ORIGINAL ARTICLE

Evaluation of five discriminating indexes to distinguish Beta-Thalassemia Trait from Iron Deficiency Anaemia

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Abstract

Objective: To assess the reliability of different red blood cell indices-based formulae in the indexes formula in differential diagnosis of beta thalassemia trait and iron deficiency anaemia.

Methods: This cross-sectional study was conducted between January and October 2015 in Dera Ismail Khan in the Khyber Pakhtunkhwa province of Pakistan. Patients of beta thalassemia trait and iron deficiency anaemia were registered irrespective of age and gender. About 5 mL of blood was taken from each patient to analyse different red cell parameters like red blood cell count, haemoglobin, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and red cell distribution width. Five formulae were used to discriminate between the two conditions. These were red cell distribution width index, Shine and Lal index, Mentzer index, Srivastava index, and the Green and King index. Sensitivity, specificity, positive and negative predictive values and Youden's index of all the indices were calculated.

Results: Of the 800 patients, 230(29%) had beta thalassemia trait and 570(71%) had iron deficiency anaemia. The red cell distribution width index appeared be a reliable index in discriminating between beta thalassemia trait and iron deficiency anaemia with sensitivity and specificity of 100% and 93% respectively.

Conclusion: The red cell distribution width index was the most consistent index for differentiating between beta thalassemia trait and iron deficiency anaemia. IIt could be used as a screening index for beta thalassemia trait in areas where haemoglobin electrophoresis facility is unavailable.

Keywords: Beta-thalassemia trait, Iron deficiency anaemia, Red cell parameters, Hb electrophoresis, Red cell distribution width. (JPMA 66: 1627; 2016)

Introduction

Beta-thalassemia trait (BTT) and iron deficiency anaemia (IDA) are the most commonly encountered disorders of hypochromic and microcytic anaemia.[1,2] A recent report by World Health Organisation (WHO) shows that out of 2 billion anaemia cases, 50% are IDA.[3] High incidence of iron deficiency in developing and under-developed countries are associated with countless morbidities. Frequently it is mild but, if not treated, can lead to maternal haemorrhage due to uterine atony, arrhythmia, pregnancy-related complications, delayed growth in infants and children, and even mortality. Blood loss, parasitosis, iron-poor diet, malabsorption, multiparity, poor health of pregnant women and socio-economic burden are the key factors for IDA.[4-6]

Thalassemia, an inherited haemoglobinopathy, is characterised by impaired synthesis of normal globin peptide chains in haemoglobin (Hb). It is the most common genetic disorder, and its prevalence and severity are population-dependent with the type of thalassemia seen dependent on racial background. About 5% of the world's population has Hb production defects and 7% of carrier for Hb disorders.[7] Annually 0.3-0.5 million children are born with severe haemoglobinopathies; 80% of these children are from developing and underdeveloped countries.[7]

About 2-18% of Eastern Mediterranean population, 0-11% Southeast Asian and 0-12% in Sub-Saharan Africa harbour the thalassemia gene.[8] Earlier, it was thought that North America and Northern Europe were non-endemic regions for thalassemia, but now these regions also have high incidence of thalassemia due to immigration of people from thalassemia-endemic regions.[9,10]

Thalassemia is a major global health problem; 10% is contributed by the Middle East, 9% by Southeast Asia, and 8% from the Mediterranean region.[11] Each year 5000 cases of homozygote thalassemia patients are born in Pakistan, making it one of the most prevalent inherited disorders of the country[11] and about 5% of Pakistanis carry the heterozygous beta thalassemia gene.[12]

Definite diagnostic parameters for BTT and IDA are serum

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iron, ferritin and haemoglobin alpha 2 (HbA2) electrophoresis.[13] Gold standards for measurement of HbA2 level for the BTT diagnosis are cellulose acetate or gel-electrophoresis and high-performance liquid chromatography techniques.[14-16]

Different red cell indices provided by electronic haematological cell counter can be used to discriminate BTT and from IDA. The purpose of using indices is to reduce unnecessary investigation cost on discriminating BTT from IDA. Various red cell indices have been proposed in the past as easy and economical tools to discriminate BTT or IDA.[17-21] Using red cell indices or parameters like mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC) and red cell distribution width (RDW), one can differentiate BTT from IDA. Best discrimination index which has high specificity and sensitivity is the one which can detect the maximum number of BTT patients (high sensitivity) while taking out patients with IDA (high specificity). The current study was conducted to check the ability of 5 different indices-based formulae by calculating their sensitivity and specificity.

