



# Utility of Red Blood Cell Parameters and Indices of Iron Homeostasis in Evaluation of Microcytosis without Anemia or with Mild Anemia: A Diagnostic Accuracy Study

Paruvathavarthini Thambiraj<sup>1</sup>  Nivedita Nanda<sup>2</sup>  Rakhee Kar<sup>1</sup> 

<sup>1</sup> Department of Pathology (Hematology Section), JIPMER, Pondicherry, India

<sup>2</sup> Department of Biochemistry, JIPMER, Pondicherry, India

**Address for correspondence** Rakhee Kar, MD (Pathology), DM (Hematopathology), Department of Pathology, JIPMER, Pondicherry 605006, India (e-mail: drrakheekar@gmail.com).

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## Abstract

**Introduction** Asymptomatic microcytosis may be a prelude to microcytic hypochromic anemia of varied causes. Evaluation of red cell indices may help delineate cases needing further investigation like hemoglobin high-performance liquid chromatography (Hb-HPLC). In addition, markers of iron homeostasis will help confirm iron-deficient erythropoiesis (IDE)/iron deficiency anemia (IDA).

**Materials and Methods** This was a single institutional hospital-based study over a period of 18 months. The sample size was 60, which include all age groups, males and females, and values of mean corpuscular volume (MCV)  $< 80$  fL, Hb  $\geq 10$ g/dL were taken as the inclusion criteria. Various derived red cell indices, Hb-HPLC, and iron parameters were assessed. Hb-HPLC and serum ferritin with transferrin saturation (TSAT) values were taken as the gold standard for diagnosis of hemoglobinopathies/thalassemia trait and IDA/IDE, respectively.

**Results** Out of 60 samples, 24 (40%) and 36 (60%) had abnormal and normal proportion of Hb variants, respectively. In total, seven indices were evaluated, which included Mentzer's index, red cell distribution width index, Ehsani's index, Sirdah's formula, Matos-Carvalho index, Shine and Lal index, and Sehgal's index. The Shine and Lal index showed better sensitivity (89%) and specificity (73%) for diagnosing pure thalassemia trait. The Sirdah index showed better sensitivity (78%) and specificity (42%) in diagnosing IDE/IDA. Ferritin showed better sensitivity (74%) and specificity (84%) in diagnosing pure IDA and TSAT showed better results in diagnosing IDA/IDE.

**Conclusion** The Shine and Lal index and Mentzer index can be used as screening tools and help detect subjects who require appropriate follow-up with confirmation by Hb-HPLC. Serum ferritin remains the gold standard for diagnosis of IDA.

## Keywords

- ▶ derived red cell indices
- ▶ diagnostic accuracy
- ▶ iron parameters
- ▶ IDA/IDE
- ▶ Hb-HPLC
- ▶ screening tool
- ▶ Mentzer index

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## Introduction

Microcytic hypochromic anemia due to varied causes is an important cause for poor performance in daily life. Mild degrees of anemia, if detected early and treated properly, can prevent significant morbidity. The main problem in the detection of these cases is that they are usually asymptomatic. Although mild iron deficiency is one of the most important causes of asymptomatic microcytosis, there are other causes that should be ruled out as their significance and treatment modalities are totally different from iron deficiency anemia (IDA). Among the differentials, hemoglobinopathies especially alpha and beta thalassemia traits ( $\beta$ TT) are significant contributors to asymptomatic microcytosis. Complete blood count, readily available from electronic counters if properly evaluated, can pick up cases with high probability of turning out to be hemoglobinopathies. Basic parameters like red blood cell (RBC) count and red cell distribution width (RDW) help in the differentiation.<sup>1</sup> Numerous indices like Mentzer's index and Ehsani's index have been introduced and their utility evaluated in the study of IDA and  $\beta$ TT. Recently the Matos-Carvalho index was introduced as a good discriminator between IDA and  $\beta$ TT.<sup>2</sup> However, none of these indices are found to have 100% sensitivity or specificity.

