1
2	10.5	5.30	63	19.1	14.2
3	8.7	4.72	58	18.4	15.7
4	8.1	4.56	56	17.7	18.7
5	7.5	4.23	53	17.7	17.3
6	9.4	4.98	59	18.8	14.2
7	8.2	4.12	57	19.9	15.3
8	9.6	4.87	60	19.7	15.9
9	7.9	4.83	53	16.3	17.0
10	10.6	5.23	62	20.2	13.5
11	9.9	4.89	59	20.2	14.2
12	7.2	3.97	55	18.1	18.3
13	9.0	4.73	58	19.0	16.2
14	8.5	4.75	52	17.8	17.6
15	10.3	5.26	61	19.5	13.7
16	7.9	4.57	54	17.2	18.7
17	7.6	4.14	59	18.3	19.3
18	11.0	5.50	62	20.0	13.6
19	8.6	4.54	51	18.9	17.1
20	9.2	4.97	59	18.5	16.1

 $\label{eq:Note: Mean Corpuscular Volume (fL), MCH: Mean Corpuscular Volume (fL), MCH: Mean Corpuscular Hemoglobin (pg), RDW: Red Cell Distribution Width (%)$ 

We build the scoring system using the previously mentioned 5 formulas, all using the 5 indices (Hb, R.B.Cs, MCV, MCH, and RDW) for evaluation.

# 3. Result and Discussion

In IDA patients, the mean value of Hb was  $9.01\pm4.5$ , the mean value of R.B.Cs was  $3.79\pm1.70$  mean



of MCV was  $65.2 \pm 23.77$ , MCH was  $23 \pm 11.60$ , and RDW  $17.84 \pm 5.6$ . While in  $\beta$ TT, the mean value of Hb was  $8.84 \pm 4.00$ , the mean of MCV  $57.15 \pm 12.00$  and MCH  $18.57 \pm 4.10$  and RDW  $16.29 \pm 5.80$ . Our results show that the mean Hb in IDA is slightly higher than  $\beta$ TT group. Also, other MCV, MCH, and RDW parameters are higher in IDA than in  $\beta$ TT. The only exception is the R.B.Cs count, which is more in  $\beta$ TT than in the IDA group. Tables 5 and 6 summarize the mean  $\pm$  standard deviation of the various hematological parameters obtained from individuals with IDA and  $\beta$ TT.

The data was then used to calculate the 5 ratios outlined in the introduction, and the outputs were recorded accordingly for each patient. A binary distribution system was then used to assign whether a case was an IDA or βTT diagnosis as per each ratio, where cases of IDA were assigned the value of 1 and BTT the value of 0. The results were then used to create a score using the proposed scoring system, and each case was assigned a test outcome. For example, in Patient No.1, where Hb is 6.8, R.B.Cs 3.65, MCV 58, MCH 17.2, and RDW is 16.9. The calculation was done as follows:

- 1- MCV/R.B.Cs= if applied 58/3.65=15, >13 = 1in the score system.
- 2- MCV-(Hb\*5)-R.B.Cs -3.4 = if applied 58-(6.8X5)-3.65-3.4 = 16.9 which is more than zero, so the score is set to 1.
- 3- MCV-(10\*R.B.Cs) = 58-(10\*3.65) = 21.5 so it is more than 15 so the score equals 1.
- 4- MCV-R.B.Cs-(3\*Hb) = 58 3.65 (3\*6.8) = 33.9 which is more than 27 so the score equals 1.
- 5- MCV\*6.9/R.B.Cs= 58\*16.9/3.65 = 268 which is more than 220 so the score equals 1.

After summing all the scores above, the final score is 5. Using the same principle shown in the above example, we applied the 5 equations to the 50 samples and get the result shows in table 7, the 30 patients Whose are diagnosed with IDA get a score more than 3 while in  $\beta$ TT group 15 patients are less than 3. We statistically analyzed the result in the following section.

#### **Data Analysis**

A sample of 50 patients with confirmed cases of IDA or  $\beta$ TT was used in conducting this analysis. The data consists of blood test results, with the descriptive statistics for each sample of the confirmed cases below Tables 5 and 6.