Material and Methods

This cross- sectional study was conducted between January and October 2015 in Dera Ismail Khan, a city situated on the west bank of the Indus River in Khyber Pakhtunkhwa province of Pakistan. After approval from the ethics committee of Gomal Medical College (GMC) and Hayat Medical Lab, and after taking proper informed consent from all patients/parents, blood samples were collected.

The study population comprised microcytic hypochromic anaemia patients referred for Hb electrophoresis to the GMC pathology lab. Patients included were aged 2-35 years of either gender with HB ranging from 8 g/dl to 11.5 g/dl. Patients having recent blood transfusion in the preceding four weeks and those who were critically ill were excluded. By using aseptic venepuncture blood collection, about 5mL of blood was collected and divided into Ethylenediaminetetraacetic acid (EDTA) and plain gel tube for biochemical and haematological analysis. Serum was collected from plain gel tube after centrifugation at 3000 RPM for 5 minutes for serum iron which was analysed by using Merck kits on Rayto (RT-9200) chemistry analyser.

Using an automated haematology analyser (Nihon Kohden, Tokyo), complete blood parameters, including Hb, red blood cell (RBC) count and RBC indices, like MCV, MCHC, MCH, packed cell volume (PCV) and RDW, were performed. For estimation of HbA2 level of BTT, alkaline cellulose acetate electrophoresis method (Wealtech, USA) was used.

SPSS 19 was used for statistical analysis. For screening performance of various indices for BTT, receiver operating characteristic (ROC) curve was used. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using previously suggested formulae.[22,23]

Results

The five discrimination indices used in the evaluation were calculated and summarised (Table-1).

Of the 800 blood samples analyzed, 570(71.3%) were labeled IDA after serum iron analysis and 230(28.8%) isolates as BTT after Hb electrophoresis (Table-2).

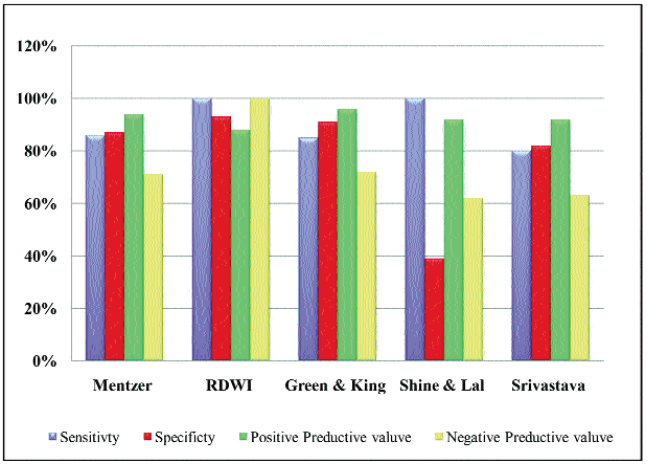

Figure-1: Sensitivity, specificity and predictive values of the five indices.

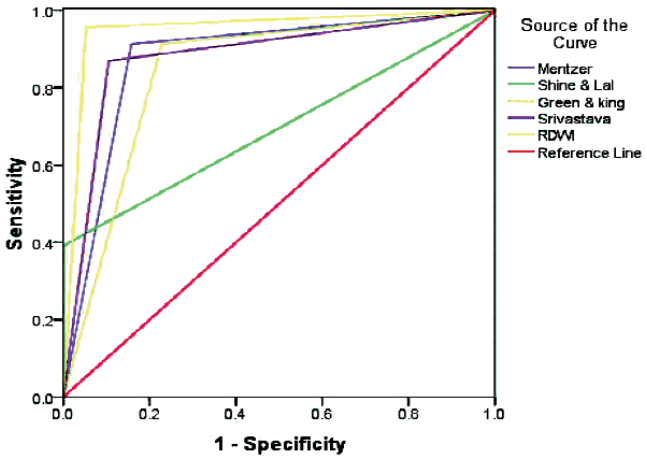

Figure-2: Receiver operative characteristic curve (ROC) of five indices.

Table-1: Red cell parameter and the five haematological indexes.

