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Role of Hematological Indices in the Screening of B-Thalassemia Minor (Trait) and Iron Deficiency Anaemia

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Abstract

Background: One of the most common causes of microcytic hypochromic anemia is either "Iron Deficiency" or "β-thalassemia trait". It is a major diagnostic consideration to distinguish between the two. There are many formulas or indices that can discriminate similar CBC entities obtained via hematological cell analyzers. The aim of our study was to differentiate β-thalassemia trait from IDA on the basis of Mentzer's Index and Red cell Distribution Width (RDW).

Method: A retrospective study of total of 255 patients samples, including children (0-12.5 years), was done (Males=56; Females=141; Children=58). Respective results were then segregated into their categories (IDA and TT) based on Mentzer's Index, and RDW.

Result: Among the true positive cases males had a higher percentage of TT followed by children and females. The patient groups were evaluated according to RDW, Mentzer's index and HbA2 levels.

Conclusion: The Mentzer index is almost equally reliable index in comparison to the newly found index- RDW. They have a sensitivity of 73.5% and 81.8% and specificity of 94.8% and 95.7% respectively. Therefore, on the basis of Mentzer' index and RDW we can diagnose β-thalassemia minor in our routine CBC. We focus on inclusion of routine CBCs in pre-marital counseling, thereby hoping for a prevention of upcoming β-Thalassemia Majors.

Keywords: β- Thalassemia Trait, Iron deficiency, CBC, Mentzer's Index, Red Cell Distribution Width, Pre-marital Counselling.

Introduction

β- Thalassemia belongs to a heterogenous group of disorders categorized by a genetically determined reduction in the synthesis rate of β- globin chains of the hemoglobin molecule. The β chains are synthesized independently in balanced amount, apart from the α-chains, under a separate genetic control. [1] Thalassemia (as a whole) can be categorized into two types. If the α-chains are affected, it is known as α- Thalassemia and if the β chains are affected, it is called β- Thalassemia. Out of the two, β-thalassemia is more prevalent in the Mediterranean countries, North Africa, the Middle East, India, Central Asia, and Southeast Asia.[2]

β- thalassemia or Iron deficiency frequently cause microcytic anemia. On the basis of certain hematological parameters only, β-thalassemia is inappropriately differentiated with microcytic hypochromic anemia. Similarly, in the case of iron deficiency, the mean cell volume (MCV), mean cell hemoglobin (MCH), hemoglobin and RBC count, tend to decrease. Therefore results in both microcytic hypochromic anemias can overlap.[2] Many formulas and indices that have been proposed, pose the ability to differentiate iron deficiency from β-thalassemia trait

by using simple formulas that include a minimum of two CBC (Complete Blood Count) parameters (Hb, MCH, MCV, RBC count, red cell distribution width [RDW]) in various combinations. A confirmatory differential diagnosis between β- Thalassemia trait and iron deficiency is based HbA2 electrophoresis and serum-ferritin iron levels. The only purpose of using CBC indices to discriminate the microcytic hypochromic anemias is to detect a high probability of requiring appropriate follow-up and to reduce unnecessary diagnostic costs. Since 1970s, it has been determined that a number of CBC indices have been proposed to act as simple and inexpensive tools to investigate whether the blood sample is more suggestive of β-thalassemia trait or iron deficincy.[3] For an index to be ideal, it is required that it should have a sensitivity (i.e, it can detect maximum number of patients) and high specificity (i.e, it should be capable of eliminating patients with iron deficiency). Our study focuses on a comparative study between Mentzer's index and RDW. The Mentzer's index is defined as Mean Corpuscular Volume per Red Cell Count (MCV/RBC Count), a value < 13 states that the patient has β-thalassemia trait, while a value >13 suggests that the patient has iron deficiency. The principle involved is as follows: In iron deficiency, the bone marrow cannot produce as many RBCs and they are microcytic, so the RBC count and MCV both will be low, as a result, the value will be >13. Conversely, in β-thalassemia, the number of RBC's produced is normal, but the cells are smaller and more fragile. Therefore, the RBC count is normal but the MCV is low, so the value will be < 13.[4] Similarly, RDW critically defines microcytic hypochromic anaemia where an index of >18 shows IDA and index <18 indicates thalassemia trait.