Some studies show that laboratory tests to assess iron homeostasis help in differentiating the cause of microcytosis. These include serum ferritin, serum iron, transferrin saturation (TSAT), and soluble transferrin receptor (sTfR) levels. Based on assessment of these parameters, iron deficiency can be confirmed.<sup>1</sup> However, it is a known fact that serum ferritin and serum iron are affected by factors like the presence of inflammation. A soluble form of transferrin receptor assay can be used as diagnostic tool for differentiating IDA and  $\beta$ TT.<sup>3</sup> The gold standard for the diagnosis of  $\beta$ TT is HbA<sub>2</sub> estimation, with a cutoff value of greater than 3.8% in the range of 3.8 to 6.2%.<sup>4</sup> This can be detected by hemoglobin high-performance liquid chromatography (Hb-HPLC).<sup>5</sup> Differentiation of these main groups is necessitated by the point that iron treatment is not needed for thalassemia trait (TT). Under-recognition of TT has the risk of children being born with thalassemia major in case the partner of the index case is also with TT. Evaluation of the red cell indices and markers of iron homeostasis will help to narrow down cases needing investigations like Hb-HPLC/capillary electrophoresis. This study was done to study the utility of derived RBC indices and indices of iron homeostasis in the evaluation of microcytosis without anemia or with mild anemia

## Materials and Methods

### Study Design, Setting, Inclusion, and Exclusion Criteria

This was a diagnostic test accuracy study done over a period of 1.5 years (2020–2021) and was carried out in the Department of Pathology, with Department of Biochemistry, JIPMER, as the collaborating department. Patients who had presented to the JIPMER hospital with a hemogram sample

showing microcytosis (mean corpuscular volume [MCV] < 80 fL) without anemia or with mild anemia were included in the study. Anemia cutoff was taken according to the WHO criteria for all the patients. As the population in the study group is Indian, the cutoff for mild anemia (10–10.9 g/dL) was taken as per the Indian Council of Medical Research (ICMR) criteria. Patients who had been started on iron treatment and for whom samples were not available for complete workup were excluded.

### Sample Size Calculation

The sample size was calculated by using the difference in the mean and standard deviation of RBC counts ( $\times 10^{12}/L$ ) in  $\beta$ TT ( $5.56 \pm 0.4$ ) and IDA ( $4.84 \pm 0.59$ ) with 95% confidence interval and 90% power. The minimum sample size required for the study was estimated as 22 in each group. This was calculated by using the OpenEpi version 3.01 software. For most of the indices of iron homeostasis, the sample size was found to be lower. Since information is not available in the literature for sTfR, the sample size was rounded off to 30 in each group.

### Study Procedure

The patients who presented to the JIPMER hospital whose routine hemogram sample were sent for hematology were recruited as per the inclusion and exclusion criteria. The patients were briefed about the study protocol and a written informed consent was obtained from them. Complete hemogram values (Sysmex XT 4000i) such as Hb, RBC count, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RDW, peripheral smear were assessed. Based on these values, derived RBC indices were calculated. In total, seven indices were evaluated, which included Mentzer's index, red cell distribution width index (RDWI), Ehsani's index, Sirdah's formula, Matos-Carvalho index, Shine and Lal index, and Sehgal's index.

### Derived Red Cell Indices

The formulae for derived red cell indices that were calculated included<sup>2,6–18</sup>:

- Mentzer's index<sup>7</sup> = MCV/RBC count.
- RDWI<sup>13</sup> = (MCV  $\times$  RDW)/RBC.
- Ehsani's index<sup>16</sup> = MCV – 10  $\times$  RBC
- Sirdah's formula<sup>14</sup> = (MCV – RBC – (3  $\times$  Hb)).
- Matos-Carvalho index<sup>2</sup> = (1.91  $\times$  RBC) + (0.44  $\times$  MCHC).
- Shine and Lal index<sup>9</sup> = MCV  $\times$  MCV  $\times$  MCH/100.
- Sehgal's index<sup>18</sup> = MCV  $\times$  MCV/RBC.

Using these formulae, derived red cell indices were calculated. Based on the literature, certain cutoffs in these indices have been used for differentiating TT and IDA. These established cutoffs were used in this study to determine the diagnostic accuracy of these indices in differentiating IDA from TT.

