Original Article

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Paediatrics Section

Diagnostic Performance of Haematological Markers in Iron Deficiency Anaemia among Children Aged Six Months to 15 Years: A Prospective Observational Study

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ABSTRACT

Introduction: Iron Deficiency Anaemia (IDA) is the most common nutritional deficiency among children and remains a major public health concern in developing countries. Although serum ferritin is considered the gold standard marker for diagnosing IDA, its levels may be falsely elevated during infections and inflammatory conditions. Hence, additional haematological and biochemical markers are required for accurate diagnosis, especially in children with microcytic hypochromic anaemia.

Aim: To compare the diagnostic utility of serum iron, Total Iron Binding Capacity (TIBC), Transferrin Saturation (TSAT) and serum ferritin in identifying IDA among children.

Materials and Methods: This hospital-based prospective observational study was conducted at the Paediatric Outpatient and Inpatient Departments of Srinivasan Medical College and Hospital, Samayapuram, Tiruchirappalli, Tamil Nadu, India, from July 2025 to November 2025. A total of 120 children with microcytic hypochromic anaemia and normal or low red blood cell counts were included. Blood samples were analysed for serum iron, serum ferritin, TIBC, TSAT, Red cell Distribution Width (RDW), and the Mentzer index. The diagnostic utility of these parameters in identifying IDA was compared. Data were analysed using Spearman's rank correlation test to assess correlations. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated. A p-value <0.05 was considered statistically significant.

Results: The mean age of the study population was five years four months. Of the 120 children, 63 (52.5%) were males, and 57 (47.5%) were females. Low serum iron was observed in 107 (89.2%) of children, while abnormal TSAT and elevated TIBC were seen in 84 (70.0%) and 76 (63.3%), respectively. TSAT showed the highest diagnostic accuracy (71.7%), whereas serum iron demonstrated the highest sensitivity (88.9%). RDW >13% was present in 111 (92.5%) of children, and Mentzer index >13, was observed in all 120 (100%) cases.

Conclusion: Serum iron and TSAT are valuable diagnostic markers for IDA and should be interpreted along with serum ferritin, particularly in hospital-based settings where inflammatory conditions may alter serum ferritin levels. An elevated Mentzer index may also prompt evaluation for possible co-existing haemoglobinopathies in children presenting with microcytic anaemia.

Keywords: Iron-binding proteins, Mentzer index, Microcytic hypochromic anaemia, Red cell distribution width, Transferrin saturation

INTRODUCTION

Iron deficiency is a serious child health issue in underdeveloped nations and one of the most common nutritional deficiency disorders [1]. According to the World Health Organisation (WHO) database on anaemia in 2005, 1.62 billion people were anaemic, with the majority of them being preschool children. According to the WHO Global Database on Anaemia, approximately 293 million preschool-aged children worldwide were affected by anaemia [1]. Because they do not consume enough iron in their diets, preschool children are more likely to develop IDA [2]. In both newborns and adolescents, the lack of iron causes delays in cognitive development, behavioural and motor problems [3].

Iron has a key function in the formation of haemoglobin, which is a major oxygen carrier. Reduced iron storage in the body has an effect on haemoglobin formation, which slows the delivery of oxygen to the body's tissues. The presence of microcytic hypochromic indices in a haemogram indicates IDA. At this point, a complete blood count will suffice in detecting IDA, but it may be normal in the early stages of iron deficiency [4,5].

IDA progresses via three stages in the body [6]. In both pre-latent and latent iron deficiency, low serum ferritin is common. T-sat, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and red blood cell distribution width are other peripheral markers that might be altered in latent iron deficiency. Although serum ferritin is the most sensitive indicator of iron shortage, it has a major drawback in that it can act as a positive acute phase reactant in inflammatory conditions. Many studies on TSAT and TIBC have yielded ambiguous results [7-10].

In hospital settings, it may be challenging to exclude clinically significant serum ferritin elevation, although it is considered the gold standard; levels may be falsely elevated in children with underlying microcytic syndromes or inflammatory states [11]. Previous studies have assessed the role of haemogram parameters and serum ferritin in diagnosing IDA in children [12,13]. However, serum ferritin can be falsely elevated in children with infections or inflammatory conditions, which may affect its accuracy [14]. Also, limited studies are available in hospitalised children with such conditions [15]. Although several studies have investigated haematological parameters and serum ferritin for diagnosing IDA in children, evidence in hospitalised paediatric patients with coexisting infections or inflammatory conditions remains scarce [16-18]. This

can complicate the diagnosis of IDA. Hence, the present study aimed to compare serum iron, TIBC, TSAT, Mentzer index, RDW, and serum ferritin to determine the most reliable diagnostic marker.

