

Original Research Article

Validation of new indices for differentiation between iron deficiency anemia and beta thalassemia trait, a study in pregnant females

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ABSTRACT

Background: The two most frequent types of microcytic hypochromic anemia in developing countries are beta thalassemia trait (β -TT) and iron deficiency anemia (IDA). Several indices using blood cell count parameters have been suggested to differentiate between two. In this study we evaluated the reliability of three indices.

Methods: The present cross-sectional study was carried out in 300 pregnant females in third trimester. Out of these, 90 microcytic hypochromic females were selected. Mentzers index, red cell distribution width index and Matos and Carvalho index (MCI) were calculated from the regular 3-part analyser haematological parameters.

Results: The Mentzer index was the most reliable index, as it had the highest sensitivity (97.62%), specificity (66.67%), and Youden's index (64%) for detecting Iron deficiency anaemia; the Matos and Carvalho Index showed higher sensitivity (98.81%) but a much lower specificity (33.3%) and Youden's index (32%) while the red cell distribution width index showed the sensitivity and specificity of 92.86% and 66.67%, respectively with Youden's index of 59%.

Conclusions: The Mentzer index provided the highest reliabilities for differentiating IDA from β -TT. The new indices have high sensitivities and positive predictive value for identifying IDA, making them useful for screening population at risk.

Keywords: Red blood cell indices, Microcytic hypochromic anaemias, Lower socio-economic class

INTRODUCTION

Iron Deficiency Anaemia (IDA) and beta thalassemia trait (β TT) are the most common causes of microcytic hypochromic anaemias. As maintained by World Health Organization (WHO) estimates in 2004, there were 2,73,000 deaths due to Iron deficiency anaemia (IDA) along with 19.7 million disability adjusted life years. Approximately 1.3% cases were recorded globally with 93% in developing countries like India.¹ The other differential diagnosis i.e. β -TT is the most common type of hemoglobinopathy transmitted by heredity. It is estimated that about 50% of the world's population with

β -TT is in Southeast Asia.² It is mandatory to differentiate between the two as prognosis and treatment are distinct.

Microcytic hypochromic anaemias are diagnosed microscopically in conjunction with red blood cell (RBC) indices. Due to overlapping features between IDA and TT, complementary lab methods are needed. Diagnosis of IDA is done by evaluating the iron metabolism, including serum iron, total serum iron binding capacity and serum ferritin measurements. Whereas diagnosis of the β thalassemia trait (β TT) rests on HPLC (high performance liquid chromatography) with HbA2 levels (>3.5%).^{3,4}

Despite their great utility, gold standard tests for the diagnosis of these microcytic and hypochromic anemias involve time consuming methodologies, high costs and are inaccessible to lower socio-economic class of people.³⁻⁶

In an attempt to simplify the differential diagnosis between IDA and TT, several indices using blood cell count parameters have been suggested.⁷⁻¹¹ Individuals with the beta thalassemia trait (β -TT) are usually asymptomatic and may be unaware of their carrier status unless diagnosed by haematological tests. Due to the migration and intermarriage of different ethnic populations, β -TT is found in people with no obvious ethnic connection to the disorder. A definitive differential diagnosis between β -TT and IDA is based on the result of HPLC, serum iron levels, and a ferritin values.¹²

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.¹³⁻¹⁵ RDWI is more advantageous as all the discriminating factors including RBC count, MCV (mean corpuscular volume) and RDW are incorporated in its formula.¹⁶ Derived indices like an index of RDW can be calculated using the automated blood cell counters for differentiation between IDA and β TT. The RDW measures the average RBC size variation, calculated by the RBCs histogram and is calculated as a standard statistical value, the coefficient of variation of the volume distribution. According to few studies, RDW is the first index to become abnormal in iron deficiency.¹⁷⁻¹⁹ A rather improvised index, RDWI has proven to be a reliable discrimination index in the differentiation of β TT and IDA.¹⁶ According to the ROC curve, the RDWI conferred with a cut off value of 220 to discriminate between IDA and TT. If the index is <220, the patient is tabulated as β TT patient, while values >220 classified as a IDA.¹⁶

The Mentzer's index is calculated using mean corpuscular volume and red blood cell count. If (MCV(fl) / RBC count (millions per microliter)) is less than 13, β TT is said to be more likely. If the result is greater than 13, then iron-deficiency anaemia is said to be more likely.

The adjusted formula of the new index was Matos and Carvalho index (MCI). According to the ROC curve, the MCI conferred with a cut off value of 23.85 to discriminate between IDA and TT. If the index is <23.85, the patient is tabulated as an IDA patient, while those with >23.85 are classified as a β TT.⁶

The aim of this study is to validate the new indices (RDWI and MCI) to discriminate between IDA and β TT, employing very simple parameters provided by all automated cell counters.

METHODS

Present cross-sectional study was conducted at Pathology department of a tertiary care hospital from July 2016 to December 2016. The subjects included all pregnant females in the third trimester of pregnancy, attending regular antenatal clinic (300). Of these subjects, 90 pregnant females with microcytic hypochromic blood picture were included in the study group for which consent was taken from all the participants.

