Introduction of new formulas and evaluation of the previous red blood cell indices and formulas in the differentiation between beta thalassemia trait and iron deficiency anemia in the Makkah region

Amal Zaghloul¹, T. A. M. A. Al-bukhari¹, Nada Bajuaifer¹, Maged Shalaby¹, H. A. AL-Pakistani¹, Saeed H. Halawani¹, Shirin H. Teama²,³, Ghada A. Wassif⁴

¹Department of Hematology and Immunology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia, ²Department of Clinical Pathology (Clinical Chemistry), King Abdulaziz Hospital, Ministry of Health, Makkah, Saudi Arabia, ³Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt, ⁴Department of Anatomy, Faculty of Medicine, Taibah University, Al Madinah Al Munawarah, Saudi Arabia

Background: Many formulas from red blood cell (RCB) indices are instructed to differentiate between iron deficiency anemia (IDA) and beta thalassemia trait. None had 100% Youden index.

Aim of the work: To introduce two new formulas and evaluate them in the differentiation between IDA and beta thalassemia trait in adults Saudi (male and female; male; female) in the Makkah region. Furthermore, to evaluate the previous formulas in our population.

Methods: A total of 249 participants, 91 with IDA, 123 with beta thalassemia trait, and 35 healthy persons. All subjected to complete blood count, measurement of iron profile, hemoglobin electrophoresis and hemoglobin A2 by column chromatography. The first new formula equal hemoglobin (Hb) + hematocrit (Hct) + RBC and second equals Hb + Hct + RBC-red cell distribution (RDW). The previous formula used is England and Fraser, Mentzer, Strivastava, Ehsani, Green and King, red cell distribution index, Ricerca, and Shine and Lal

Results: In both men and women, the England and Fraser was the best with a Youden’s index of 70.4%, followed by Green and King 67.4%. In men, the England and Fraser and our new formula 1 had the highest Youden’ index 84.7% and 84.1%, respectively. In women, the England and Fraser and RDW index had the highest Youden’ index 74% and 69.2%, respectively.

Conclusion: The England and Fraser and our new formula 1 are the best formulas in men. The England and Fraser and RDW index are the best formulas in women.

Keywords: Red blood cell indices, Iron deficiency anemia, Beta thalassemia trait

Introduction
Iron deficiency anemia (IDA) and beta thalassemia trait (β-TT) are two of the most common causes of microcytic hypochromic anemia worldwide. They have similar red blood cell (RCB) indices. Therefore, it is important to develop reliable measures, which can easily diagnose and differentiate between these two conditions.

Serum ferritin, total iron-binding capacity (TIBC), transferrin saturation, serum iron, serum transferrin receptor levels, and hemoglobin electrophoresis may be helpful in differentiating IDA from β-TT.¹ In general, IDA is associated with low ferritin, low transferrin saturation, low serum iron, and high TIBC.¹ In addition, erythrocytosis with very low mean cell volume (MCV), irrespective of hemoglobin level is interpreted as a beta thalassemia carrier state which confirmed by hemoglobin electrophoresis and normal iron profile.²

Different workers have instructed several mathematical formulas as simple, fast and inexpensive means for
differentiating IDA and β-TT.3 These are England & Fraser which equals MCV+RBC-5XHb-3.4; Mentzer equals MCV/RBC; Strivastava = mean corpuscular hemoglobin (MCH)/RBC; Ehsani et al. = MCV-(10XRBC); Green and King = MCV X red cell distribution (RDW)/100XHb; Ricerca = RDW/RBC.9 Shine and Lal = MCV×MCHX0.01.10 and RDW index = MCVXRDW/RBC.11 In addition, to the RBC count. Moreover, we introduce two new formulas, first one equal hemoglobin(Hb)+ hematocrit (Hct)+ RBC and second one equals Hb+Hct+RBC–RDW. The aim of the work: the aim of our study was to introduce new formulas and to evaluate them in the differentiation between iron deficiency anemia (IDA) and beta thalassemia trait in adults Saudi (males, females, and both male and female) in the Makkah region. In addition, to evaluate the previous formulas in our Makkah region and to determine the best one.