Measure Hb **RBCs** MCV MCH **RDW** 9.01 3.79 23.77 Mean 65.20 17.84 Mode 8.70 3.78 67.00 19.60 16.90 9.05 24.30 17.90 Median 3.80 65.00 4.50 1.70 23.00 Range 11.60 5.60 **Standard Deviation** 2.89 1.40 0.46 6.78 1.27 8.34 Variance 1.95 0.21 46.03 1.60

Table 5: Descriptive statistics of the IDA group

 $Note: Hb-Hemoglobin, R.B.Cs-Red \ Blood \ Cell \ Count, \ MCV-Mean \ Corpuscular \ Volume, \ MCH-Mean \ Corpuscular \ Hemoglobin, \ RDW-Red \ Cell \ Distribution \ Width.$ 



The above table 5 shows descriptive statistics for a group of individuals with Iron Deficiency Anemia (IDA). Hb (Hemoglobin): The average hemoglobin level is 9.01 g/dL, below the normal range, indicating anemia. The standard deviation is 1.40 g/dL, indicating a moderate spread of hemoglobin levels in the group. RBCs (Red Blood Cell Count): The average red blood cell count is 3.79 million/μL, which is also below the normal range, indicating anemia. The standard deviation is 0.46 million/μL, suggesting a relatively small variation in red blood cell counts. MCV (Mean Corpuscular Volume): The average red blood cell size is 65.20 fL, which is below the normal range, indicating microcytosis (small red blood cells). The standard deviation is 6.78 fL, suggesting a moderate variation in red blood cell sizes. MCH (Mean Corpuscular Hemoglobin): The average amount of hemoglobin in each red blood cell is 23.77 pg, which is low. However, when combined low MCH with the low MCV, it indicates a microcytic hypochromic anemia. The standard deviation is 2.89 pg, suggesting a moderate variation in hemoglobin content per red blood cell. RDW (Red Cell Distribution Width): The red blood cell size variation is 17.84%, which is high. This suggests that the anemia is anisocytosis (having a wide variation in cell size). Mode is the most frequent value in the data.8.70 g/dL (Hb), 3.78 million/μL (RBCs), 67.00 fL (MCV), 19.60 pg (MCH), 16.90% (RDW). The median shows the middle value in the data when arranged in order 9.05 g/dL (Hb), 3.80 million/µL (RBCs), 65.00 fL (MCV), 24.30 pg (MCH), 17.90% (RDW). Ranges of the difference between the largest and smallest values.4.50 g/dL (Hb), 1.70 million/μL (RBCs), 23.00 fL (MCV), 11.60 pg (MCH), 5.60% (RDW.) Variance is the square of the standard deviation.1.95 g/dL (Hb), 0.21 million/µL(RBCs), 46.03 fL(MCV), 8.34 pg(MCH), 1.60% (RDW). The descriptive statistics indicate that the individuals in this group have iron deficiency anemia, characterized by low hemoglobin levels, and microcytic hypochromic anemia.

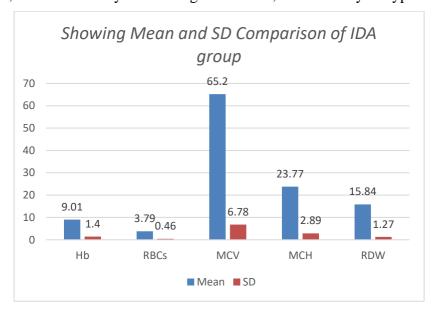


Figure 1: Showing the mean and SD of IDA group

Figure 1 presents a comparative analysis of mean and standard deviation (SD) values for various hematological parameters in an Iron Deficiency Anemia (IDA) group. The parameters assessed include Hemoglobin (Hb), Red Blood Cell count (RBCs), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Red Distribution Width (RDW).

Table 6: Descriptive statistics of βTT group

Measure	Hb	RBCs	MCV	MCH	RDW
Mean	8.84	4.72	57.15	18.57	16.29
Mode	7.90	4.97	59.00	17.70	14.20
Median	8.65	4.74	58.00	18.65	16.15
Range	4.00	1.53	12.00	4.10	5.80
Standard Deviation	1.20	0.42	3.70	1.20	1.96



Variance   1.43   0.18   13.71   1.45   3.84
--

Note: Hb – Hemoglobin, R.B.Cs – Red Blood Cell Count, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Hemoglobin, RDW – Red Cell Distribution Width.