**Hemoglobin High-Performance Liquid Chromatography**  
Using HPLC (Bio-Rad D10 analyzer), hemoglobin variants were assessed. The principle is that it is a form of liquid

**Table 1** Distribution of patients based on the cutoffs of derived red cell indices for suggestive diagnosis of thalassemia trait and iron deficiency anemia

Sl. no.	Derived red cell indices	Thalassemia trait		Iron deficiency anemia cutoff results	
		Cutoff <sup>14-21</sup>	Results		
1	Red cell distribution index	<220	25/60 (42%)	>220	35/60 (58%)
2	Mentzer index	<14	37/60 (62%)	>14	23/60 (38%)
3	Matos-Carvalho index	>23.85	25/60 (42%)	<23.85	35/60 (58%)
4	Sirdah index	<27	20/60 (33%)	>27	40/60 (67%)
5	Shine and Lal index	<1,530	57/60 (95%)	>1,530	03/60 (05%)
6	Ehsani index	<15	24/60 (40%)	>15	36/60 (60%)
7	Sehgal index	<972	36/60 (60%)	>972	24/60 (40%)

chromatography used to separate compounds that are dissolved in solution and high pressure is used to push the mobile-phase solution (liquid) through a column of stationary phase (solid) allowing separation of complex mixtures with high resolution based on physical and chemical interactions. HPLC was done for all patients ( $n = 60$ ) and hemoglobin fractions such as HbA<sub>2</sub>, HbA<sub>0</sub>, HbF, and Hb variants, if any, were assessed.

Patients were divided into two groups: group 1 having microcytosis with normal proportion of Hb variants and group 2 having abnormal proportion of Hb variants.

#### Iron Parameters

Serum ferritin, serum iron (Fe), serum transferrin (TF), and sTfR were measured. The ferritin levels were assessed by chemiluminescence assay. Iron was estimated colorimetrically in Beckman Coulter clinical chemistry analyzer by spectrometry. Transferrin and sTfR assays were assessed by enzyme-linked immunosorbent assay (ELISA). TSAT was calculated by using the following formula:  $TSAT = (Fe/TF) \times 70.9$ . The sTfR ferritin (sTfR-F) index was calculated by the following formula:  $sTfR-F \text{ index} = sTfR/\log\text{-ferritin}$ .

Based on all these values, the patients ( $n = 60$ ) were categorized as IDA and iron-deficient erythropoiesis (IDE). IDE was defined by no anemia but with serum ferritin values less than 15  $\mu\text{g/L}$ , and if the ferritin values were in the range of 15 to 99  $\mu\text{g/L}$ , then TSAT values less than 16% was taken. If it was associated with anemia, then it was labeled as IDA. Normal iron store is defined as serum ferritin in the range of 15 to 300  $\mu\text{g/L}$  and TSAT of 16 to 50% and increased iron store is defined as serum ferritin greater than 300  $\mu\text{g/L}$ .

Reference ranges for iron parameters according to the literature was obtained, and various cutoffs as given in the literature were used in this study to determine the diagnostic accuracy of these parameters in diagnosing IDA.

#### Statistical Analysis

Mean with standard deviation was calculated for normally distributed data such as Hb, RBC count, RDW, MCV, MCH, MCHC, and hematocrit. Median with range was calculated for non-normally distributed data. Diagnostic test accuracy parameters such as sensitivity, specificity, positive predictive

value (PPV), and negative predictive value (NPV) of various derived red cell indices and indices of iron homeostasis were calculated taking Hb-HPLC as the gold standard for diagnosis of  $\beta$ TT/hemoglobinopathies and ferritin/TSAT values as the gold standard for the diagnosis of IDA/IDE.

## Results

#### Demographic Details

A total of 60 patients were enrolled in the study, of which 36 (60%) were males and 24 (40%) were females with a median (range) age of 31 (1-75) years. Seven of 60 patients (11.6%) were under the age of 5 years, 4 (6.6%) were in the 5- to 11-year age group, 1 (1%) in the 12- to 14-year age group, and 48 (80%) patients were older than 15 years.