Subjects and methods

Subjects

The analytical methods of this study were carried out in the laboratory of the Hematology and Immunology Department, Faculty of Medicine, Umm Al-Qura University in accordance with the approved guidelines. The Umm AlQura University ethics committee approved the protocol of this study. All participants gave informed consent according to the declaration of Helsinki. This study was carried out from January 2012 to June 2012; it included 249 subjects. They were 91 patients with IDA, 123 cases with β-TT and 35 healthy subjects as a control group. All participants were recruited at the outpatient clinic of medicine of Al-Noor Hospital, Heraa Hospital, King Abdul Aziz Hospital and Obstetric and Pediatrics Hospital; Makkah, Saudi Arabia for performing the premartial tests done before marriage.

Sample collection

4 ml of blood sample was collected from each participant under complete aseptic conditions. 2 ml of blood was dispensed into a tube containing EDTA as anticoagulant substances for performing complete blood count, hemoglobin electrophoresis and measurement of hemoglobin A2 by column chromatography. The other 2 ml was collected in plain tubes, left to clot, then separation of serum that was used for determination of serum iron, serum ferritin, and TIBC.

Inclusion criteria

Cases with low hemoglobin, low serum iron, low ferritin, high total iron-binding capacity, and normal electrophoresis were classified as IDA. Cases with normal serum iron, serum ferritin, total iron-binding capacity, and high hemoglobin A2 by electrophoresis and column chromatography were considered as thalassemia trait. In IDA, the cut off in males, were hemoglobin less than 13 g/dl, serum iron less than 65 μg/dl and serum ferritin less than 30 ng/ml. In females, the cut off, were hemoglobin less than 12 g/dl, serum iron less than 50 μg/dl and serum ferritin less than 13 ng/ml. In thalassemia trait cases, the hemoglobin A2 cut off was more than 3.5%.

Exclusion criteria

Any participant with a hemoglobin level less than 9 g/dl was excluded from the study. Mixed cases of IDA and beta thalassemia trait were excluded from the study. Microcytic hypochromic cases with normal iron profile and normal hemoglobin electrophoresis were excluded from the study as it may be alpha thalassemia trait.

Methods

All cases and control group were subjected to the followings:

1. A full clinical history.
2. Complete hemogram analysis on Sysmex XT2000i which included the following parameters RCBs, hemoglobin concentration, packed cell volume, MCH, MCV, MCH concentration, and RDW.
3. Estimation of Hb A2 by column chromatography (Helena laboratories, Beaumant, TX).
4. Hemoglobin electrophoresis (Helena Laboratories, Beaumant, TX).
5. Determination of serum iron and TIBC on Dimension Max Siemens.
6. Measurement of serum ferritin on Cobas e 411, Roche Hitachi.

Statistical analysis

The statistical analysis of this study was done using SPSS program version 20. Quantitative data were described in the form of mean ± SD for the normally distributed data. The median and range were used for the data that was not normally distributed. The comparison between groups was performed by using student t test. The Mann–Whitney U test and the Kruskal–Wallis test were used for the data that was not normally distributed. The validity of the different indices and formulas used in the study was assessed by using sensitivity, specificity, and Youden’s index. The Youden’s index was calculated. Y1 = sensitivity + specificity – 100. The Gaussian curve for each formula was constructed to see the degree of overlap between IDA and beta thalassemia trait. A P > 0.05 was considered statistically not significant while a P < 0.05 and <0.001 were considered significant and highly significant respectively.

Results

The results of this study are summarized in tables from one to eight and Figs. 1 and 2.
This study included 214 subjects with IDA and beta thalassemia trait as well as 35 healthy persons serving as a control group. The IDA patients (91) were 21 men and 70 women with male to female ratio of 1:3.3. Their mean age was 30.4 ± 15.1 years. The β-TT cases (123) were 58 men and 65 women with male to female ratio of 1:1.1. Their mean age was 31.5 ± 13.8 years. The control group was 18 men and 17 women and with male to female ratio of 1.1:1.0. Their mean age was 28.4 ± 11.5 years. There were no significant differences between the control group, IDA, and beta thalassemia trait groups with regard to age (Table 1). In addition, we divide the cases into female and male group. The female group was 70 with IDA and 65 with β-TT whereas, the male group was 21 with IDA and 58 with β-TT. The ratio of IDA to β-TT in the female group was 1.07:1. The ratio of IDA to β-TT in the male group was 1.0:2.8. The criteria to define IDA in males were, hemoglobin less than 13 g/dl, serum iron less than 65 μg/dl, serum ferritin less than 30 ng/ml, and saturation index less than 15%. In females, hemoglobin less than 12 g/dl,
serum iron less than 50 µg/dl, serum ferritin less than 13 ng/ml, and saturation index less than 15%. Beta thalassemia trait was diagnosed when the Hb A2 more than 3.5% (Table 2).