Five ml of venous blood was taken, out of which three ml was taken in EDTA vial and remaining two ml blood was collected in plain vial. The haematological parameters, haemoglobin along with RBC indices (PCV, MCV, MCH and MCHC) were analysed by three-part haematology analyser. Two peripheral blood smears were also made and stained by Leishman's stain for each case. Serum ferritin and HPLC (High Performance Liquid Chromatography) was done on all the samples with microcytic hypochromic blood picture. The cationic exchange column chromatography enables quantitative determination of HbA₂, HbF and abnormal haemoglobins. 10 μ l haemolysis mixture prepared by diluting 5 μ l of whole blood with 1 ml haemolysis reagent was injected into HPLC system. A flow rate of 1.5 ml/min, with an analytical run time of 6.5 min as recommended in kit protocol, was set. A cut off Hb A₂ level of $\geq 3.5\%$ was used for diagnosing thalassaemia trait.

Serum ferritin was done by the principle of microplate immunoenzymometric assay. Serum ferritin level of <10 μ g/dl was taken as cut off for diagnosis of iron deficiency anaemia.

We used the following cell counter-based formulas in our study:

Mentzer's index: MCV/RBC count;

RDWI: MCV \times RDW/RBC

MCI: (1.91 \times RBC)+(0.44 \times MCHC).

Youden's index (YI): (sensitivity+specificity)-100

The results were analysed statistically by using SPSS version 16.0.

RESULTS

The Mentzer index was the most reliable index, as it had the highest sensitivity (97.62%), specificity (66.67%), and Youden's index (64%) for detecting Iron deficiency anaemia (Table 1).

Red cell distribution width index showed the sensitivity and specificity of 92.86% and 66.67%, respectively with Youden's index of 59% (Table 2).

Table 1: Mentzer's index.

Statistic	Value (IDA)	95% CI (IDA)	Value (β TT)	95% CI (β TT)
Sensitivity (%)	97.62	91.66-99.71	66.67	22.28-95.67
Specificity (%)	66.67	22.28-95.67	97.62	91.66-99.71
Positive likelihood ratio	2.93	0.94-9.08	28.00	6.36-123.20
Negative likelihood ratio	0.04	0.01-0.16	0.34	0.11-1.06
Disease prevalence (%)	93.33	86.05-97.51	6.67	2.49-13.95
Positive predictive value (PPV) (%)	97.62	92.97-99.22	66.67	31.25-89.80
Negative predictive value (NPV)(%)	66.67	31.25-89.80	97.62	92.97-99.22
Youden's index	+ 0.64			

Table 2: Red cell distribution width index (RDWI).

Statistic	Value (IDA)	95% CI (IDA)	Value (β TT)	95% CI (β TT)
Sensitivity (%)	92.86	85.10-97.33	66.67	22.28-95.67
Specificity (%)	66.67	22.28-95.67	92.86	85.10-97.33
Positive likelihood ratio	2.79	0.90-8.65	9.33	3.59-24.29
Negative likelihood ratio	0.11	0.04-0.28	0.36	0.12-1.11
Disease prevalence (%)	93.33	86.05-97.51	6.67	2.49-13.95
Positive predictive value (PPV) (%)	97.50	92.62-99.18	40.00	20.39-63.43
Negative predictive value (NPV) (%)	40.00	20.39-63.43	97.50	92.62-99.18
Youden's index	+0.59			

Table 3: Matos and Carvalho index (MCI).

Statistic	Value (IDA)	95% C.I (IDA)	Value (β TT)	95% C.I (β TT)
Sensitivity (%)	98.81	93.54-99.97	33.33	4.33-77.72
Specificity (%)	33.33	4.33-77.72	98.81	93.54-99.97
Positive likelihood ratio	1.48	0.84-2.61	28.00	2.94-266.48
Negative likelihood ratio	0.04	0.00-0.34	0.67	0.38-1.19
Disease prevalence (%)	93.33	86.05-97.51	6.67	2.49-13.95
Positive predictive value (PPV) (%)	95.40	92.17-97.34	66.67	17.37-95.01
Negative predictive value (NPV) (%)	66.67	17.37-95.01	95.40	92.17-97.34
Youden's index	+0.32			

Matos and Carvalho index showed higher sensitivity (98.81%) but a much lower specificity (33.3%) and Youden's index (32%) (Table 3).

DISCUSSION

The requirement of simple distinguishing parameters between IDA and β TT in a patient presenting with hypochromic microcytic blood picture is needed since long, as several studies have pointed out the direct effect of coexisting IDA on HbA2 synthesis resulting in confusing levels of HbA2 in β TT and the MCV in such patients would not improve on continued iron therapy.¹⁷ The differentiation between β TT and IDA, requires Hb A2 estimation by HPLC, examination of a peripheral blood film, serum ferritin, iron, TIBC, and transferrin saturation. But being relatively expensive and time-consuming, it is preferred to rely on simple and already available information. Many investigators have used different mathematical indices to distinguish β -TT from IDA using complete blood count. This process helps to

select appropriate individuals for a more detailed examination; however, no study has found 100% specificity or sensitivity for any of these RBC indices. This problem urged us to compare the value of popular discrimination indices.