MATERIALS AND METHODS

This hospital-based prospective observational study was conducted at the Paediatric Outpatient and Inpatient Departments of Srinivasan Medical College and Hospital, Samayapuram, Tiruchirappalli, Tamil Nadu, India, from July 2025 to November 2025. This study was approved by the Institutional Ethics Committee (SMCH/MRB/2025/08/021) on 19.08.2025. The parents of the children signed a consent form before the study began. Strict confidentiality was maintained in the analysis and presentation of the data.

Inclusion criteria: Children aged six months to 15 years with microcytic hypochromic anaemia on peripheral smear with normal or low RBC count were included in the study.

Exclusion criteria: Children who were using iron supplements, those who had received blood transfusion within the previous three months, haemodynamically unstable children, and those with elevated RBC counts were excluded.

Sample size calculation: Since the primary objective of the study was to find the sensitivity and specificity of different iron parameters in diagnosing IDA, the sample size was calculated based on the sensitivity of serum ferritin (73.7%) reported by Jonker FA et al., [19].

$n = (Z^2 \times p \times q)/d^2$

Where Z = standard normal variate value = 1.96

P= sensitivity of serum ferritin 73.7%

q= 1-p (100-73.7) = 26.3%

d= clinical allowable error (8%)

n= (1.96×1.96×73.7×26.3)/(8×8)= 116.3

As a result, the study included a total of 120 children. Children fulfilling the inclusion criteria during the study period were included by convenience sampling.

Study Procedure

The lead investigator acquired the patient's medical history and conducted a clinical evaluation. Following that, a complete blood count with indices and RDW was taken, as well as serum iron, serum ferritin, TSAT, and TIBC. For the diagnostic-performance analysis, serum ferritin <18 ng/mL was used as the prespecified reference standard for iron deficiency anaemia; serum iron, TSAT and TIBC were evaluated as index tests. Haemoglobin and red blood cell indices were estimated using an automated haematology analyser. Serum iron and TIBC were measured by a colourimetric method, while serum ferritin was estimated using an immunoassay method. TSAT was calculated from serum iron and TIBC values. RDW, RBC count, and Mentzer index were obtained from complete blood count parameters. In the present study, for operational definition, normal serum iron levels range from 50 to 120 microgram/deciliter [20], TIBC was between 250 and 425 microgram/deciliter [20], and TSAT was 15.2-49.3%. TSATs greater than 50% suggest iron overload, whereas less than 15% indicates IDA [21]. Serum ferritin<18 ng/mL is indicative of iron insufficiency [22]. RDW values >13 and Mentzer index values >13 were considered abnormal and suggestive of IDA for analysis in the present study. All of the data were entered into a spreadsheet.

STATISTICAL ANALYSIS

For analysis, the data from the MS Excel spreadsheet were imported to Statistical Package for Social Sciences (SPSS) version 20.0. Spearman's rank was used to evaluate the association between the variables. A Scatter plot was used to represent the correlation. Sensitivity, specificity, PPV, NPV, positive likelihood ratio, and negative likelihood ratio were utilised to determine efficacy. A p-value < 0.05 was considered statistically significant in all analyses.

RESULTS

In the present study, a total of 120 children with proven microcytic hypochromic anaemia were enrolled and tested with available laboratory parameters for diagnosis of IDA, with a mean age of five years four months. There was a slight male predominance (52.5%) compared to females (47.5%). Most participants belonged to the lower middle socioeconomic class (45.0%), followed by the upper middle class (31.7%). Pallor was the most common presenting complaint (80.0%), followed by fatigue (56.7%) and loss of appetite (43.3%), while fever was less frequently reported (12.5%) [Table/Fig-1].

Variables	Values n (%)
Total participants	120
Age (Mean)	5 years 4 months
Gender	
Female	57 (47.5%)
Male	63 (52.5%)
Socioeconomic status	
Upper class	5 (4.2%)
Lower middle class	54 (45.0%)
Upper middle class	38 (31.7%)
Lower class	5 (4.2%)
Presenting complaint	
Pallor	96 (80.0%)
Fatigue	68 (56.7%)
Fever	15 (12.5%)
Loss of appetite	52 (43.3%)

[Table/Fig-1]: Demographic characteristics of the study participants (N=120).

