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EVALUATION OF THE MOST RELIABLE FORMULA BASED ON RBC INDICES TO DIFFERENTIATE BETWEEN β-THALASSAEMIA TRAIT AND IRON DEFICIENCY ANAEMIA IN TERTIARY CARE TEACHING HOSPITAL - CROSS-SECTIONAL OBSERVATIONAL STUDY

Hematology

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ABSTRACT

Introduction: Hemoglobinopathies are the most common genetic disorders reported worlwide. According to WHO, 5% of the world population is a carrier for hemoglobin disorders. **Aims and objective:** The aim of the study was to determine the most reliable formula based on RBC indices for screening β-Thalassemia Trait (βTT) and iron deficiency anaemia (IDA).Materials and Methods: During 1.5 year period, a cross-sectional study was carried out on 110 diagnosed cases of IDA and βTT of all age groups. All the samples were tested for complete blood count and HPLC study. **Results:** The Mentzer index showed good sensitivity, specificity, with Youden's index values of 98.7%, 82.3%, and 81%, respectively. Mentzer index> Srivastava index> Green and King index> England and Fraser index> Ricerca index> Shine and Lal index. **Conclusion:** The percentage of correctly diagnosed patients of ß-TT as well as IDA was the highest with the Mentzer index (87.27%). Cell-count-based parameters and formulas, particularly the MCV and RBC counts and their related indices (Mentzer index), have good discrimination ability in distinguishing ß-TT from IDA.

KEYWORDS

Hemoglobinopathies, HPLC, *β*-Thalassemia trait, Iron deficiency anaemia.

INTRODUCTION:

Anaemia is defined as the reduction in circulating red-cell mass below normal levels which lead to decrease in the oxygen carrying capacity of blood resulting in tissue hypoxia.[1][2]Among anaemia, microcytosis is a common microscopic findings which observed in iron deficiency anaemia (IDA), β- thalassemia trait (β-TT), anemia of inflammation (chronic disease), lead poisoning and sideroblastic anaemia. The most frequent microcytic anemia is IDA and β-TT. The differentiation between thalassemia and non-thalassemia microcytosis is important in clinical management. For this purpose, High performance liquid chromatography (HPLC) is important investigation but because of its limited availability, differentiation on microscopy and automated hematology analyzer is crucial.[3] According to World Health Organization (WHO), 5% of the world population is a carrier for Hemoglobin disorders.[4]The incidence of βTT in India is 3.3% with 1%–7% of couples being affected annually.[5]

In various parts of India, the prevalence of β-Thalassemia is different: 1.9% in Maharashtra, 2.6% in Mumbai, 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal.[6] Diagnosis of βTT often becomes perplexing due to its overlapping and similar features with iron deficiency anaemia as below.

There are similarities in red cell indices between the two disorders such as reduced hemoglobin (Hb), Mean Cell Volume (MCV), Mean Cell hemoglobin (MCH) and varying degree of microcytosis with hypochromia seen in both conditions on peripheral smears. Clinical features do not help much in their discrimination from each other. The identification of βTT is essential for two reasons. Firstly, since treatment for both conditions is different. Secondly, for prevention of β- thalassemia major by parental genetic counselling. The screening of β-TT can be done by naked-eye single tube red cell osmotic fragility (NESTROF) test, various cell counter based formulas, HbA2 by elution following cellulose acetate electrophoresis and HPLC. In β-TT HbA2 is more than 4%.[7]

Alternatively, use of various formulas has been proposed to differentiate β-TT from IDA.

Table 1- Threshold values of the indices used to discriminate between IDAandβ-TT

	Name of Formula	Formula	Cutoff βTT	Cutoff IDA
1.	Shine and Lal index[8]	$(MCV)^2 \times MCH/100$	<1530	≥1530
2.	Mentzer index[9]	MCV/RBC COUNT	<13	≥13
3.	Srivastava index[10]	MCH/RBC COUNT	<3.8	≥3.8
4.	England and Fraser index[11]	MCV-(5×HB)- RBC-3.4	<0	≥0
5.	Ricerca index[12]	RDW/RBC COUNT	<4.4	≥4.4
6.	Green and King index[13]	$(MCV)^2 \times RDW/100 \times HB$	<72	≥72

AIMS AND OBJECTIVE:

To find out the most reliable formula, based on RBC parameters for screening β-TT & IDA. To reduce cost to patient by predicting β-TT based upon RBC indices in CBC report.