Materials and Methods

This study was done retrospectively for the samples collected from Rajasthan since last one year (January 2015-December 2015) for Hb Electrophoresis. EDTA blood samples collected from 255 patients for Hb electrophoresis and CBC test were evaluated. Automated cell counter was used for CBC test and Capillary electrophoresis for Hb electrophoresis. The blood samples were processed in the central laboratory of Dr. B. Lal Clinical Laboratories at Malviya Nagar Industrial Area, Jaipur (Rajasthan). Mentzer's index was then calculated (MCV/RBC count) for each case. Individual reports of the patients were then analyzed on the basis of parameters given in the table below:

Table1. *Differentiation of Iron Deficiency and Thalassemia Minor (trait)*

Parameters	Normal Range	Thalassemia Trait	Iron Deficiency
Hb (gdl^{-1})	13-17(M) 12-15(F)	11-13 (M) 10-12 (F)	<9.3 (M or F)
MCV (fL)	83-101	Very decreased (<<70)	Decreased
RBC (μl^{-1})	4.5-5.5	Normal or Increased(>5.5)	Usually decreased (<4.2)
Mentzer's index	-	<13	>13
Hb A_2 (%)	2.2%-3.2%	>3.2%	<3.2%
RDW (%)	11.4%-15%	Normal or slightly increased	Increased

Results

A total study of 255 patients was done, out of which 130 were completely normal. 114 patients had symptoms of either thalassemia trait or IDA. Among these, patients having sure-shot IDA were excluded from the study. Also 11 other cases which included Hb D Punjab, Persistent Hb F and Heterozygous Hb E were not considered. On behalf of Mentzer's Index and RDW following was the study:

Table2. *Cases classified*

	Mentzer's index	RDW
True positive	25	27
False positive	11	9
True negative	202	202
False negative	9	6

Further, the prevalence of disease, sensitivity and specificity, positive and negative predictive values and accuracy of Mentzer's index and RDW were calculated for the conformation and verification of the present study.

Prevalence of disease= [(True positive + False negative)/Total cases]*100

Sensitivity= [True positive/ (True positive + False negative)]*100

Specificity= [True negative/ (True negative + False positive)]*100

Positive predictive Value= [True positive/ (True positive + False positive)]

Negative predictive Value= [True negative/ (True negative + False negative)]

Accuracy= [(True positive+ True negative)/ (True positive+ False positive+ False negative+ True negative)]

Table3. *Results*

Parameters	Range	Sensitivity	Specitivity	PPV	NPV	Youden's Index	Accuracy	Prevalence of Disease
Mentzer's Index	<13	73.5%	94.8%	69.4	95.7	0.67	91.9%	13.3%
RDW	<18	81.8%	95.7%	75.0	97.1	93.8	93.8%	12.9%
Hb A_2	2.2-3.2	-	-	-	-	-	-	-

Discussion

The discrimination between iron deficiency and thalassemia has an important clinical implication. Therefore, a reliable diagnosis is needed in order to reduce unnecessary laboratory testing and avoid inappropriate treatment. A wide range of parameters are available to facilitate this differentiation between iron deficiency and thalassemia. However, no single marker or any combination of tests will be optimal for this discrimination. [5] Iron deficiency often occurs in combination with other diseases that complicate the differential diagnosis. It regulates the Hb A2 synthesis, resulting in reduced HbA2 levels in patients with iron deficiency. An increment in the HbA2 level is, so far, the most significant parameter for identifying β-thalassemia carriers.[6] On the other hand, patients with thalassemia and concomitant iron deficiency may show normal or low HbA2 levels. Hence, diagnosing patients with concomitant thalassemia and iron deficiency is even more challenging. Thalassemias are common recessive autosomal disorders. β-thalassemias posses heterogeneity at the molecular level, with > 150 molecular defects identified till date.[7] Despite of this, every "at-risk" population has its own spectrum of some common mutations, usually from 5 to 10.[10] A homozygosity for β thalassemia leads to THALASSEMIA MAJOR, which is often "Transfusion-Dependent" and, rarely "Non-Transfusion Dependent" in mild conditions (molecular diagnosis is used to define genotypes with mild forms). Application of the CBC indices is recommended for screening iron deficiency and β-thalassemia. The main idea of using different indices in microcytic hypochromic anemia discrimination is to screen the patients having a high probability of requiring appropriate follow-up to reduce unnecessary investigations and costs. Reduction of healthcare budgets and increasing parameters available in hematological analyses make it necessary to provide support