Serum iron was low in 107 (89.2%) children and normal in 13 (10.8%) children. In this cohort of 120 children, 76 (63.3%) had abnormal TIBC. TSAT was found to be low in 84 (70.0%) children and normal in 36 (30%). Serum ferritin levels were reported as abnormal in 72 (60.0%) children and normal in 48 (40%). RDW and Mentzer Index were included as supportive screening markers for IDA. Diagnostic performance of RDW and the Mentzer index was not evaluated against the ferritin reference standard; therefore, these measures are reported descriptively only. Delete the statement about absence of a non-IDA group because the ferritin-based analysis itself classified 48 participants as non-IDA. Therefore, sensitivity, specificity, and ROC-based diagnostic utility could not be reliably calculated for these parameters. Comparison of cases based on serum ferritin levels and normal and abnormal findings of serum iron, TSAT, and TIBC [Table/Fig-2].

A total of 120 children were included, of whom 72 had low serum ferritin (<18 ng/mL), 48 had high/ normal (≥18 ng/mL) serum ferritin [Table/Fig-3].

Taking serum ferritin as a gold standard, sensitivity, specificity, PPV, NPV, and accuracy of serum iron were analysed. Serum iron

Variables	Serum ferritin		Total
	Low (<18 ng/mL) n (%)	Normal/High (≥18 ng/mL) n (%)	
Serum iron			
<50 µg/dL	64 (53.3)	43 (35.8)	107 (89.2)
>50 µg/dL	8 (6.7)	5 (4.2)	13 (10.8)
Transferrin Saturation (TSAT)			
<15.2%	61 (50.8)	23 (19.2)	84 (70)
>15.2%	11 (9.2)	25 (20.8)	36 (30)
Total Iron Binding Capacity (TIBC)			
>425 µg/dL	54 (45)	22 (18.3)	76 (63.3)
<425 µg/dL	18 (15)	26 (21.7)	44 (36.7)
Total	72 (60)	48 (40)	120 (100)

[Table/Fig-2]: Association between serum ferritin status and other iron parameters.

Variables	Iron Deficiency Anaemia (IDA)		Total
	Yes	No	
Serum iron			
<50 µg/dL	64	43	107
>50 µg/dL	8	5	13
Transferrin Saturation (TSAT)			
<15.2%	61	23	84
>15.2%	11	25	36
Total Iron Binding Capacity (TIBC)			
>425 µg/dL	54	22	76
<425 µg/dL	18	26	44
Total	72	48	120

[Table/Fig-3]: Association of serum iron, Transferrin Saturation (TSAT), and Total Iron Binding Capacity (TIBC) with Iron Deficiency Anaemia (IDA).

has 88.9% sensitivity and 10.4% specificity, with an accuracy of about 57.5%. TSAT had 84.7% sensitivity and 52% specificity with an accuracy of 71.7%, and TIBC had a sensitivity of 75.0% and a specificity of 54.2% with an accuracy rate of 66.7% in this cohort [Table/Fig-4]. Diagnostic performance analysis for RDW and Mentzer index was not performed, as no independent non-IDA comparison group was available for these parameters. Sensitivity, specificity, PPV, NPV, and diagnostic accuracy for serum iron, TSAT, and TIBC were calculated using serum ferritin (<18 ng/mL) as the reference standard and are presented in [Table/Fig-4].

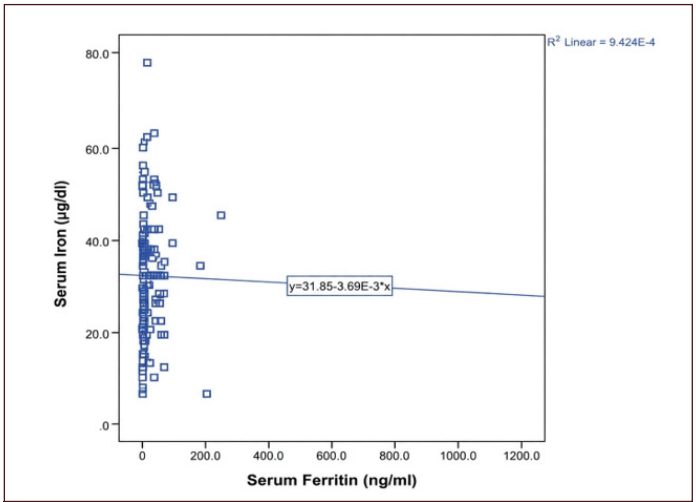
Parameters	Serum Iron	Transferrin Saturation (TSAT)	Total Iron Binding Capacity (TIBC)
Sensitivity	88.9%	84.7%	75.0%
Specificity	10.4%	52.0%	54.2%
Positive predictive value	59.8%	72.6%	71.1%
Negative predictive value	38.5%	69.4%	59.1%
Diagnostic accuracy	57.5%	71.7%	66.7%
Positive likelihood ratio	9.4	1.7	1.4
Negative likelihood ratio	0.1	0.6	0.7

[Table/Fig-4]: Diagnostic efficacy of different investigations. Likelihood ratios are dimensionless measures.