#### Derived Red Cell Indices

Derived red cell indices were calculated based on the formulae given in the "Materials and Methods" section. Based on the literature, the cutoff values were used to presumptively discriminate IDA and TT. The cutoff values and distribution of the cases based on these cutoff values irrespective of their final diagnosis are given in ►Table 1.

#### Subclassification of the Enrolled Patients Based on Hb-HPLC

Of 60 the patients, 24 (40%) had hemoglobinopathies and 36 (60%) had normal Hb-HPLC. Of the 24 patients, 21 (87.5%) were  $\beta$ TT, while 1 (4%) each was alpha-TT, sickle beta thalassemia, and HbE trait.

#### Iron Parameters Used in This Study

Iron parameters were analyzed in all the 60 patients included in the study. Based on these, derived iron parameters such as TSAT and sTfR-F index were calculated. The median (range) values for the various iron parameters were as follows: serum ferritin 18.25 (4.2-897.4)  $\mu\text{g/L}$ , serum iron 42 (10-326) mcg/dL, TSAT 12.9 (2.86-109.6)%, sTfR 7.85 (2.1-35.9) mg/L, and sTfR-F index 5.65 (1.59-176.3).

IDE was based on a decrease in serum ferritin (<15  $\mu\text{g/L}$ ).<sup>22</sup> If the ferritin values were in the range of 15 to 99  $\mu\text{g/L}$ , then TSAT less than 16%<sup>22</sup> was taken. If it was associated with

**Table 2** Distribution of patients based on the cut-offs of iron parameters

Sl. no.	Iron parameters	Cutoff for IDA/IDE <sup>22,23</sup>	Results	Cutoff for non-IDA/IDE	Results
1	Serum ferritin	< 15 µg/L	23/60 (38%)	> 15 µg/L	37/60 (61%)
2	Serum iron	< 60 mcg/dL	41/60 (68%)	> 60 mcg/dL	19/60 (31%)
3	Transferrin saturation	< 16%	36/60 (60%)	> 16%	24/60 (40%)
4	Soluble transferrin receptor	> 5 mg/L (adult males) > 4.4 mg/L (adult females) > 3 mg/L (<18 y)	49/60 (82%)	< 5 mg/L (adult males) < 4.4 mg/L (adult females) < 3 mg/L (<18 y)	11/60 (16%)
5	Soluble transferrin receptor ferritin index	> 1.5	60/60 (100%)	< 1.5	0/60

Abbreviations: IDA, iron deficiency anemia; IDE, iron-deficient erythropoiesis.

**Table 3** Final diagnosis of patients based on both Hb-HPLC and iron parameters

Sl. no.	Categories	Number
<b>Distribution of patients based on normal proportion of Hb variants (n = 36)</b>		
1	Iron deficiency anemia (IDA)	23 (64%)
2	Iron deficiency erythropoiesis (IDE)	04 (11%)
3	Increased iron stores	02 (05%)
4	Normal iron stores	07 (19.4%)
<b>Distribution of patients based on abnormal proportion of Hb variants (n = 24)</b>		
1	Thalassemia trait	09 (37.5%)
2	TT + IDE/IDA	13 (54.1%)
3	Sickle beta thalassemia + IDA	01 (04%)
4	HbE + IDA	01 (04%)

Abbreviations: HbE, hemoglobin E; HPLC, high-performance liquid chromatography; TT, thalassemia trait.

anemia, then it was labeled as IDA. The cutoffs of various iron parameters for diagnosing IDA and IDE based on the literature and as mentioned earlier and the distribution of cases are given in ►Table 2.

#### Distribution of the Patients Based on Both Iron Parameters and Hb-HPLC

Considering both Hb-HPLC findings and iron studies (serum ferritin and TSAT) as the gold standard, the final diagnosis of the patients is given in ►Table 3.

#### Diagnostic Accuracy of Derived Red Cell Indices in Presumptively Diagnosing Thalassemia Trait and IDA

The diagnostic accuracy measures of the various derived red cell indices in diagnosing the overall thalassemia/hemoglobinopathies, pure TT, and TT in combination with IDA/IDE, and in the diagnosis of IDA/IDE are given in ►Table 4.