The above table 6 shows descriptive statistics for a group of individuals with Beta thalassemia trait (βTT). Hb (Hemoglobin): The average hemoglobin level is 8.84 g/dL, slightly below the normal range, indicating mild anemia. The standard deviation is 1.20 g/dL, indicating a moderate spread of hemoglobin levels in the group. RBCs (Red Blood Cell Count): The average red blood cell count is 4.72 million/μL, within the normal range. The standard deviation is 0.42 million/μL, suggesting a relatively small variation in red blood cell counts. MCV (Mean Corpuscular Volume): The average red blood cell size is 57.15 fL, below the normal range, indicating microcytosis (small red blood cells). The standard deviation is 3.70 fL, suggesting a moderate variation in red blood cell sizes. MCH (Mean Corpuscular Hemoglobin): The average amount of hemoglobin in each red blood cell is 18.57 pg, which is low. However, when combined low MCH with the low MCV, it indicates a microcytic hypochromic anemia. The standard deviation is 1.20 pg, suggesting a moderate variation in hemoglobin content per red blood cell. RDW (Red Cell Distribution Width): The variation in red blood cell size is 16.29%, which is high. This suggests that the anemia is anisocytosis (having a wide variation in cell size). Mode showing the most frequent value in the data. 7.90 g/dL (Hb), 4.97 million/µL (RBCs), 59.00 fL (MCV), 17.70 pg (MCH), 14.20% (RDW). Median: The middle value in the data when arranged in order.8.65 g/dL (Hb), 4.74 million/μL (RBCs), 58.00 fL (MCV), 18.65 pg (MCH), 16.15% (RDW). Ranges also elaborate the difference between the largest and smallest values.4.00 g/dL (Hb), 1.53 million/μL (RBCs), 12.00 fL (MCV), 4.10 pg (MCH), 5.80% (RDW). Variance of the square of the standard deviation.1.43 g/dL, 0.18 million/µL, 13.71 fL, 1.45 pg, 3.84% (RDW). The descriptive statistics indicate that the individuals in this \( \beta TT \) group have low hemoglobin levels, and microcytic hypochromic anemia

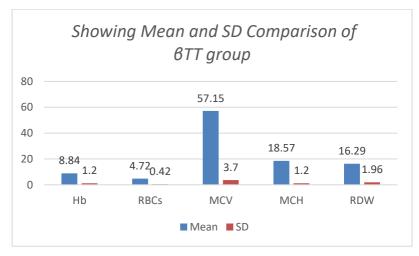


Figure 2: Showing the mean and SD of βTT group

Figure 2 presents a comparative analysis of mean and standard deviation (SD) values for various hematological parameters in Beta thalassemia trait ( $\beta$ TT) group. The parameters assessed include Hemoglobin (Hb), Red Blood Cell count (RBCs), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Red Distribution Width (RDW).

Table 7: 2x2 Contingency table of Actual condition

Tested Actual	Positive	Negative
Positive	30	0



Negative	2	18
----------	---	----

The tested outcomes were then compared to actual conditions. The table 7 above shows a 2x2 contingency table, which compares the actual condition of individuals to the results of a diagnostic test. Actual: This column represents the true condition of the individuals, whether they are positive or negative for the condition being tested. Tested: This column describes the results of the diagnostic test, whether it was positive or negative. 30 individuals who were actually positive for the condition were correctly identified as positive by the test (True Positive). 0 individuals who were actually negative were incorrectly identified as positive by the test (False Negative). 18 individuals who were actually negative were correctly identified as negative by the test (True Negative).

Table 8: F1 Score

Precision	0.94
Recall	1.00
F1	0.97

Note: The p-value for this test was < 0.05, indicating statistical significance.