MATERIALS AND METHODS:

This Cross sectional observational study was carried out in Pathology Department, PCMC's PGI YCMH, PIMPRI, Pune from Jan 2021 to June 2022 on 110 diagnosed cases of IDA(N=55) and β-TT(N=55).

2ml blood will be collected in K2 EDTA (di-potassium **E**thylene **D**iamine **T**etraacetic **A**cid)

Peripheral blood Smear (PBS), Complete blood count (CBC) including red blood cell (RBC) Indices by 5-part automated hematology analyzer-CELL DYN Ruby. HPLC was done on fully automated HPLC analyzer BIO-RAD D-10 as per manufacturer's instructions. Various formulae as per Table 1 were applied on all samples with RBC indices obtained from hematology analyzer and values from the formulae were correlated with HPLC findings. Special investigations such as iron studies including serum iron and serum Ferritin were performed wherever needed, to confirm IDA. Statistical tests were applied to the data with calculation of Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV), Youden's index for each Formula as per Table1.

RESULTS:

We calculated the number/percentage of correctly diagnosed cases of β-TT and IDA based on following criteria:

1. For β-TT - HPLC as a gold standard to diagnose β-TT. All patients having HbA2 > 4 were diagnosed as β-TT.
2. For IDA- Serum ferritin and serum iron were done. Diagnosis of IDA on following criteria:

- Serum Ferritin <10 ng/L
- Serum Iron <30 µg/dL

The distribution of cases was studied in 55 each β-TT, and IDA.

ß-TT was commonly diagnosed in 2nd decade of life with almost 32.72% of them observed in same age. The age group in IDA was commonly observed in 2nd to 4th decade of life with almost 72.71% of them observed in same age.

Table 2: Age distribution in studied groups

Age Groups in years	ßTT		IDA		Total	
	N	%	N	%	N	%
01-10	15	27.27	04	07.27	19	17.27
11-20	14	25.45	02	03.63	16	14.54
21-30	18	32.72	15	27.27	33	30.00
31-40	06	10.91	12	21.81	18	16.36

41-50	01	1.81	13	23.63	14	12.72
51-60	01	1.81	04	07.27	05	04.54
61-70	00	00	03	05.45	03	02.72
71-80	00	00	01	1.81	01	00.91
81-90	00	00	01	1.81	01	00.91
Total	55	100	55	100	110	100

- There were 28 males (50.91%) and 27 females (49.09%) out of 55 β-TT cases. The male to female ratio was 1:1.
- There were 10 males (18.18%) and 45 females (81.82%) out of 55 IDA cases. The male to female ratio was 1: 4.5. Females were predominant in IDA group.

Table 3: Gender wise distribution of IDA&β-TT

Gender	ßTT		IDA		Total	
	N	%	N	%	N	%
Male	28	50.91	10	18.18	38	34.54
Female	27	49.09	45	81.82	72	65.46
Total	55	100	55	100	110	100

Table 4: Laboratory values in the IDA& β-TT groups (mean ± SD)

Parameters	ßTT (N = 55)	IDA (N = 55)
RBC count(×106/L)	05.35 ± 1.09	04.28 ± 0.72
Hb (g/dl)	10.36 ± 1.97	8.60 ± 1.77
MCV (fL)	64.16 ± 6.46	66.33 ± 7.86
MCH (pg)	19.64 ± 2.29	20.15 ± 3.03
MCHC	30.56 ± 1.04	30.20 ± 1.49
RDW	18.11 ± 3.52	21.03 ± 4.60

The percentage of diagnosed cases based on different formulas were calculated and was highest for Mentzer index (87.27%), followed by Srivastava index & Green and King index (80%) and third highest Ricerca index (72.72%).

Table 5: Differential values in IDA& β-TT with respect to discriminatory indices

FORMULA	ß -TT (N=55)	IDA (N=55)	Correctly diagnosed cases	Correctly identified cases (%)
Shine and Lal index ß- TT <1530 IDA>1530	 55 00	 49 06	61	55.45
Mentzer index ß -TT <13 IDA>13	 53 02	 12 43	96	87.27
Srivastava index ß-TT <3.8 IDA>3.8	 48 07	 15 40	88	80.00
England and Fraser index ß-TT < 0 IDA>0	 38 17	 18 37	75	68.18
Ricerca index ß-TT <4.4 IDA >4.4	 55 00	 30 25	80	72.72
Green and King index ß-TT < 65 IDA > 65	 40 15	 08 47	87	79.09

The Mentzer index showed good sensitivity, specificity, and Youden's index values of 98.7%, 82.3%, and 81%, respectively. Mentzer index> Srivastava index> Green and King index> England and Fraser index> Ricerca index> Shine and Lal index.