#### Diagnostic Accuracy of Indices of Iron Homeostasis in Diagnosing IDA

The diagnostic accuracy measures of the various indices of iron homeostasis in diagnosing IDA and IDA/IDE taking the

diagnostic criteria based on serum ferritin in combination with TSAT as given in the earlier gold standard of diagnosis are given in ►Table 5.

## Discussion

IDA and TT are common causes of microcytic hypochromic anemia. Non diagnosis of carriers of TT in parents has the potential for birth of affected homozygous children. Adequate screening, early detection of subjects, and counseling of high-risk couples are the essential measures for the reduction of disease-related morbidity and mortality. A simple red cell index based test with a combination of high sensitivity and specificity for detecting TT would be a useful screening tool in the evaluation of microcytic hypochromic anemia in countries that show higher prevalence of nutritional deficiencies and thalassemia syndromes.

The purpose of using various derived red indices to discriminate microcytosis is to detect subjects who have high probability of requiring appropriate follow-up with further tests like Hb-HPLC, thereby reducing unnecessary investigative costs. Some studies show that laboratory tests to assess iron homeostasis help in differentiating the cause of microcytosis. Iron deficiency can be confirmed based on the assessment of these parameters. This mainly tells the importance of these diseases and these should be evaluated readily for proper detection and management. In this study, we evaluated the basic hematological and iron parameters and their significance as a screening tool in diagnosing and distinguishing these diseases. Utility of derived red cell indices and iron parameters was evaluated.

Out of 60 patients studied, 24 patients (40%) had hemoglobinopathies, of which 9 (15%) patients were pure TT, 13 (22%) were overlap cases (TT with IDA/IDE), and 2 (03%) had other hemoglobinopathies such as HbE trait and sickle beta thalassemia, which were associated with IDA.

In our study, among the seven derived RBC indices, the Shine and Lal index showed a higher sensitivity of 92%, followed by the Mentzer index (68%), for diagnosing hemoglobinopathies. Sirdah's index showed a better specificity of 72%, followed by Matos-Carvalho index and RDWI with a specificity of about 61% each for diagnosing hemoglobinopathies.

**Table 4** Diagnostic accuracy of derived red cell indices in diagnosis of TT, TT with IDA and IDA

Sl. no.	Diagnostic accuracy	Mentzer index	Ehsani index	Sirdah index	Matos–Carvalho index	RDWI	Sehgal index	Shine and Lal index
<b>Diagnostic accuracy of derived red cell indices for diagnosis of thalassemia trait/hemoglobinopathies (n = 24)</b>								
1	Sensitivity (%)	63	38	42	46	46	58	92
2	Specificity (%)	38	58	72	61	61	33	03
3	Positive predictive value (PPV; %)	40	38	50	44	44	38	39
4	Negative predictive value (NPV; %)	60	58	65	63	63	58	33
<b>Diagnostic accuracy of derived red cell indices for diagnosis of pure TT (n = 9)</b>								
1	Sensitivity (%)	67	56	67	67	67	67	89
2	Specificity (%)	82	92	92	90	90	84	73
3	PPV (%)	40	56	60	55	55	43	36
4	NPV (%)	93	92	94	94	94	93	97
<b>Diagnostic accuracy of derived red cell indices for diagnosis of overlap cases (TT + IDA/IDE; n = 13)</b>								
1	Sensitivity (%)	62	31	31	31	31	54	92
2	Specificity (%)	85	89	87	85	85	85	79
3	PPV (%)	53	44	40	36	36	50	55
4	NPV (%)	89	82	82	82	82	87	97
<b>Diagnostic accuracy of derived red cell indices for diagnosis of IDA/IDE (n = 27)</b>								
1	Sensitivity (%)	41	56	78	63	63	41	0
2	Specificity (%)	64	36	42	45	45	60	91
3	PPV (%)	48	42	53	49	49	46	0
4	NPV (%)	57	50	70	60	60	56	53

Abbreviations: IDA, iron deficiency anemia; IDE, iron-deficient erythropoiesis; NPV, negative predictive value; PPV, positive predictive value; RDWI, red cell distribution width index; TT, thalassemia trait.