Hematological Index	Formulae	BTT Cut off value	IDA Cut off value
Mentzer[28]	MCV/RBC	< 13.0	> 13.0
RDWI[14]	MCV x RDW/RBC	< 220	> 220
Shine & Lal[29]	MCV x MCV x MCH/100	< 1530	>1530
Srivastava[30]	MCH/RBC	< 3.8	> 3.8
Green & King[17]	MCV x MCV x RDW/Hb x 100)	< 72	> 72

BTT: Beta thalassemia trait
IDA: Iron deficiency anaemia
Hb: Haemoglobin
RBC: Red blood cell
MCV: Mean cell volume
MCH: Mean cell haemoglobin
RDW: Red cell distribution width.

Table-2: Results range of biochemical and haematological parameters in BTT and IDA declared patients.

Parameters	IDA (n=570) Mean ± SD	BTT(n=230) Mean ± SD
Hb (g/dL)	9.25 ± 1.1	10.7 ± 1.2
RBC ($\times 10^6$/L)	4.0 ± 0.3	6.3 ± 0.7
HbA2 (%)	2.0 ± 0.3	6.1 ± 0.8
MCV (fl)	55.3 ± 5.2	67.2 ± 6.2
MCH (pg)	21.1 ± 3	19 ± 1
RDW (%)	20.2 ± 1.9	14.2 ± 1.4
Serum Iron (ng/mL)	7.54 ± 3.2	33.75 ± 22.9

BTT: Beta thalassemia trait
IDA: Iron deficiency anaemia
Hb: Haemoglobin
RBC: Red blood cell
HbA2: Hamoglobin alpha 2
MCV: Mean cell volume
MCH: Mean cell haemoglobin
RDW: Red cell distribution width.

RDW Index (RDWI) established the highest sensitivity and specificity of 100% and 93% respectively. Not a single index provided 100% sensitivity and specificity (Figure-1). The highest diagnostic values based on the area under the ROC curve were related to the RDWI (Figure-2).

Discussion

Pakistan, an agricultural country, is struggling to defeat IDA, the most prevalent nutritional deficiency all over the globe. In Pakistan, prevalence of IDA among child-bearing age women is 50%, in children it is 65-78% while about 39% of adolescents are suffering from this deficiency.[11]

The two most common aetiologies of microcytic anaemia i.e., BTT and IDA, are seen in both children and adults. It is mandatory to differentiate BTT from IDA, because diagnosis of BTT often becomes confusing due to its overlapping and similar features with IDA in routine laboratory results.[24] However, the prognosis, causes and treatment of these clinical conditions are entirely different.[22] There are several similarities in red cell indices of these two groups, for instance reduced Hb, MCV, MCH, microcytosis and hypochromia.[14,25]

A number of RBC indices and formule have been devised. The purpose of this study was to pick the most accurate formula which could show highest sensitivity and specificity in discriminating BTT from IDA. This formula would help to classify the patients with hypochromic microcytic anaemia into two most important categories, so specific tests can be asked for i.e., serum iron (SI) or Hb electrophoresis. It will save time and reduce diagnostic expenses.[26,27]

Different mathematical formulae based on red cell indices have been used by researchers to differentiate BTT from IDA, but none has been found to be 100% sensitive and specific. In this study, we checked the sensitivity, specificity, PPV and NPV of various RBC indices-based formulae. We applied five formulae on each enrolled case of microcytic hypochromic sample to differentiate BTT from IDA.[14,17,28-30] RDWI index came up with the most dependable index with sensitivity of 100% and specificity of 93%. Our results are consistent with previous studies which showed that RDWI is the most reliable formula to differentiate these two conditions.[23,24,31] A Spanish group of haematologists also reported RDWI as the most reliable index.[15] A study[32] reported Shine and Lal formula as the best index for BTT, although in our study Shine & Lal had 100% sensitivity but only 39% specificity.

One study.[33] suggested that Green & King's index is the best discriminator, but in our study it was the second best index after RDWI. Another study found that formulae presented by Shine and Lal, Srivastava and Mentzler had good discriminative function.[34]

Many studies have shown that these formulae are only 61-91% successful in properly classifying whether hypochromic and microcytic anaemia is due to BTT or IDA. All the formulae showed overlapping results.[26] These results could be due to different values in different studies so there is a need to define separate cut-off values for each population.

We recommend future studies with a larger sample size to accurately determine the sensitivity and sensitivity of various formulae in discriminating BTT from IDA.

Conclusion

RDWI appeared to be the most reliable formula in differentiating BTT from IDA in patients with hypochromic microcytic anaemia. For mass screening of microcytic hypochromic anaemia, this formula can be used in areas lacking facilities for Hb electrophoresis.

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