To validate the results, two tests were conducted: the F1score, and the diagnostic odds ratio (DOR). The F-Score uses the precision (ratio of true positives to all predicted positives) and the recall (ratio of true positives to samples that were meant to be positive). The F1 score represents precision and recall in one metric using the harmonic mean of both measures. As shown in above table (8), the results displayed an F1 score of 0.97, which is on the higher end of precision, as a score of 1.0 indicates perfect precision of the proposed scoring system.

The table 8 presents the results of a diagnostic test, including the F1 score, precision, and recall. These metrics are commonly used to evaluate the performance of classification models. Precision= 0.94 indicates that 94% of the test's positive predictions were correct. In other words, out of all the individuals the test predicted as positive, 94% truly had the condition. Recall 1.00; this indicates that 100% of the individuals who actually had the condition were correctly identified by the test. In other words, the test did not miss any positive cases. F1 Score= 0.97, the F1 score is a harmonic mean of precision and recall, providing a metric that balances both measures. In this case, the F1 score of 0.97 suggests that the test has good overall performance, with high precision and recall. The results suggest that the diagnostic test has high sensitivity (ability to identify positive cases correctly) and specificity (ability to identify negative cases correctly). This is indicated by the high recall and precision values, respectively. The F1 score further confirms the good overall performance of the test.

To support our findings, the diagnostic odds ratio was also calculated. Because there were 0 false negatives, a value of 0.5 was added to all figures in the contingency table 7, the reasoning for which will be discussed further in the limitations of the data.

Table 9: DOR Metrics

True Positive Rate	0.98
False Negative Rate	0.02
True Negative Rate	0.88
False Positive Rate	0.12

Building a Score to Discriminate Between Iron Deficiency Anemia and Beta Thalassemia Trait. SEEJPH 2024 Posted: 11-09-2024

Positive Predictive Value	0.92
False Discovery Rate	0.08
Negative Predictive Value	0.97
False Omission Rate	0.03

Note: Diagnostic Odds Ratio (DOR), the p-value for this test was < 0.05, indicating statistical significance.

The above table 9 metrics were calculated to aid with formulating the DOR. Diagnostic Odds Ratio (DOR) is a metric used to evaluate the performance of diagnostic tests. It is calculated as the ratio of the odds of a positive test result in individuals with the condition to the odds of a positive test result in individuals without it. A high DOR indicates a strong association between the test result and the presence or absence of the condition. Dividing the ratio of true positive to false positive by the ratio of false positive to false negatives yields a DOR of 451, which can be interpreted as the scoring system proposed is effective at 451:1. To test for significance, a 95% confidence interval was calculated which yielded a confidence interval of 9,929 to 21.

Table 9 shows various performance metrics for a diagnostic test. True Positive Rate (TPR) = 0.98; this represents the proportion of individuals who actually have the condition and were correctly identified by the test (sensitivity). A high True Positive Rate (TPR) indicates that the test is good at detecting individuals with the condition. False Negative Rate (FNR) = 0.02; this represents the proportion of individuals who actually have the condition but were incorrectly identified as negative by the test. A low False Negative Rate (FNR) indicates that the test is good at avoiding false negatives. True Negative Rate (TNR) = 0.88, this represents the proportion of individuals who do not have the condition and were correctly identified as negative by the test (specificity). A high TNR indicates that the test is good at avoiding false positives. False Positive Rate (FPR) = 0.12; this represents the proportion of individuals who do not have the condition but were incorrectly identified as positive by the test. A low FPR indicates that the test is good at avoiding false positives.

Positive Predictive Value (PPV) = 0.92, this represents the probability that an individual who tests positive actually has the condition. A high PPV indicates that a positive test result strongly predicts the condition. False Discovery Rate (FDR) = 0.08; this represents the proportion of positive test results that are actually false. A low FDR indicates that the test is good at avoiding false positives. Negative Predictive Value (NPV) = 0.97; this represents the probability that an individual who tests negative does not have the condition. A high NPV indicates that a negative test result is a strong predictor of not having the condition. False Omission Rate (FOR) = 0.03. This represents the proportion of individuals who have the condition but were incorrectly identified as negative by the test. A low FOR indicates that the test is good at avoiding false negatives. These metrics suggest that the diagnostic test performs well regarding sensitivity, specificity, and predictive values. It can accurately identify individuals with and without the condition, with relatively low rates of false positives and negatives.