and interpretation for a correct clinical diagnosis. All algorithms used for screening purpose should have good sensitivity scores to detect a maximum number of patients. On the other hand, these should be able to eliminate as many "other" patients (high specificity) as possible to avoid false positive results. A variety of formulae and indices have been described to facilitate the screening procedure of iron deficiency and β-thalassemia, that combine certain parameters obtained from a routine blood count. Well known conventional algorithms are those of Mentzer, England & Fraser, Shine & Lal, Ehsani and Shrivastava formulae. Comparing to these, we have found that Mentzer's index and RDW are much more specific indices in comparison to others (Table 4) [8]

Table4. *Comparison of different discrimination indices for differentiating β-TT from IDA according to the previously published standard cut-off values.*

Indices	Cut-off	PPV	NPV	Sensitivity	Specificity	Youden's index
Mentzer Index	<13	**69.44**	**95.7**	**73.5%**	**94.8%**	**0.67**
RDW	<18	**75.0**	**97.11**	**81.8%**	**95.7%**	**0.76**
England & Fraser Index	<0	96	66	74	94	0.68
Shine and Lal formula	<1530	90	100	100	91	0.91
Ehsani formula	<15	94	44	63	89	0.52
Srivastava formula	<3.8	95	29	57	84	0.41

In our study, Mentzer's Index, RDW and Hb A2 were taken into consideration. The prevalence of disease being 13%, the sensitivity, specificity and accuracy of our Mentzer's Index and RDW index were appreciatively high in comparison to other studies regarding the two. Some have reported a lower sensitivity of 67% in Mentzer's index while other studies have been higher with this index (82–95%) [9-12]. Talking about RDW, some have reported a sensitivity and specificity of 81.0% and 53.4% and a positive and negative predictive value of 63.0% and 72.2% respectively. This study has concluded that RDW has a limited specificity for screening purpose. Complementary to this study, our study says that RDW along with a combination of other index (Mentzer's index) is best capable of ruling out β-TT from IDA. Significantly higher RDW values are a known observation in iron-deficient patients, and these findings can increase the discrimination accuracy between thalassemia and IDA [13]. According to our study, these indices can be taken as the most reliable, most common and predictive for differentiating β-TT from IDA, by simply considering a routine CBC. Considering this can be helpful in not just preventing a very fatal disease, known as β-thalassemia major, but also eliminating the cause of this genetic disorder in the long run.

Conclusion

The present study was performed to promote screening programs to detect thalassemic heterozygotes (β-thalassemia trait). We used common hematological indices (included in a routine CBC). These indices were found to be highly specific, sensitive and most accurate method for detecting β-thalassemia trait. This study also disclosed that males are at the highest risk of being a carrier and this might be transferred to their progeny. The main idea of our study was to create awareness and to focus on pre-marital counseling so that any two individuals before getting married should undergo thalassemia screening in order to have a healthy progeny. It is reported that annually, over 9 million β-thalassemia carriers give birth to a new one. The risk that their partner is also a carrier varies from 0.1%–40% (global average being 14%). Also, there are minimum 948 000 new carrier couples and over 1.7 million pregnancies to carrier couples that happen annually and out of them about 75% are actually at stake. [14] They all need some expert risk assessments and genetic counseling. Two thalassemia minors may produce a thalassemia major which is a fatal disease, but on the other hand, if one of the partners is thalassemia minor and the other is negative, the coming offspring can either be a carrier or negative. Hence to prevent this life threatening disease and produce healthier offsprings each individual should undergo a routine CBC test.

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