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Determination of Efficacy and Reliability of Various Discrimination Indices in Screening Beta Thalassemia Trait

Saima Khan[1]*, Sayeedul Hasan Arif[1], Zeeba Zaka-ur-Rab[2] and Mahboob Hassan[1]

1 Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

2 Department of Pediatrics, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

***Corresponding author:**
Saima Khan

saima.khan1501@gmail.com

Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

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Abstract

Background: Beta-thalassemia trait (βTT) is a common hemoglobinopathy and imposes a significant burden on the global healthcare. Screening of this disorder is immensely important as it can significantly reduce the future incidence of thalassemia major. This study was conducted in order to evaluate the role of discrimination indices to differentiate cases of βTT from other causes of microcytic anemia.

Materials and Methods: A single-center study was carried out on 1600 subjects reported during November 2015 to November 2017.Tests for serum iron and ferritin were also conducted in individuals showing low MCV. All the selected samples were subjected to complete blood counts and blood morphology. Cellulose acetate alkaline electrophoresis and iron profile were carried out to differentiate IDA and beta thalassemia trait. Comparison of various parameters and discrimination indices were calculated for all the samples.

Results: Shine and Lal index, Mentzer index and red cell distribution width index had revealed better discriminative function compared to England and Fraser index, Srivastava index, and Green and King Index.

Conclusion: Though HbA2 estimation is the gold standard for diagnosing βTT, in developing countries, discrimination indices are rapid, reliable and easy tools to screen cases of βTT and send the subject for further electrophoresis and high performance liquid chromatography evaluation.

Keywords: β-Thalassemia trait; Hemoglobinopathy; Discrimination index

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Introduction

The most common cause of anemia in India and other developing countries is Iron Deficiency Anemia (IDA) and the most common nutritional deficiency worldwide. Lack of sufficient iron to synthesize hemoglobin leading to anemia is the most common hematological disease. As iron is an important constituent in the synthesis of hemoglobin, a large number of women suffer from anemia throughout their lives. Poverty, Vegetarianism, lack of nutrition, high parity, bare foot walking leading to hookworm infestations all play roles in the prevalence of anemia [1]. Recent estimations state that about 30% of the global population suffers from iron deficiency anemia, most of them living in developing countries.

Among the most frequently encountered cases of microcytic hypochromic anemia, the major contributors are Iron deficiency anemia and thalassemia. Microcytic anemia in a case of thalassemia which is the most common hereditary hemoglobinopathy results from unpaired synthesis of globin chain and reduced hemoglobin synthesis causing microcytosis and hypochromia [2].

Although the cases of beta thalassemia major are easy to diagnose, milder forms such as β thalassemia trait go unrecognized or misdiagnosed as iron deficiency anemia most of time [3].

It is very difficult to differentiate between these two conditions clinically therefore, a battery of investigations like serum ferritin, serum iron, Total Iron Binding Capacity (TIBC), transferrin saturation, bone marrow studies and HBA2 levels are required for the same [4]. Even the peripheral blood film serves no help and creates great confusion when it comes to differentiate these disease states as they very closely mimic each other morphologically. But it is not feasible to perform these investigations in each case for diagnosis in poor and developing countries like India due to financial reasons, time constrains and unnecessarily invasive procedures. So there is need for an efficient diagnostic approach which can easily rule in or out the diseases with required accuracy and minimize testing in a cost-effective manner [4].

With the availability of such tests the cost of anemia workup would drop. Further, misdiagnosis of Thalassemia as IDA and unnecessary overloading the patients with iron therapy thereby worsening the condition could be avoided. On the other hand, chances of a potential homozygous offspring may also decrease leading to reduced thalassemia burden on the society.

Not many studies are conducted in the Northern Indian where the incidence of β-TT is high (3-15%) [5,6]. Seven Different discrimination indices were studied to find out the best to differentiate between β-TT and IDA by calculating their sensitivity, specificity and Youden's index values.

Materials and Methods

A total of 1600 cases of all age group and sex were included in the study after taking informed consent strictly following the inclusion and exclusion criteria. Institutional ethical clearance was taken for the study.

Inclusion and exclusion criteria

Cases which had hemoglobin level below their reference range for age as per WHO guidelines and MCV below 80 fl are included in the study.