A highly significant increase of RCB counts, hemoglobin concentration, hematocrit, hemoglobin A2 by hemoglobin electrophoresis and column chromatography, serum iron, and serum ferritin were found when the cases of β-TT were compared to IDA patients (P < 0.01) (Tables 1 and 2). In addition, cases of β-TT showed highly significant decrease of RDW and MCV when compared to the IDA group (P < 0.01). Moreover, significant decrease of total iron-binding capacity and MCH were found when the β-TT cases compared to IDA patients (Table 1). The comparison between control group and each of IDA and β-TT is shown in Tables 1 and 2.

The area under the curve, sensitivity, specificity, Youden’ index, and cutoff values of all RCB indices of our work are shown in Table 3. The cut off values of RCB count, hematocrit, and hemoglobin in males were 5.1, 35.5, and 11.4, respectively. In females, the cut off values of RCB count, hematocrit, and hemoglobin were 5.2, 35.5, and 11.4, respectively. In both male and female, the RCBs count had the best Youden index (65.7%) followed by hematocrit (57.4%), then hemoglobin (50.6%), then RDW (36.3%), then MCV (31%) and the last one was the MCH (22.1%). In male the RCBs count had, the best Youden index (81.4%) followed by hematocrit (78.8%), then hemoglobin (74.8%). In female the hematocrit had, the best Youden’ index (43.2%) followed by RCBs count (31.9%), then hemoglobin (30.2%). The sensitivity, specificity, and area under the curve were obtained from SPSS version 20. The Youden’s index was calculated. We instruct two new formulas from our indices to improve the Youden’ index of each red cell indices and to differentiate between IDA and beta thalassemia trait. The new formula one was RCBs + hematocrit + hemoglobin. The second formula was RCB + hematocrit + hemoglobin − RDW. The Gaussian curve for each formula was constructed to see the degree of overlap between IDA and beta thalassemia trait (Fig. 1). The new formulas and their cut off values in males, females, and both male and female are shown in Table 4 and Fig. 2. The cut off values of our new formula one in males were 55.7 whereas in females 51.6. Values higher than these cut off indicate beta thalassemia trait. The cut off values of our new formula

### Table 1 Clinical and laboratory findings of patients

| Parameters | IDA (n = 91) Mean ± SD | β-TT (n = 123) Mean ± SD | Control (n = 35) Mean ± SD | Significance |
|------------|------------------------|--------------------------|---------------------------|--------------|
| Age (years) | 30.4 ± 15.1 p3 = 0.758 | NS 31.5 ± 13.8 p2 = 0.288 | NS 28.4 ± 11.5 p1 = 0.587 | NS           |
| Red blood cells (x10^6/µl) | 4.5 ± 0.43 p3 = 0.000 | HS 5.7 ± 0.7 p2 = 0.000 | HS 5.0 ± 0.5 p1 = 0.000 | HS           |
| Hemoglobin (g/dl) | 10.4 ± 1.1 p3 = 0.000 | HS 12.5 ± 1.6 p2 = 0.000 | HS 15.0 ± 1.7 p1 = 0.000 | HS           |
| Hematocrit (%) | 32.9 ± 3.4 p3 = 0.000 | HS 39.0 ± 4.5 p2 = 0.000 | HS 42.5 ± 4.3 p1 = 0.000 | HS           |
| Red cell distribution (%) | 17.7 ± 2.7 p3 = 0.000 | HS 16.3 ± 2.6 p2 = 0.000 | HS 13.7 ± 0.6 p1 = 0.000 | HS           |
| Mean cell volume (fl) | 72.8 ± 5.9 p3 = 0.000 | HS 68.6 ± 6.9 p2 = 0.000 | HS 84.8 ± 3.9 p1 = 0.000 | HS           |
| Mean corpuscular hemoglobin (pg/ml) | 23.0 ± 2.4 p3 = 0.004 | S 22.0 ± 2.6 p2 = 0.000 | HS 29.9 ± 1.5 p1 = 0.000 | HS           |
| TIBC (µg/dl) | 454.2 ± 58.1 p3 = 0.007 | S 398.2 ± 47.4 p2 = 0.000 | 268.0 ± 54.29 p1 = 0.000 | HS           |

IDA: iron deficiency anemia, β-TT: beta thalassemia trait, TIBC: total iron-binding capacity, HS: highly significant.