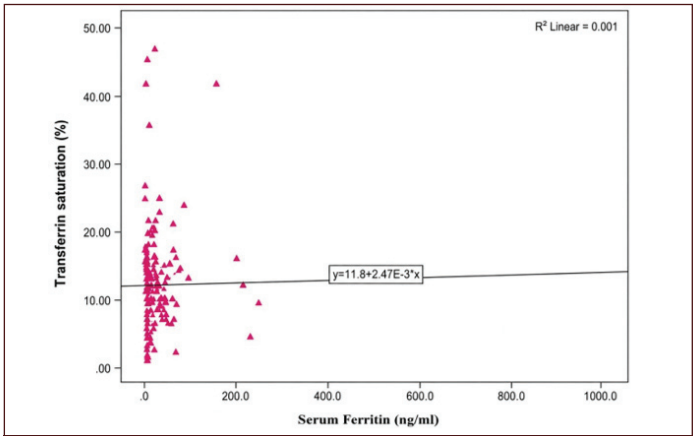
Spearman's rank correlation coefficient (rho) was 0.197 (p=0.031) for serum iron, 0.214 (p=0.019) for TSAT and -0.406 (p=0.0005) for TIBC, respectively, which shows a statistically significant positive correlation between serum ferritin and serum iron and serum ferritin and TSAT; however, there was a significant negative correlation observed between serum ferritin and TIBC levels [Table/Fig-5-8].

Variables	Parameter	Values
Correlation between Serum ferritin and serum iron	Correlation	0.197
	p-value	0.031*
Correlation between Serum ferritin and Transferrin Saturation (TSAT)	Correlation	0.214
	p-value	0.019*
Correlation between Serum ferritin and TIBC	Correlation	-0.406
	p-value	0.0005*

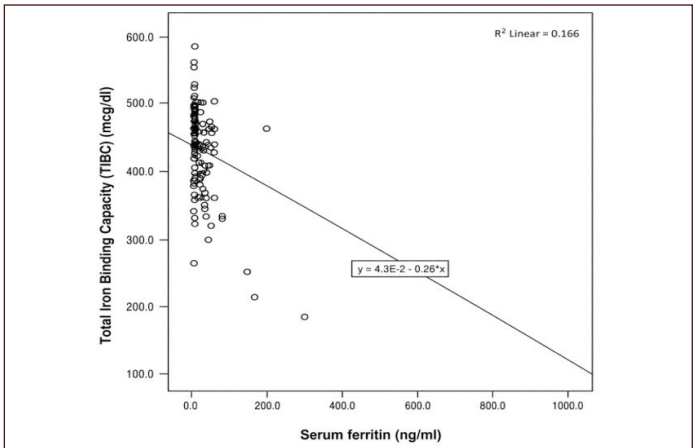
[Table/Fig-5]: Correlation between serum ferritin and other investigations among study participants (N=120).
* Statistically significant at p < 0.05



[Table/Fig-6]: Correlation between serum iron and serum ferritin.



[Table/Fig-7]: Correlation between Transferrin Saturation (TSAT) and serum ferritin.



[Table/Fig-8]: Correlation between serum ferritin and TIBC.

Correlation between serum ferritin and serum iron by Spearman's rank correlation was ρ-value=0.197 (p=0.031), which shows a statistically significant positive correlation between serum ferritin and serum iron [Table/Fig-6].

Correlation between serum ferritin and TSAT by Spearman's rank correlation: ρ-value=0.214 (p=0.019), which shows a statistically significant positive correlation between serum ferritin and TSAT [Table/Fig-7].

Correlation between serum ferritin and TIBC by Spearman's rank correlation ρ-value=-0.406 (p=0.0005), which shows a highly statistically significant negative correlation between serum ferritin and TIBC.

DISCUSSION

In most cases, IDA is diagnosed easily in the setting of anaemia with microcytic, hypochromic indices. Empirical iron therapy is often prescribed, and improvement in haemoglobin supports the diagnosis. However, disorders such as thalassemia trait may present similarly; therefore, confirmatory testing is useful. The present study aimed to identify the most effective diagnostic modality for IDA among tests available at the facility. Several studies with similar objectives are compared below [Table/Fig-9]. Most had sample sizes comparable with the current study, except Asif N et al., which included both adults and children and therefore had a larger cohort [7].