Cases with history of fever within the last four weeks, those suffering from any chronic illness, those who were subjected to iron therapy in the past twelve weeks or those who had prior blood transfusion/s as well as pregnant females were excluded from the study.

Study design

1. Subjects with suspected discrimination indices for Thalassemia were taken for alkaline agarose gel hemoglobin electrophoresis (pH- 8.5).
2. Subjects with suspected discrimination indices for other causes of microcytic anemia were taken for Iron profile.
3. Subjects with normal iron profile, who had discrimination indices in favor of IDA, were taken up for alkaline agarose gel hemoglobin electrophoresis (pH- 8.5).

Venous blood was drawn taking all aseptic precautions after the patient had been lying quietly for at least 20 minutes. A fully automated hematological analyzer – Lab Life H3D Premier (RFCL Limited, New Delhi) was used to obtain complete blood cell counts and leukocyte differential counts. The semi-automated HYDRASYS HEMOGLOBIN (E) instrument by Sebia was used for agarose gel alkaline electrophoresis (pH 8.5) for estimation of HbA2 Serum iron (SI), serum iron binding capacity (SIBC), was carried by fully automated chemical analyser (Agappa Diagnostic Ltd). Iron saturation was calculated as the ratio of serum iron and total iron binding capacity. Serum ferritin values were determined by chemiluminescence by VitrosEci by orthodiagnostic (Johnson & Johnsons)

The cut off value for HbA2 was kept at 3.5%. Patients with HbA2 levels more than 3.5% were labelled as β-TT group and those with value less than 3.5% were labelled as IDA group.

Discrimination Indices

Seven discrimination indices were applied as defined in the original published reports [7-12]. England and Fraser, Mentzer, Srivastava, Shine and Lal, Ricerca, RDWI & Green and King were computed in the present study.

Hematological index formula

Mentzer index (MI) (1973)	MCV/RBC
RDWI (1987)	MCV × RDW/RBC
Shine and Lal (S and L) (1977)	MCV × MCV × MCH/100
Srivastava (1973)	MCH/RBC
Green and King (G and K) (1989)	MCV × MCV × RDW/Hb × 100
England and Fraser (E and F) (1973)	MCV − (5 × Hb) − RBC − 3.4
Ricerca (1987)	RDW/RBC

Sensitivity, Specificity, Positive predictive value, Negative predictive value and Youdens index were calculated as Sensitivity: True positive / (true positive + false negative), Specificity: True negative / (true negative + false positive), Positive predictive value: True positive / (true positive + false positive), Negative predictive value: True negative/ (true negative + false negative), Youden's index = (sensitivity + specificity) – 100 [13].

Statistical software SPSS 16 version was used for statistical analysis. P Values < 0.05 were considered significant.

Results

In the present study of 1600 subjects, the maximum number of patients belonged to the 21-30 years age group (496, 31.0%). Complete blood counts along with general blood picture were done and discrimination indices calculated. The mean values for hematological parameters were calculated **(Table 1).** Cases were subjected to Iron studies and alkaline haemoglobin electrophoresis.

On the basis of discrimination indices, 345 cases favored beta thalassemia trait, out of which 310 cases emerged as definite beta thalassemia traits having HbA2 levels of more than 3.5% **(Table 2).** On the other hand, 1255 cases selected for iron profile on the basis of discrimination index. Out of which 1220 were diagnosed as IDA **(Table 3).**

As is evident from the results shown in **Table 4**; none of the indices showed 100% efficiency in recognizing beta thalassemia trait. The Mentzer and RDWi indices exhibited the highest sensitivity of 90.3 % and 83.8% each but when it came to specificity, Shine and Lal index was the most specific (96.7%) followed by Mentzer (93.4%).

The highest and the lowest PPV were found for Shine and Lal index (85.1%) and England and fraser (26%) respectively. The Mentzer index demonstrated the highest NPV of 97.4%. The highest and the lowest values for Youden's index were shown by Mentzer (83.7%) and Green & King (10.6%) respectively. None of the indices was completely sensitive and specific in differentiating between BTT and IDA (**Table 5**).

Table 1: Showing the Mean Values and Standard Deviation of Various Hematological Parameters.