**Table 5** Diagnostic accuracy of indices of iron homeostasis in diagnosis of IDA and IDA/IDE

<b>Diagnostic accuracy of iron parameters for diagnosis of IDA (n = 23)</b>						
Sl. no.	Diagnostic accuracy	Serum ferritin	Serum iron	Transferrin saturation	Soluble transferrin receptor	sTfR ferritin index
1	Sensitivity (%)	74	91	83	78	100
2	Specificity (%)	84	46	55	16	0
3	PPV (%)	74	51	53	37	38
4	NPV (%)	84	89	83	55	0
<b>Diagnostic accuracy of iron parameters for diagnosis of IDA/IDE (n = 27)</b>						
1	Sensitivity (%)	63	88	82	74	–
2	Specificity (%)	83	48	58	12	–
3	PPV (%)	74	59	61	41	–
4	NPV (%)	73	84	79	36	–

Abbreviations: IDA, iron deficiency anemia; IDE, iron-deficient erythropoiesis; NPV, negative predictive value; PPV, positive predictive value; sTfR, soluble transferrin receptor.

The Shine and Lal index showed higher sensitivity (89%) and specificity (73%) in diagnosing pure TT compared to the other derived red cell indices. For diagnosis of overlap cases (TT with IDA/IDE), the Shine and Lal index showed better sensitivity (92%) and specificity (79%) followed by the Mentzer index with sensitivity and specificity of about 62 and 85%, respectively.

Overall the Shine and Lal index showed better sensitivity and specificity in the diagnosis of pure TT and overlap cases (TT with IDE/IDA) compared to other derived red cell indices. Overall, the Mentzer index showed better sensitivity (63%), specificity (38%), PPV (40%), and NPV (60%) for diagnosing hemoglobinopathies.

In 2016, Ullah et al<sup>19</sup> conducted a study on 50 cases of diagnosed TT and found that the Shine and Lal index could correctly identify TT in about 92% cases, which is similar to our study.

In 2015, according to Sehgal et al,<sup>18</sup> the combination of indices such as the Sehgal and Mentzer indices showed better sensitivity and specificity in diagnosing TT and other causes of microcytic hypochromic anemia.

In 2010 study, Ferrara et al<sup>20</sup> reported that RDWI showed the highest sensitivity (78.9%). In 2009, Ehsani et al<sup>16</sup> showed that the best discrimination index was the Mentzer index (90.1%), followed by the Ehsani index (85.5%). Similarly in their study, the Mentzer and Ehsani indices were able to correctly diagnose 94.7 and 92.9% of cases, respectively. Other studies also show similar results such as the Mentzer index with sensitivity of 90.9% and specificity of 80.3% in Ghafouri et al.<sup>21</sup>

As mentioned earlier, none of the derived red cell indices proved to be 100% sensitive and specific. In addition, most studies have used HbA<sub>2</sub> levels for the identification of TT and it is well known that there are silent TT cases with normal HbA<sub>2</sub> levels.

In addition, the diagnostic accuracy of derived red cell indices for diagnosing iron deficiency was also evaluated. Sirdah's index showed better sensitivity of 78% and the Shine and Lal index showed specificity of 91% in diagnosing IDE/IDA. However, the Shine and Lal index showed very poor sensitivity. Moreover, overall, comparing with other derived red cell indices, Sirdah's index showed better sensitivity (78%), specificity (42%), PPV (53%), and NPV (70%) in diagnosing IDE/IDA.

None of the studies have evaluated the utility of derived red cell indices in diagnosing IDA or overlap cases. This is probably the first study for evaluation of derived red cell indices in diagnosing IDA and overlap cases.

The diagnosis of thalassemia minor involves measurement of HbA<sub>2</sub> concentration by Hb-HPLC, which is the gold standard for diagnosis.<sup>24</sup> Some of studies have mentioned that iron deficiency affects the rate of iron synthesis; hence, iron therapy should be given for 16 to 20 weeks before diagnosis. Repeat HPLC should be done after 3 months of iron treatment. If there is persistent microcytosis after recovery from IDA, HbA<sub>2</sub> should be measured again. If TT is associated with IDA, it lowers the HbA<sub>2</sub> values and it can be wrongly diagnosed as IDA. In our study, overlap cases (TT

with IDA/IDE) were higher at up to 59% (13/22) compared to pure TT, which was 41% (9/22).

