Discriminant Functions In Distinguishing Beta Thalassemia Trait and Iron Deficiency Anemia: The value of the RDW-SD
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Abstract
The most commonly encountered disorders with mild 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. Red blood cell counts red blood cell distribution (RDW) and other discriminant functions calculated by blood cell analyzers have been helpful in distinguishing between the two disorders. In this study we evaluated ten discriminant functions to determine their ability to distinguish between IDA and BTT. A total of 216 cases of microcytic hypochromic anemia were studied. We were especially interested in comparing the effectiveness of the RDW-SD, a recently introduced function, and the RDW-CV, currently used in most clinical laboratories. The RBC count and E & F Index were found to be very useful functions while the RDW-CV was ineffective. However, the study revealed that the RDW-SD was the most useful discriminant function for distinguishing IDA from BTT.

INTRODUCTION
The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and β-thalassemia trait (BTT) (1,2). The gene frequency of β-thalassemia, however, is high and varies considerably from area to area, having its highest rate of more than 10% around the Caspian Sea and Persian Gulf. The incidence of β-thalassemia trait is particularly high in Jamnagar district, Gujarat, India with over 250 cases of β-thalassemia major registered in our hospital.

The differential diagnosis between IDA and BTT is important to avoid unnecessary iron therapy. Iron therapy would be indicated in IDA but usually contraindicated in BTT. Standard tests used for the differentiation between IDA and β-thalassemia trait include: complete blood count (CBC), serum iron, serum ferritin, total iron binding capacity (TIBC), bone marrow iron stores, HbA2 levels, free erythrocyte protoporphyrin and zinc protoporphyrin levels (3-8). Despite their usefulness, these standard tests are often expensive and time consuming.

Routine electronic red blood cell counts and other discriminant functions derived from modern blood cell analyzers can be rapidly obtained, are inexpensive, and can help distinguish between IDA and BTT. There is controversy about which discriminant functions are best in identifying IDA and BTT. Also, there are reasonable questions as to whether discriminant functions are sufficiently accurate to warrant not ordering serum ferritins and other standard tests to detect iron deficiency (5, 6).

In this study we evaluated ten discriminant functions for their ability to distinguish between IDA and BTT in 216 cases of microcytic hypochromic anemia. We were especially interested in determining the value of a recently introduced discriminate function, RDW-SD.

MATERIALS AND METHODS
The study was carried out at Outdoor Patient Hematology Laboratory, Pathology Department, M P Shah Medical College, Jamnagar in co-operation with two voluntary organizations named Indian Red Cross Society, Jamnagar Branch and Jamnagar Thalassemic Society.

The study groups included were: 1) The relatives of known thalassemia Major Patients (as a part of family screening); 2) Antenatal patients coming to Gynecology and Obstetrics Department of our Hospital. Screening for thalassemia is compulsory for antenatal cases in our hospital; 3) Thalassemia screening camps held in high risk communities in our area.

A total of 216 subjects (age<30 years) with confirmed microcytic anemia were included in the study. Anemia was
defined as a hemoglobin concentration of at least 2 standard deviations lower than age- and sex-specific average. IDA and BTT were diagnosed by the following tests: serum iron levels, serum TIBC levels and HbA2 levels performed by chromatography.

We did not test for serum ferritin levels since we do not have facilities to perform this assay. We retested the patients having borderline HbA2 values and low serum iron levels after giving them iron replacement therapy.

The following hematological data were obtained: Hemoglobin, Red cell count, Hematocrit, MCV, MCH, MCHC, RDW-CV, RDW-SD, Serum iron, HbA2.

**Figure 1**

Figure 1: RDW-CV and RDW-CV (From Sysmex - Operating Manual)

Figure 1 demonstrates how RDW-CV and RDW-SD were calculated (CV = coefficient of variance, SD = one standard deviation). A Sysmex KX-21, three-part differential analyzer, (India, Pvt, Ltd., Mumbai, India) was used to obtain data.

The data were analyzed individually using the ten mathematical discriminant functions: Mentzer Index, MI, Shine and Lal (S & L) Index, England and Fraser (E & F) Index, Green and King (G & K) Index, RDW, Index (RDWI), Srivastava Index (SI), Ricerca Index (RI).

The sensitivity, specificity, positive predictive values, negative predictive values and the Youden’s indices for each of the 10 discriminant functions were calculated. The Youden’s index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity (7,8).

**RESULTS**

The mean and standard deviations of various hematological and biochemical parameters of all patients are shown in Table 1a. Table 1b displays the cut-offs for RBC Indices and RDW. All parameters for IDA and BBT except serum iron were significantly different as determined by an independent t test analysis as shown in Table 2. A serum ferritin might have been a more reliable parameter.