Parameter	Male	Female
	Mean ± SD	Mean ± SD
Hemoglobin (g/dl)	8.60 ± 2.11	8.04 ± 1.96
RBC (million/µl)	4.33 ± 5.00	3.76 ± 0.86
MCV (fl)	71.73 ± 7.31	71.30 ± 6.48
MCH (pg)	22.76 ± 7.88	22.17 ± 5.59
MCHC (g/dl)	30.25 ± 2.39	29.74 ± 3.02
RDW (%)	16.97 ± 2.18	18.66 ± 3.46

Table 2: Cases Selected For Alkaline Hemoglobin Electrophoresis On The Basis Of Discrimination Index / Indices.

No of Cases	Screened as βTT	Screened as non-βTT
1600	345	1255

Table 3: Distribution of total cases.

Total no of cases	Conclusively diagnosed as βTT	Conclusively diagnosed as IDA	Variants -HPFH	Other causes of microcytic anemia	Inconclusive (excluded from the study)
1600	310	1220	5	45	20

Table 4: Differential Values of Discrimination Indices and the Correctly Identified Cases.

S. No	Indices	β-TT (n= 310)	IDA (n= 1220)	Correctly diagnosed cases (TP + TN)	% of correctly identified patients
1	Mentzer β-TT<13 IDA>13	280(TP) 30 (FN)	80(FP) 1140(TN)	280+1140= 1489	92.8 %
2	England & Fraser β-TT<0 IDA>0	130 (TP) 180 (FN)	370 (FP) 850 (TN)	130 + 850=980	64.0%
3	Srivastava β-TT<3.8 IDA>3.8	240 (TP) 70 (FN)	320(FP) 900 (TN)	240 + 900= 1140	74.5 %
4	Shine and Lal β-TT<1530 IDA>1530	230 (TP) 80 (FN)	40 (FP) 1180 (TN)	230 + 1180 = 1410	85.6%
5	RDWi β-TT<220 IDA>220	260 (TP) 50 (FN)	320 (FP) 900(TN)	260 + 900 = 1160	75.8 %
6	Ricerca β-TT<4.4 IDA>4.4	180 (TP) 130 (FN)	240 (FP) 980 (TN)	180 + 980= 1160	75.8 %
7	Green and King β-TT<65 IDA>65	140 (TP) 170 (FN)	420 (FP) 800 (TN)	140 + 800 = 1160	61.8 %
True positives –TP, True negative – TN, False positive- FP, False negative – FN					

Table 5: Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Youdens Index of Each Discrimination Index.

S.no	Indices	Sensitivity	Specificity	PPV	NPV	Youdens Index
1	Mentzer	90.3%	93.4 %	77.7%	97.4 %	83.7
2	England & Fraser	41.9%	69.6 %	26.0 %	82.5%	11.5
3	Srivastava	77.4%	73.7 %	42.8 %	92.7 %	51.5
4	Shine and Lal	74.1%	96.7%	85.1%	93.6 %	70.8
5	RDWi	83.8%	73.7 %	44.8 %	94.7 %	57.5
6	Ricerca	58.0%	80.3 %	42.8%	88.2 %	38.3
7	Green and King	45.1%	65.5 %	46.4%	82.4 %	10.6

Discussion

Hemoglobinopathies constitute not a very unimportant causative factor for anemia. So the need for proper awareness and meticulous detection is over emphasized. The presumptive identification of hemoglobin disorders must rely on cost effective methods of detection, to allow an efficient use of the available resources. Additionally the diagnosis of hemoglobinopathies becomes important in situations where early detection and diagnosis can ameliorate the chances of the resultant homozygous offspring. It is therefore fruitful to recognize such screening tests which could be easy and reliable for diagnostic purposes in a nation where financial constraints are widely prevalent.

In Northern India, not much work has been done, especially Uttar Pradesh. The aim for present study is to find out best discrimination index so that microcytic hypochromic anemia can be screened to select out the possible case of βTT, so that only

such cases are subjected for further study for final diagnosis in a cost effective manner.

Initial studies done showed that a 100% sensitivity and specificity can be achieved when two or more discrimination indices are summed up. However subsequent studies done proved otherwise and estimated these indices sensitivity to be between 61-91% [14-18].

Combination of various discrimination indices, lead to a great improvement in discriminating IDA and βTT [19]. Mentzer and Shine & Lal index was the best discrimination index while minimum number of cases was compatible with Green and King, This result is ompatible with Piplani et al. [13].