N.B.: the Mann–Whitney U test and the Kruskal–Wallis test were used for comparison. The data between brackets for females.
Table 3 The sensitivity and specificity of all red blood cell indices in our work

| Indices | AUC & CI | Cut off | Sensitivity | Specificity | Youden’ index |
|---------|---------|---------|-------------|-------------|---------------|
| RBC count, male and female | 0.935 (0.904–0.967) | 5.2 | 93.5 | 72.2 | 65.7 |
| RBC count, male | 0.949 (0.902–0.996) | 5.1 | 95.5 | 87.9 | 81.4 |
| RBC count, female | 0.905 (0.851–0.958) | 5.2 | 90.2 | 59.7 | 31.9 |
| Hematocrit, male and female | 0.866 (0.889–0.990) | 35.8 | 86.0 | 71.4 | 57.4 |
| Hematocrit, male | 0.940 (0.820–0.912) | 37.6 | 90.9 | 87.9 | 78.8 |
| Hematocrit, female | 0.785 (0.707–0.863) | 35.5 | 80.3 | 62.9 | 43.2 |
| Hemoglobin, male and female | 0.854 (0.806–0.902) | 11.7 | 83.9 | 66.7 | 50.6 |
| Hemoglobin, male | 0.930 (0.876–0.985) | 12.3 | 95.5 | 79.3 | 74.8 |
| Hemoglobin, female | 0.775 (0.695–0.855) | 11.4 | 77 | 53.2 | 30.2 |
| Red cell distribution | 0.690 (0.619–0.762) | 16.6 | 68.8 | 67.5 | 36.3 |
| Mean cell volume | 0.868 (0.615–0.757) | 71.3 | 69.9 | 61.1 | 31 |
| Mean corpuscular hemoglobin | 0.616 (0.540–0.692) | 23 | 60.2 | 61.9 | 22.1 |

AUC: area under the curve; CI: confidence intervals; RBC: red blood cell.

two in males were 40.4 whereas in females 35.6. Values higher than these cut off indicate beta thalassemia trait.

The others discrimination formulas and indices used in this study with their cut off values (previous published and of us) that differentiate between IDA and β-TT are summarized in Table 5.

In both male and female, the England and Fraser had a Youden’ index of 70.4%, sensitivity 95.6%, and specificity 74.8%. The Green and King had a Youden index of 67.4%. The RDW index had a Youden’ index of 65.8 and the RCBs count had a Youden’ index of 65.7%. Our new formula 1 had a Youden’s index of 62.7%, sensitivity 87.9%, and specificity 74.8%. The

Table 4 The new formulas and their cut off

| Formula | Gender | IDA | β-TT |
|---------|--------|-----|------|
| Our new formula | Male and females | <52.5 | >52.5 |
| (1) = Hb + Hct + RBC | | |
| Our new formula | Male | <55.7 | >55.7 |
| (2) = Hb + Hct + RBC - RBC | | |
| Red cell distribution index | 0.854 (0.801–0.907) | 4.6 | 84.6 | 77.2 |
| RBC count (x10^6/μl) | <5 | 5 | |
| Ehsani et al. = MCV-(10xRBC) | >15 | 15 | |
| England & Fraser = (5xHb)-MCH/RBC | >13 | 13 | |
| Montzer = MCH/RBC | >3.8 | 3.8 | |
| Strivastava = MCV/RBC | >3.8 | 3.8 | |
| RDW index = MCV/RBC/RBC | >220 | 220 | |
| Shine & Lal = MCV×MCHX0.01 | >1530 | 1530 | |