Eighty one patients were diagnosed IDA based low serum iron levels, high serum TIBC levels, response to iron therapy and absence of HbA2 elevation (<3.5%). One hundred and thirty five patients were diagnosed to have beta thalassemia trait based on absence of evidence of iron deficiency and elevated HbA2 (>3.5 %). Alpha thalassemia cases could be missed since HbA2 not elevated in this condition. However, we do not believe that any of the patients in the study had alpha thalassemia.
The ability of 10 discriminant functions to identify IDA and BTT is shown in Table 2. Formulas for discriminant function that were used are shown in Table 3. The number and percent of IDA and BBT identified by each discriminant function is shown. The percent of IDA and BBT identified by discriminant functions was based on the number identified by the discriminant function/ the total number of IDA and BBT. Only 4 of the 10 discriminant functions detected more that 90% of IDA (E&F, RBC count, RDW-CF and RDW-SD). Only 3 discriminant functions detected more that 90% BBT (S&K, SI and RDW-SD).

Table 4 lists the effectiveness of each discriminant function to distinguish BTT and IDA. The following were analyzed: sensitivity, specificity, positive and negative predictive value and the Youden index. All of the functions showed overlapping in the patients with BTT and IDA. None of the functions was completely sensitive or specific in differentiation between BTT and IDA. Youden's index in decreasing order was as follows: RDW-SD > RBC COUNT > E & F > MI > RI > RDWI > G & K > SI > S & L > RDW-CV.

Discussion

The most frequently encountered conditions presenting with mild microcytic anemia are BTT and IDA. It is important to distinguish between BTT and IDA to avoid unnecessary iron therapy and to offer genetic counseling to individuals with BTT. The diagnosis of BTT can be established by family history of HbA$_2$ levels (9). Decreased serum iron, increased levels of TIBC and serum ferritin are the standard diagnostic criteria for IDA (10). Discriminant functions such as the RBC count and RDW can
be used to help identify IDA and BTT. These functions that are calculated when routine blood counts are done by blood analyzers are less time-consuming methods and less expensive than the standard test for diagnosing these disorders.

In 2007, Suad et al. (11) compared nine well-documented discriminant functions in a population of 153 confirmed cases of microcytic anemia. They concluded that the E&F index was the best discriminant function to differentiate between IDA and thalassemia minor cases.

In 2007, Beyen et al. (12) attempted to evaluate the predictive value of the following discriminant functions in the differential diagnosis of IDA and BTT in adults: RBC Counts, RDW, MI, S&L, E&F, SI, G&K, RDWI, and Ricerca index. They concluded that none of these formulations was superior to RBC Count. The authors suggested that total body iron status and HbA2 levels should be obtained to diagnose IDA and BTT until more efficient tools are developed.

We carried out an comprehensive evaluation of the ability of discriminant functions to distinguish IDA and BTT. In addition to calculating the sensitivity, specificity, positive predictive value and negative predictive value of each discriminant function, we also calculated Youden's index. The Youden's index takes into account both sensitivity and specificity and therefore provides a reliable measure to determine that validity of a technique. The Youden's Index introduces the least bias to the measure of effect (13).

Positive and negative predictive values are independent of the Youden Index and depend strongly on the underlying prevalence of the disease.

Our data indicates that the 3 discriminant functions were the extremely very useful for distinguishing IDA and BTT. RDW-SD had the highest rating, RBC Count was 2nd best and E & F is 3rd best in identifying these disorders. This was based on the the Youden index results as well as positive and negative predictive values. Our data was consistent with the study of Saud et al. (11) who found that the E & F and with the study of Beyen et al. (12) who reported that the RBC Count was the best discriminate function for distinguishing IDA and BTT.

Our study is unique in that it found that RDW-SD was the most useful functions to distinguish between IDA and BTT while the RDW-CF was ineffective. When calculating RDW-CV and RDW-SD, the same particle size distribution of RBC sizes are analyzed, but the functions are calculated by a different formulas as shown in Figure 1. The RDW-SD calculation includes a wider range of red blood cell sizes as shown in Figure 1.

Our findings are consistent with the previously reported observation that there are differences in the sensitivities of the RDW-SD and the RDW-CV and that the RDW-SD more accurately reflects the actual blood cell size variation as measured by the red blood cell distribution curve (14).

Since RDW-CV has been used in virtually all previously studies, it is not surprising that that there is considerable controversy about the value of the RDW-CV as a discriminate function for differentiating between IDA and thalassemia minor (7,8,15-20).

CONCLUSION

The RDW-SD appears to be the best discriminant function for distinguishing between IDA and BTT. The RBC count and E & F Index are useful functions for that purpose. However, the RDW-CV was ineffective for this purpose. The fact that RDW-SD is calculated differently than the RDW-CV most likely accounts for it apparent greater value in distinguishing between IDA and BTT.

It would be important to continue studying the value of the RDW-SD not only in distinguishing IDA and BTT but also in characterizing other anemias. Ideally, our study should have serum ferritin levels to identify IDA. Nevertheless, we believe our findings are meaningful and should stimulate the evaluation of the RDW-SD in clinical and research setting.

Most medical centers report RDW based on RDW-CV calculations.

The decision to rely solely on discriminant functions to detect IDA and BTT is a clinical decision. However, as we obtain more experience with the RDW-SD and other new discriminant functions, they might have major roles in these clinical decisions. Discriminant functions would be helpful in distinguishing alpha-thalassemia for IDA since HbA2 levels are not elevated in this disorder.

References
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