Table 6:Sensitivity, specificity, PPV, NPV and Youden's index of indices to differentiate between ß-TT and IDA.

Formula	Sensitivity	Specificity	PPV	NPV	Youden's index
Shine and Lal index ß-TT IDA	 100 10.2	 10.2 100	 52 100	 100 52	10.2
Mentzer index ß-TT IDA	 98.7 82.3	 82.3 98.7	 85 98	 98 85	81
Srivastava index ß-TT IDA	 84.5 69	 69 84.5	 73 81	 81 73	55.7
England and Fraser index ß-TT IDA	 64.3 82.1	 82.1 64.3	 80 69	 69 80	49.8
Ricerca index ß-TT IDA	 100 14.5	 14.5 100	 56 100	 100 56	14.5
Green and King index ß-TT IDA	 83.1 71.5	 71.5 83.1	 75 78	 78 75	56.1

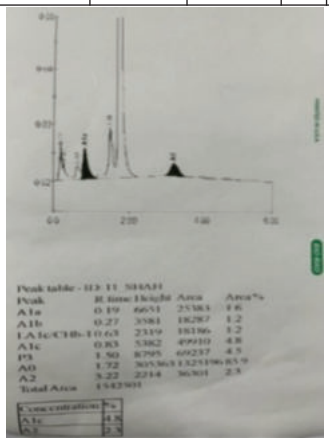

Figure 1: Chromatogram of iron deficiency anaemia

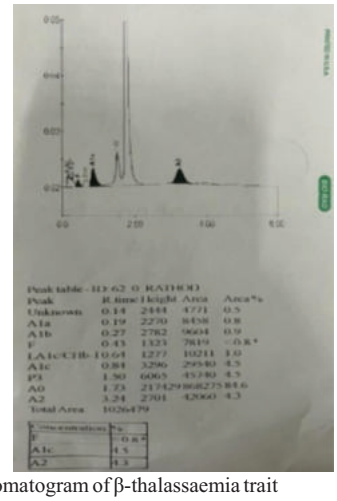

Figure 2: Chromatogram of β-thalassaemia trait

DISCUSSION

In our study, we found, 28 males (50.91%) and 27 females (49.09%) in 55 β-TT cases. The male to female ratio was around 1:1.There were 10 males (18.18%) and 45 females (81.82%) in 55 IDA cases. The male to female ratio was 1:4.5. Females were predominant compared to males in IDA. β-TT was prevalent in 2nd decade while IDA was in 2nd to 4th decade of life

After calculations of formulas, highest percentage of correctly diagnosed cases was with Mentzer index (87.27%), followed by Srivastava index & Green and King Index (80%) and third highest Ricerca index (72.72%). The Ricerca index and the Shine and Lal indices demonstrated the highest sensitivity (100%) but had low specificities for correctly identifying IDA (14.5%) and ß-TT (10.2%). Therefore, according to our results, these indices cannot be used as screening tools for ß-TT, as using them could result in a significant number of false-negative results.

England and Fraser index had lowest sensitivity of 64.3%, and identification was wrong in about 28% of ß-TT cases. England and Fraser as well as Mentzer demonstrated highest specificities of 82.1% and 82.3%, respectively.

Furthermore, above table showed highest and lowest PPV, which were found for Mentzer index (98.1%) and Shine and Lal (52%) respectively. The Shine and Lal and the Ricerca indices demonstrated highest NPV of 100%.

Additionally, highest and lowest Youden's index values belonged to Mentzer index (81%) and Shine and Lal (10.2%) for IDA and ß-TT. However, none of the indices was 100 % sensitive or 100 % specific in distinguishing ß-TT and IDA.

The Mentzer index showed good sensitivity, specificity, and Youden's index values of 98.7%, 82.3%, and 81%, respectively. Mentzer index> Srivastava index> Green and King index> England and Fraser index > Ricerca index> Shine and Lal index.

CONCLUSIONS

In conclusion, Present study revealed the cell-counter-based calculated indices, particularly Mentzer index, is easily available and relatively reliable method for detecting ß-TT as well as IDA. According to our results, the percentage of correctly diagnosed patients of ß-TT and IDA, was highest with Mentzer index (87.27%). Cell-count-based parameters particularly MCV and RBC counts and formulas like Mentzer index, have good discrimination ability in distinguishing ß-TT from IDA.

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