Table 5 Other discrimination formulas and indices used in the study with their cut off in our work and previous published work

| Indices | Previous cut off | Our cut off |
|---------|-----------------|-------------|
| IDA | β-TT | IDA | β-TT |
| England & Fraser = (5xHb)-3.4 | >0 | <0 | >4.1 | <4.1 |
| Montzer = MCV/RBC | >13 | 13 | >13.7 | <13.7 |
| Strivastava = MCV/RBC | >3.8 | 3.8 | >4.6 | <4.6 |
| RBC count (x10^6/μl) | <5 | 5 | <5.2 | >5.2 |
| Ehsani et al. = MCV-(10xRBC) | >15 | 15 | >18.3 | <18.3 |
| Green & King = MCVXRDW/100XHb | >65 | 65 | >69.3 | <69.3 |
| RDW index = MCVXRDW/RBC | >220 | 220 | >222.2 | <222.2 |
| Shine & Lal = MCV×MCHX0.01 | >1530 | 1530 | >1154 | <1154 |

Table 6 Sensitivity, specificity, and Youden’s index of all formulas in males and females

| Indices and formulas | *AUC & CI | Cut off | Sensitivity | Specificity | Youden’s index |
|---------------------|----------|---------|-------------|-------------|---------------|
| England & Fraser | 0.935(0.902–0.970) | 69.3 | 93.4 | 74.0 | 67.4 |
| Green & King | 0.895(0.809–0.980) | 4.1 | 95.6 | 74.8 | 70.4 |
| Red cell distribution index | 0.933(0.906–0.971) | >222.2 | 93.4 | 72.4 | 65.8 |
| Our new formula (1) | 0.888(0.847–0.930) | 52.5 | 87.9 | 74.8 | 62.7 |
| Mentzer | 0.878(0.829–0.926) | 13.7 | 87.9 | 74.8 | 62.7 |
| Ricerca | 0.912(0.875–0.950) | 3.1 | 91.2 | 70.7 | 61.9 |
| Strivastava | 0.854(0.801–0.907) | 4.6 | 84.6 | 77.2 | 61.8 |
| Our new formula (2) | 0.877(0.831–0.922) | 37.1 | 87.9 | 70.7 | 58.6 |
| Ehsani | 0.868(0.820–0.917) | 18.3 | 86.8 | 69.9 | 56.7 |
| Shine & Lal | 0.657(0.582–0.731) | 1154 | 65.9 | 61.8 | 27.7 |

*AUC: area under the curve; CI: confidence intervals.
Mentzer, Ricerca, Strivastava, our new formula 2, Ehsani and Shine and Lal had a Youden’s index of 62.7, 61.9, 61.8, 58.6, 56.7 and 27.7%, respectively (Table 6).

In males, the cut off values of England and Fraser, Green and King, RDW index Mentzer, Ricerca, Ehsani, Strivastava, and Shine and Lal were 4.7, 65.7, 214.9, 13.4, 2.9, 40.4, 19.6, 95.2, respective. The England and Fraser had a Youden’s index of 84.7% and our new formula 84.1%. The RCBs count had a Youden’s index of 81.4%. Both the Green and King and the RDW index had a Youden’s index of 77.7%. The Mentzer and Ricerca had a Youden’s index of 75.9. Our new formula 2 and Ehsani had a Youden’s index of 75.0 and 72.4%, respectively. The Strivastava, and Shine and Lal had a Youden’s index of 65.9 and 36.9% (Table 7).

In females, the cut off values of England and Fraser, RDW index, Green and King, Mentzer, Ricerca, Strivastava, Ehsani, and Shine and Lal were 7.1, 240.5, 74.1, 14.5, 3.4, 4.6, 19.4, and 1150.8, respectively. The England and Fraser had a Youden’s index of 74.0% and the RDW index had a Youden’s index of 69.2%. The Green and King, Mentzer and Ricerca had a Youden’s index of 66.1, and 54.6%, respectively. The Strivastava, Ehsani, our new formula 1 and our new formula 2 had a Youden’s index of 54.5, 49.7, 46.5, and 46.4%, respectively.

The RCBs count and Shine and Lal had a Youden’s index of 31.9 and 25.2%, respectively (Table 8).