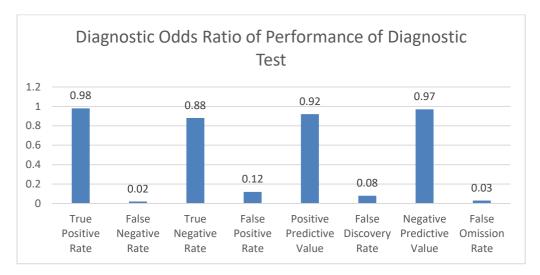


Figure 3: Showing the DOR metrics

The figure 3 presents a comparison of different DORs related to a diagnostic test. The specific values in each cell represent the DOR associated with the corresponding rate. A higher DOR indicates a better diagnostic accuracy. A high DOR for the True Positive Rate suggests that the test is good at correctly identifying individuals with the disease. A low DOR for the False Positive Rate indicates that the test is less likely to incorrectly identify individuals without the disease as positive.

**Table 10:** Results of the score system

Patient	Hb	RBCs	MCV	МСН	RDW	Type	Score
1	6.8	3.65	58	17.2	16.9	IDA	5
2	7.5	3.82	60	19.6	19.3	IDA	5
3	8.8	4.01	64	21.1	18.2	IDA	5
4	7.2	3.71	56	19.4	17.2	IDA	5
5	10.6	4.1	73	25.8	19.4	IDA	5
6	8.7	3.02	63	28.8	18.3	IDA	5
7	7	3.78	60	18.5	16.7	IDA	5
8	9.1	3.51	64	25.9	17	IDA	5
9	7.7	3.92	59	19.6	18.8I	IDA	5
10	9.3	3.92	66	23.7	17.7	IDA	5
11	10.9	4.1	72	26.5	16.6I	IDA	5
12	8.9	3.12	67	28.5	19.6	IDA	5
13	7.3	3.45	55	21.1	19.5	IDA	5
14	9.5	3.56	68	26.6	18.1	IDA	5
15	10	4.2	71	23.8	17.1	IDA	5
16	7.6	3.32	54	22.8	18.7	IDA	5
17	10.2	4	72	25.5	17.3	IDA	5
18	10.7	4.52	74	25.1	17	IDA	5
19	9	3.32	62	27.1	17.4	IDA	5



20	7.9	3.12	59	25.3	19.1	IDA	5
21	8.7	3.91	63	22.2	17.9	IDA	5
22	9.6	3.78	67	25.3	18.4	IDA	5
23	9.7	3.99	66	24.3	19.1	IDA	5
24	7.1	3.01	57	23.5	19.0	IDA	5
25	10.5	4.32	75	24.3	16.8	IDA	5
26	9.3	3.78	67	24.6	17.9	IDA	5
27	7.4	3.21	58	23	19.4	IDA	5
28	10.7	4.12	73	25.9	16.9	IDA	5
29	11.2	4.71	77	23.7	16.0	IDA	5
30	11.3	4.61	76	24.5	14	IDA	5
31	7	4.34	52	16.1	19.1	βТТ	2
32	10.5	5.3	63	19.1	14.2	βТТ	1
33	8.7	4.72	58	18.4	15.7	βТТ	2
34	8.1	4.56	56	17.7	18.7	βΤΤ	3
35	7.5	4.23	53	17.7	17.3	βΤΤ	1
36	9.4	4.97	59	18.8	14.2	βΤΤ	1
37	8.2	4.12	57	19.9	15.3	βΤΤ	4
38	9.6	4.87	60	19.7	15.9	βΤΤ	1
39	7.9	4.83	53	16.3	17	βΤΤ	1
40	10.6	5.23	62	20.2	13.5	βΤΤ	1
41	9.9	4.89	59	20.2	14.2	βΤΤ	1
42	7.2	3.97	55	18.1	18.3	βΤΤ	5
43	9.0	4.73	58	19	16.2	βΤΤ	1
44	8.5	4.75	52	17.8	17.6	βΤΤ	1
45	10.3	5.26	61	19.5	13.7	βΤΤ	1
46	7.9	4.57	54	17.2	18.7	βΤΤ	2
47	7.6	4.14	59	18.3	19.3	βΤΤ	5
48	11.o	5.5	62	20	13.6	βΤΤ	0
49	8.6	4.54	51	18.9	17.1	βΤΤ	1
50	9.2	4.97	59	18.5	16.1	βΤΤ	1