Sensitivity and specificity are two of the very important parameters required by a screening test to be validated. Diagnostic ability of a test method highly depends on these parameters. In Mentzer index (Table 1), sensitivity (Sn) and positive predictive value (PPV) of the index was very high (97.62%) for identifying IDA patients. However, specificity (Sp) and negative predictive value (NPV) of the index for IDA was low (66.67%). Specificity (Sp) and negative predictive value (NPV) of the index was very high (97.62%) for identifying β TT patients. However, sensitivity (Sn) and positive predictive value (PPV) of the index for β TT was low (66.67%). Overall the validity of the index, according to Youden's index was +0.64. The YI takes into account both sensitivity and specificity and gives an appropriate

measure of the validity of technique.^{18,19} Ehsani et al reported a sensitivity and specificity of 95.5% and 94.6% respectively for Mentzer index with overall validity, by Youdens index, of +0.91.²⁰ Ntaios et al reported a Sn and Sp of 59.78% and 99% respectively with YI of +0.58.²¹ Hence Mentzer index showed high validity in this study and these results are comparable to other studies.

Ehsani et al showed that the best discrimination index according to Youden's criteria was the Mentzer index (90.1%).²⁰ In their study, the Mentzer indices were able to correctly diagnose 94.7% and 92.9% of cases, respectively, and both are easy to calculate.²¹ Similar results (Mentzer index: sensitivity, 90.9%; specificity, 80.3%) were found by Ghafouri et al.¹⁹ Their results overlapped those of our study. In the present study, YI evaluated the discriminating function of the red cell indices and their derived formulae. In our study, the highest YI was obtained for Mentzer's index which provided with the most important discriminating index between IDA and β TT.

Red cell distribution width (RDW) (Table 2) is provided in CBCs by the automated analysers and can be utilized in the association with a derived value RDWI to distinguish IDA and β TT.²² RDWI denotes anisocytosis. Its value is increased in IDA, and it is near normal or mildly increased in β TT. RDWI came out as good discriminator between β TT and IDA. The sensitivity and specificity of RDWI in the detection of β TT were found 66.67% and 92.86%, respectively and the sensitivity and specificity for the detection of IDA were 92.86% and 66.67%, respectively. RDWI also showed a high PPV in IDA. These results are consistent with the findings of other relevant studies.^{19,20} The Youdens index was calculated as +0.59.

MCI (Table 3) was also evaluated for discriminating between IDA and TT. This formula produced high sensitivity and excellent diagnostic accuracy. MCI also revealed high PPV (95.40%), showing good applicability as a screening tool in the clinical practice. An important result obtained for MCI is the high sensitivity to detect IDA (98.81%). As sensitivity is a parameter that indicates the proportion of individuals correctly identified with the disease, the high sensitivity of the MCI in the detection of IDA indicates that it is a good tool to identify this disorder.

One may argue that a discriminative index that correctly diagnoses IDA would clinically be more valuable compared to those that correctly diagnose TT. This is because one month of iron supplementation to a patient mistakenly classified as having IDA who in fact has β TT, causes less damage than the lack of this supplement to a patient with IDA who was mistakenly classified as a β TT. Iron and erythropoietin are required for the formation of hemoglobin. Furthermore, iron is a component required in critical cellular processes such as the transport and utilization of oxygen, production of adenosine

triphosphate (ATP), DNA synthesis, metabolism of catecholamines, mitochondrial electron transport and other physiological processes. Thus, biological systems including the immune and neurological systems, are all affected by the lack of iron. For these reasons the lack of treatment for IDA would be very harmful to the individual. It should be noted that these three parameters are obtainable from most simple cell counters, therefore sophisticated automatic counters are not required. Hence, the MCI can be applied in areas where advanced technologies are not available in clinical labs. Despite the advantages and simplicity of the implementation of the MCI in the laboratory practice, there is a limitation of MCI and other discriminating formulas since they are not able to differentiate all cases of IDA from TT.

However, the new index may be a suitable alternative, if not a replacement, to the Mentzer index because the difference between the performance of these two indices in our series was attributable to the lower specificity of the new index in diagnosing IDA, a problem which can be partly resolved by IDA patients' suggestive history and manifestations in physical examination. Also, two indices can be collaborated to provide a more accurate discrimination decision. Due to remarkable inconsistencies between the results obtained so far, it is not possible to choose one discrimination index as the most appropriate and the issue awaits future large studies.

CONCLUSION

In closure, the automated cell-count-based indices, particularly the Mentzer index, RDWI and MCI are easily available and reliable methods for detecting IDA & differentiating it from β -TT. According to our results, the sensitivity and Youdens index was the highest with the Mentzer index. Both RDWI and MCI, the new indices also showed very high sensitivity and PPV hence appearing to be a reliable and useful index for initial screening of microcytic hypochromic anemia. This would result in a significant cost saving for the health system, especially advantageous in underdeveloped and developing countries with limited financial resources.

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