### Table 7 Sensitivity, specificity, and Youden’s index of all formulas in males

| Indices and formulas | *AUC & CI | Cut off | Sensitivity | Specificity | Youden’s index |
|----------------------|-----------|---------|-------------|-------------|----------------|
| England & Fraser     | 0.976(0.947–1.0) | 4.7     | 95.2        | 89.5        | 84.7           |
| Our new formula (1)  | 0.955(0.914–0.997) | 55.7   | 95           | 89.1        | 84.1           |
| Green & King         | 0.961(0.924–0.998) | 65.7   | 95.2        | 82.5        | 77.7           |
| Red cell distribution index | 0.966(0.932–1.00) | 214.9  | 95.2        | 82.5        | 77.7           |
| Mentzer              | 0.956(0.914–0.998) | 13.4   | 95.2        | 80.7        | 75.9           |
| Ricerca               | 0.950(0.907–0.993) | 2.9    | 95.2        | 80.7        | 75.9           |
| Our new formula (2)  | 0.954(0.911–0.996) | 40.4   | 95           | 80          | 75.4           |
| Ehsani                | 0.947(0.900–0.994) | 19.6   | 95.2        | 77.2        | 72.4           |
| Strivastava           | 0.929(0.873–0.985) | 4.5    | 90.5        | 75.4        | 65.9           |
| Shine & Lal           | 0.783(0.682–0.885) | 1127.4 | 77.3        | 59.6        | 36.9           |

*AUC: area under the curve; CI: confidence intervals.

### Table 8 Sensitivity, specificity, and Youden’s index of all formulas in females

| Indices and formulas | *AUC & CI | Cut off | Sensitivity | Specificity | Youden’s index |
|----------------------|-----------|---------|-------------|-------------|----------------|
| England & Fraser     | 0.926(0.880–0.973) | 7.1     | 93.4        | 80.6        | 74             |
| Red cell distribution index | 0.907(0.851–0.962) | 240.5  | 91.8        | 77.4        | 69.2           |
| Green & King         | 0.912(0.858–0.967) | 74.1   | 91.8        | 74.2        | 66             |
| Mentzer              | 0.811(0.732–0.890) | 14.5   | 80.3        | 75.8        | 56.1           |
| Ricerca               | 0.870(0.806–0.935) | 3.4    | 86.9        | 67.7        | 54.6           |
| Strivastava           | 0.784(0.698–0.870) | 4.6    | 78.7        | 75.8        | 54.5           |
| Ehsani                | 0.799(0.719–0.878) | 19.4   | 80.3        | 69.4        | 49.7           |
| Our new formula (1)  | 0.821(0.750–0.892) | 51.6   | 82          | 64.5        | 46.5           |
| Our new formula (2)  | 0.804(0.728–0.881) | 35.6   | 80.3        | 66.1        | 46.4           |
| Shine & Lal           | 0.634(0.535–0.734) | 1150.8 | 63.9        | 61.3        | 25.2           |

*AUC: area under the curve; CI: confidence intervals.

### Discussion

Microcytic anemias are the most common types of anemia. The two most causes of microcytic anemia are IDA and beta thalassemia trait (β-TT). It is important to discriminate between these two entities because they are encountered too frequently. Many workers used different RCB indices obtained from the complete blood count to discriminate between IDA and β-TT. The aim of our study was to introduce new formulas and to evaluate them in the differentiation between IDA and beta thalassemia trait in adults Saudi (males, females, and both male and female) in the Makkah region. Furthermore, to evaluate the previous formulas in our Makkah region and to determine the best one.

In our study, in the group of IDA the ratio of male to female was 1:3.3, whereas in the group of beta thalassemia trait was 1:1.1. This was attributed to the higher incidence of IDA in females.

In the present study, significant higher values of hemoglobin (Hb), hematocrit (Hct) and RCBs count were found in beta thalassemia trait when compared to IDA. This is in agreement with previous authors. In both male and female, the Youden’s index of RBC, Hct and Hb were 65.7, 57.4, and 50.6%, respectively. When separating into two groups...
males and females the values of Youden’ index of RBC, Hct, and Hb were higher in males than in females (Table 3). The high number of IDA in the female group may be the cause of reduction in the Youden’ index as approximately around half of the cases in the female group had IDA. In contrast, in the male group it was 1:2.8.

The β-TT group had significant lower values for MCH and MCV (P < 0.05 and P < 0.000), respectively. Similar results have been reported earlier.14-16 They reported that, the β-TT cases showed reductions in both MCV and MCH values and the values did not correlate with the degree of anemia.14 Thus MCV and MCH values were found to be important parameters for detecting β-TT.14-16 In our population, the Youden’ index of MCV was 31% and of MCH was 22%. The Youden’ index of both MCV and MCH is poor.