The diagnostic accuracy of iron parameters in diagnosing IDA/IDE was assessed. Cook et al conducted a study involving 1,564 patients and found that IDA was diagnosed in about 9.3% based on serological evidence, whereas only 4.3% were diagnosed based on clinical features.<sup>25-27</sup>

Another study of 1,053 patients studied various iron parameters, in which serum ferritin was found to be decreased in about 23% of the study population, whereas a combination of TSAT and TIBC in diagnosing IDA was about 6%. This indicates that serum ferritin is the better marker for diagnosis of IDA and is accepted as a better indicator of decreased body iron stores<sup>27</sup>

Ferritin showed overall better sensitivity (63%), specificity (83%), PPV (74%), and NPV (73%) for the diagnosis of IDE/IDA compared to the other iron parameters. Serum iron showed a better sensitivity of 88%, followed by TSAT with 82% sensitivity. Similarly for diagnosis of pure IDA, serum iron showed a better sensitivity of 91%, followed by TSAT of about 83% sensitivity. Overall, serum ferritin showed better sensitivity (74%), specificity (84%), PPV (74%), and NPV (84%) in the diagnosis of only IDA.

For diagnosis of IDA/IDE, TSAT showed better sensitivity (82%), specificity (58%), PPV (61%), and NPV (79%). Based on the literature, serum ferritin being an acute phase reactant, values will be affected by inflammation, but sTfR remains unaffected by inflammation. As we have taken hospitalized patients, the ferritin values were slightly on the higher side. However, it still showed overall good sensitivity and specificity. sTfR showed good sensitivity (78%) but showed poor specificity (16%) for diagnosis of pure IDA in our study probably because of lack of standardization of the ELISA kits.

In the study by Infusino et al,<sup>28</sup> overall sensitivity, specificity, and positive and negative likelihood ratios of sTfR were 86%, 75%, 3.85, and 0.19, respectively. The lack of standardization of this sTfR values limits the diagnostic utility of the test because it is difficult to compare the values of sTfR among other studies.

However, in our study, 82% of the patients showed higher values, whereas only 18% showed normal values and none of them showed decreased values.

Lin et al<sup>29</sup> found the measurement of both sTfR and ferritin levels to be significant. In other studies, the diagnostic efficacy of the sTfR values and sTfR-F index in diagnosing IDA were found to be 83 and 99%, respectively. In contrast to serum transferrin and serum ferritin, sTfR values have been shown to be unaffected by inflammation.

Goyal et al<sup>30</sup> found that the sTfR-F index was significantly high with higher cutoffs and that the sTfR-F index can clearly distinguish between IDA and normal subjects.

In our study, the sTfR-F index showed 100% sensitivity and 0% specificity for the diagnosis of IDA, which indicate that all 60 patients showed positive values for this index. The reason for these erroneous values is probably because the sTfR values were unreliable due to lack of standardization of the ELISA kits. Hence, the sTfR-F index remains unreliable.

## Conclusion

To conclude, among the seven derived red cell indices, the Shine and Lal index showed better sensitivity and specificity for diagnosing pure TT and overlap cases (TT with IDE/IDA). Mentzer's index showed better sensitivity, specificity, PPV, and NPV for diagnosing hemoglobinopathies. Hence, the Shine and Lal and Mentzer indices can be used as screening tools to help detect subjects who have high probability of requiring appropriate follow-up with confirmation by Hb-HPLC. In addition, Sirdah's index helps IDA and it can be confirmed with iron parameters. Serum ferritin remains the gold standard for diagnosis of IDA.

### Contribution of Authors

TPV and RK designed the study and framework of study protocol. TPV had enrolled the cases and performed hematological and biochemical parameters under supervision of RK and NN respectively. NN had analyzed the biochemical parameters. RK had analyzed the hematological parameters. TPV wrote the manuscript which was edited and corrected by RK.

### Ethical Approval

Ethical clearance was obtained from the Institute's Ethics Committee (Human Studies; approval number: JIP/IEC/2018/394).

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None.

### Conflict of Interest

None declared.

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