**Note:** Hb refers to hemoglobin (g/dL), RBCs refers to red blood cells (millions/ $\mu$ L), MCV refers to mean corpuscular volume (fL), MCH refers to mean corpuscular hemoglobin (pg), RDW refers to red cell distribution width (%), Type denotes Iron Deficiency Anemia (IDA) or Beta-Thalassemia Trait ( $\beta$ TT), and Score is an assigned severity score.



#### **Limitations**:

The sample size has proved some limitations, mainly due to the lack of false negatives which required an adjustment to the contingency table in order to make the DOR ratio meaningful (24). Further, the confidence interval range can be perceived to be too wide to be representative. However, the calculated DOR ratio after adjustment falls within the confidence interval, and since the DOR ratio has no upper bounds, the result is still acceptable. To support the DOR ratio, the F1 score displayed 97% precision. Future studies can refine the results by incorporating a much larger sample size covering a more diverse demographic.

#### **Discussion:**

IDA and βTT are the most common types of hypochromic microcytic anemia present in the Middle East. The blood picture is so similar that the deferential between them is not easy, and to discriminate between them pass through many investigations, including SerumIon, TIBC, and ferritin levels, also have to measure HBA2 by HPLC or hemoglobin electrophoresis or even use molecular technology (13). All these methods are expensive and time consuming. Throughout history, many scientists have tried to use different equations depending on R.B.Cs, Hb, MCV, MCH, and RDW (14, 15, 16).

No equation was better or more accurate than others; there was variation in the sensitivity and specificity of this equation; this study proposes to make a score to differentiate between IDA and  $\beta$ TT this score depends on the sum of the most sensitive equations made many years ago to discriminate between IDA and  $\beta$ TT (17, 18). If the result of the equation shows that the result is giving the diagnosis of IDA, we give it a score of 1, and if the result of the equation shows that the patient is  $\beta$ TT, we give it a score of 0. Then, we apply this to the five equations, and if the result is three or more, this is a case of IDA; if the result is less than three, it is  $\beta$ TT.

The results for all 30 patients of IDA were four or more. While in the  $\beta$ TT group, one patient got a score of 4, one had a score of 3, and two showed a result of 5. This may be due to the combination of IDA and  $\beta$ TT. However, it is recommended to repeat this score for these four patients after treatment of iron deficiency (19, 20). These results confirm that this method and scoring system show higher sensitivity and specificity than any individual equation, and we recommend using this score to differentiate between IDA and  $\beta$ TT. For future improvements to this system, we hope to use Artificial Intelligence (AI) to make the result of the score fast and easier.

#### 4. Conclusion and future scope

In conclusion, the proposed scoring system in this study provides a site and time-efficient method of discriminating between IDA and  $\beta$ TT based on routinely accessible hematological parameters. The scoring system was shown to have significantly higher sensitivity and specificity than all the models of individual equations, making it a relevant tool for clinicians where these conditions coexist. This approach could help reduce costly and time consuming tests, such as hemoglobin electrophoresis or genetic analysis, during the diagnosis. Further research is needed to verify this score for populations that include significantly more participants of different demographics and ages and further investigate the prospect of using artificial intelligence to improve the accuracy and availability of the necessary diagnostics.

#### **Funding:**

This study is a self-funded by the author.