In this work, the RDW width showed highly significant decrease in β-TT group when compared to the IDA group and it could be a good discriminator between IDA and β-TT. This is in agreement with previous authors.17,18 The sensitivity of RDW in our work was 68.8%, specificity 67.5%, cut off value was 16.6% and the Youden’ index was 36.3%. Therefore, the RDW alone is not sufficient specific or sensitive enough to differentiate between IDA and thalassemia trait. This is in accordance with previous author.3,19

We try to increase the sensitivity, specificity, and Youden’ index of our indices by a combination of the indices and we plotted two new formulas. The new formula one was RCBs + hematocrit + hemoglobin. The second formula was RCB + hematocrit + hemoglobin – RDW. As hemoglobin, hematocrit, and RCB count differed in male and female, we measured the sensitivity, specificity, and a Youden’ index of both new formulas and old formulas in both male and female, in male only and in female only.

The cut off used in our new formulas and the old formulas are presented in Tables 4 and 5. In the old formulas used, there were differences in our cut off than the previous published formulas. This may be because of different thalassemia mutations present in different population and each population must obtain their cut off and not depend on the previous published cutoff.

In both male and female, the England and Fraser4 was the best with sensitivity of 95.6%, specificity of 74.8%, and with a Youden’ index of 70.4%. The Green and King,8 RDWI, RCB count, our new formula one, Mentzer,5 Ricerca,9 Straivasta,6 new formula two, Ehsani,2 and Shine and Lal10 indices have a Youden’s index of 67.4, 65.8, 65.7, 62.7, 62.7, 61.9, 61.8, 58.6, 56.7, and 27.7%, respectively. Many studies were done on different populations with variations in the results. Some reported that the best reliable index was Green and King.20-23 Other found that the best was England and Fraser.3,24 Moreover, other authors demonstrated that the best index was Mentzer and Shine and Lal.1,25-27 The Shine and Lal formula in our work, showed the lowest value for Youden’s index, suggesting that this formula is least effective in discrimination studies for microcytic anemia.3,13 The variation in the results may be due to different population studied with different genes mutations.

In males, the best three formulas are, the England and Fraser, our new formula one and RCB count. The England and Fraser had a Youden’ index of 84.7% and a cut off value of 4.7. Values less than 4.7 indicate beta thalassemia trait. Our new formula one had a Youden’s index of 84.1% and the RCBs count 81.4%. Their cut off values were 55.7 and 5.1, respectively. Values higher than 55.7 and 5.1 indicate beta thalassemia trait. In females, the England and Fraser had a Youden’s index of 74.0% and a cut off value of 7.1. Values less than 7.1 indicate beta thalassemia trait. It was followed by the RDW index which had a Youden’s index of 69.2% and a cut off value 240.5. Values less than 240.5 indicate beta thalassemia trait. The 3rd one was Green and King with a Youden’s index of 66% and a cut off value 74.1. Values less than 74.1 indicate beta thalassemia trait. In all formulas and indices, the Youden’ index was higher in men than women. This is in accordance with previous authors.24 The Youden’ index is a function of sensitivity and specificity that depends on the underlying distributions of the diseased and non-diseased populations, i.e. overall diagnostic effectiveness. It equals sensitivity + specificity – 100 or minus 1 according to the way of writing sensitivity and specificity. Its value ranges from 0 to 1, a value of 1 or 100 indicates that there are no false positives or false negatives, i.e. the test is perfect. In our work, the top formulas in males or in females had Youden index range from 74 to 84.7% which is of excellent utility.

Conclusion
The England and Fraser and our new formula 1 are the best formulas in men with a Youden’ index of 84.7 and 84.1%, respectively. The England and Fraser and RDW index are the best formulas in women with a Youden’ index of 74 and 69.2%. They are acceptable in discrimination between IDA and beta thalassemia trait. All formulas in men have a higher Youden’ index than in women. None of the different formulation have 100% sensitivity and specificity. Nevertheless, they can help in thalassemia screening with high precision in male than in female.
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Disclaimer statements

Contributions A.Z.: design of the study, clinical selection, performance of laboratory investigations, statistical analysis, preparing tables and figures, writing and revising of the main manuscript text. T.A.M.A., N.B., S.H., M.S., H.A., S.T., and GW.: performance of the laboratory investigations, preparing tables and figures and revising of the main manuscript text.

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