Other causes of microcytic hypochromic anemia may misled the things as there are few cases which were not confirmed by either electrophoresis or iron profile, although sensitivity and specificity of discrimination indices are quite promisible as Shine & Lal showed 96.7 % specificity and Mentzer index showed 90.3% sensitivity [20].

Moreover, literature has documented more than 200 mutations in βTT so far, and according to the extent of the reduction of beta chain output, mutations have been divided into severe, mild and silent. It is these silent mutations (measured only be genetic studies) which are characterized by near normal hematological indices and borderline HbA2 levels leading to mislabeling of these individuals as normal [21].

Agarose gel alkaline electrophoresis (pH – 8.5), although the best module to confirm the diagnosis of βTT, but it is usually seen that when there is coexisting IDA, the HbA2 level is in boarder line range (3 – 3.8). In such cases iron deficiency must be corrected first and electrophoresis should be repeated [22].

Raised HbF levels were seen in 05 cases, who were the parents of thalassemia major children taken from thalassemia clinic in our center diagnosed as hereditary persistence of fetal hemoglobin (HPFH). HPFH patients pass their life asymptomatically but when they marry a thalassemia trait partner, there is a higher chance of developing β thalassemia major in offspring. When HPFH are found with β-thalassemia mutations, they have an ameliorating effect in the disease because of increased production of gamma chains [23].

Patients with thalassemia trait are characterized by an increase in RBC count as a result of the chronic increase in erythropoiesis with slightly low hemoglobin levels, low MCV and low RDW whereas higher MCHC were noted as compared to patients with IDA [24].

To find out the best discrimination index among the seven used in the study, validity was calculated, using true and false positives, true and false negatives cases of βTT and IDA. Mentzer index came out to be more valid discrimination index to differentiate between the above two conditions whereas England and Fraser and Green and King Indices had the least. Similar observation was also reported by Sehgal K et al. [25].

The ideal index must have high sensitivity and high specificity, as it detect maximum number of thalassemic trait patients and eliminate maximum non thalassemic patients as much as possible. Sensitivity, specificity, Positive and Negative predictive value and Youdens index of all seven discriminatory indices were calculated in present study. This observation of Mentzer index being the most reliable index because it had high sensitivity as well as high specificity was also observed in other studies [26]. As far as positive and negative predictive value is concerned the observation in present study is very much in accordance with Piplani et al. [13]. Although in some other study it was concluded that Mentzers index were among the most reliable hematological index for screening of βTT [26].

Youdens Index provides an appropriate measure of validity of a particular question or technique. Mentzer index showed the best Youden index followed by Shine and Lal index in differentiating Iron deficiency anemia from β-thalassemia trait. Green and King and England & Fraser index revealed poor accuracy in terms of Youdens index which was 10.6 and 11.5 respectively in the study. Similar result was also reported by other workers [13,22].

None of the formulae and parameters was 100% sensitive and specific, and various studies have shown different results with one formula to be better than the other. There are many reasons for the discrepancies as the values of hematological parameters are affected by a number of factors even in apparently healthy populations. These factors include ethnic background, age, and sex, and physique, social, nutritional and environmental factors like attitude. The studies mentioned above also discussed the similar observation that there is no perfectly reliable discrimination index to differentiate between β thalassemia trait and iron deficiency anemia.

Another limitation that was highlighted that a high prevalence of conditions like subclinical infections and latent inflammatory disorders in our population subset studied, unlike the western population, can falsely alter the serum iron profile analysis, thereby masking the underlying IDA, suggesting that probably we need to redefine the cut off for serum ferritin levels as well [21].

Conclusion

Among the various discriminatory indices Mentzer Index and Shine and Lal index are considered to be the good indicators to differentiate βTT and IDA, but there is no single indicator that can differentiate these two entities. Thus we conclude, although HbA2 estimation continues to be the gold standard for diagnosing βTT either by HPLC or electrophoresis. A practical approach can be established to find out such a module so that Beta thalassemia trait cases may be screened out early on simple CBC by deriving a potent discrimination index in developing countries with limited facility for hemoglobin electrophoresis, poor health care resources and prevalence of financial constraints. Thus, genetic and premarital counseling can be done to minimize the cases of Beta thalassemia, so that disease burden on the society can be reduced especially in developing and underdeveloped countries.

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