#### **Conflicts of Interests:**

There are no conflicts of interests



#### Reference

- [1] Rathod, D. A., Kaur, A., Patel, V., et al. (2007). Usefulness of cell counter-based parameters and formulas in detection of β-thalassemia trait in areas of high prevalence. *American Journal of Clinical Pathology*, 128(4), 585–589. https://doi.org/10.1309/R1YL4B4BT2WCQDGV
- [2] Urrechaga, E., Borque, L., & Escanero, J. F. (2011). The role of automated measurement of RBC subpopulations differential diagnosis of microcytic anemia and β-thalassemia screening. *American Journal of Clinical Pathology*, 135(3), 374–379.
- [3] Zaini, R. G. (2016). Sickle-cell anemia and consanguinity among the Saudi Arabian population. *Archives of Medicine*, 8(3), 3–15.
- [4] McLean, E., Cogswell, M., Egli, I., et al. (2009). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutrition*, 12, 444–454.
- [5] *Mediterranean Journal of Hematology and Infectious Diseases*, 7.
- [6] Olivieri, N. F. (1999). The beta-thalassemias. New England Journal of Medicine, 341(2), 99–109.
- [7] Goonewardene, M., Shehata, M., & Hamad, A. (2012). Anaemia in pregnancy. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 26, 3–24.
- [8] Piperno, A., Mariani, R., Arosio, C., et al. (2000). Haemochromatosis in patients with beta-thalassaemia trait. *British Journal of Haematology*, 111(3), 908–914.
- [9] Zhao, J., Li, J., Lai, Q., & Yu, Y. (2020). Combined use of gap-PCR and next-generation sequencing improves thalassaemia carrier screening among premarital adults in China. *Journal of Clinical Pathology*. https://doi.org/10.1136/jclinpath-2019-206339
- [10] Rund, D. (2016). Thalassemia 2016: modern medicine battles an ancient disease. *American Journal of Hematology*, 91, 15–21.
- [11] Hashemieh, M., Timori Naghadeh, H., Tabrizi Namini, M., Neamatzadeh, H., & Hadipour Dehshal, M. (2015). The Iran Thalassemia prevention program: success or failure? *Iranian Journal of Pediatric Hematology & Oncology*, 5, 161–166.
- [12] Mentzer, W. C. Jr. (1973). Differentiation of iron deficiency from thalassaemia trait. *The Lancet*, 1(7808), 882.
- [13] Shine, I., & Lal, S. (1977). A strategy to detect β-thalassaemia minor. The Lancet, 1(8013), 692–694.
- [14] England, J. M., & Fraser, P. M. (1973). Differentiation of iron deficiency from thalassaemia trait by routine blood-count. *The Lancet*, 1(7801), 449–452.
- [15] Srivastava, P. C. (1973). Differentiation of thalassemia minor from iron deficiency. *The Lancet*, 2, 154–155.
- [16] Green, R., & King, R. (1989). A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. *Blood Cells*, 15(3), 481–495.
- [17] Ricerca, B. M., Storti, S., d'Onofrio, G., et al. (1987). Differentiation of iron deficiency from thalassaemia trait: a new approach. *Haematologica*, 72(5), 409–413.
- [18] Sirdah, M., Tarazi, I., AlNajjar, E., & Al Haddad, R. (2008). Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of β-thalassaemia minor from iron deficiency in Palestinian population. *International Journal of Laboratory Hematology*, 30(4), 324–330.
- [19] Ehsani, M. A., Shahgholi, E., Rahiminejad, M. S., Seighali, F., & Rashidi, A. (2009). A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. *Pakistan Journal of Biological Sciences*, 12(5), 473–475.



# Building a Score to Discriminate Between Iron Deficiency Anemia and Beta Thalassemia Trait. SEEJPH 2024 Posted: 11-09-2024

- [20] Telmissani, A., Khalil, S., & George, T. R. (1999). Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function. *Laboratory Haematology*, 5, 149–152.
- [21] Batebi, A., Esmailian, P. A., & Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices. *Turkish Journal of Medical Sciences*, 42(2), 275–280.
- [22] Rahim, F. (2010). Correlation of beta-thalassemia mutations with alpha-thalassemia: an experience of the southwestern region of Iran. *Hematology*, 15(6), 430–437. https://doi.org/10.1179/102453310X12719010991821
- [23] Rathod, D. A., Kaur, A., Patel, V., et al. (2007). Usefulness of cell counter-based parameters and formulas in detection of beta-thalassemia trait in areas of high prevalence. *American Journal of Clinical Pathology*, 128(4), 585–589. https://doi.org/10.1309/R1YL4B4BT2WCQDGV