Serum ferritin was used as the reference standard for diagnosing iron deficiency anaemia in the present study. Low serum ferritin (<18 ng/mL) was observed in 72 (60%) participants [22]. Because serum ferritin served as the reference standard, its frequency in the present cohort should not be interpreted as evidence of inferior diagnostic performance [7,13,27]. Rather, the study demonstrated that serum iron, TSAT, and TIBC may provide complementary diagnostic information, particularly in patients in whom serum ferritin concentrations may be influenced by inflammatory conditions. Further studies incorporating independent reference standards, such as bone marrow iron assessment or composite diagnostic criteria, are required to determine the comparative diagnostic performance of these markers.

The TIBC was the third most reliable marker, identifying IDA in 63% of participants (76/120). Its sensitivity and specificity were 75% and 54.17%, respectively. TIBC showed a significant negative correlation with serum ferritin (p=0.0005). Similar lower sensitivity and specificity were reported by Asif N et al., [7]. TIBC should not be used alone for diagnosis.

The present study cohort also included the RDW and Mentzer index. RDW >13% was seen in 92.5 % of the study group, which was on the lower end of the range reported in prior research. Düzenli Kar Y et al., found that an RDW cut-off value of > 15% was the most sensitive measure for diagnosing IDA in the context of anaemia [3]. When the diagnosis is in doubt, RDW should be utilised to suspect IDA and cross-checked with more proven IDA markers, such as serum iron, serum ferritin, and so on. The Mentzer index (MCV/RBC) helped differentiate IDA from β-thalassemia trait. All participants had a Mentzer index >13, supporting IDA [28]. However, haemoglobin electrophoresis was not performed in all participants; therefore, haemoglobinopathy traits could not be conclusively excluded.

Study	Place/year of the study	Sample size	Mean age	Parameters assessed	Finding
Jonker FA et al., [19]	Netherland 2014	87	37 months	Ferritin, Hb, MCV, RDW	Ferritin showed good diagnostic utility for IDA
Aydogan G et al., [15]	Turkey 2019	200	48 months	Ferritin, Serum Iron, TIBC, TSAT	TSAT and TIBC were useful markers for iron deficiency
Asif N et al., [7]	Pakistan 2016	1815	29 years	CBC indices, Ferritin	Haematological indices correlated with iron deficiency
Present study	Trichy, India 2026	120	5 years and 4 months	Ferritin, Serum Iron, TIBC, TSAT, RDW, Mentzer Index	TSAT and TIBC showed better diagnostic utility than ferritin; RDW >13% in 92.5%; Mentzer Index >13 in 100%

[Table/Fig-9]: Comparison of sample size and mean age of different studies.

Peripheral iron indicators- including serum ferritin, serum iron, TIBC, and TSAT- are commonly used to diagnose iron deficiency, although their interpretation may be affected by disorders of iron metabolism [7]. Serum ferritin was used as the gold standard because it is widely regarded as a valuable and frequently used marker for IDA [14,23].

These findings were comparable with those of Sazawal S et al., who reported serum iron sensitivity of 85% and specificity of 15% in a similarly sized sample [12]. Uen WC reported higher specificity and sensitivity for serum iron (74% and 91%, respectively) in a larger Taiwanese study [24]. In the present study, serum iron was useful for identifying IDA, although only 60% of children had low serum ferritin levels. Obesity can also raise serum ferritin levels, reducing its usefulness in detecting IDA [19]. Height and weight were not recorded in this study, so this effect could not be assessed.

Among the index tests evaluated against the ferritin reference standard, TSAT showed the highest diagnostic accuracy and specificity. TSAT had a positive likelihood ratio of 1.77, a negative likelihood ratio of 0.29 and diagnostic accuracy of 71.7%, compared with 57.5% for serum iron and 66.7% for TIBC. Its association was statistically significant. Powers JM and Buchanan GR. Cacoub P et al., and Elsayed ME et al., similarly reported that TSAT may better reflect iron available to the marrow than serum ferritin [6,25,26].

Limitation(s)

The current study has certain limitations. Serum inflammatory markers such as C-Reactive Protein (CRP) or procalcitonin were not assessed; hence, the possibility of subclinical inflammation contributing to elevated serum ferritin levels in some cases cannot be excluded. Furthermore, haemoglobin electrophoresis was not performed in all cases; therefore, the ability of the Mentzer index to conclusively exclude haemoglobinopathy traits could not be established.

CONCLUSION(S)

The most helpful peripheral blood measure for detecting iron-deficient anaemia was serum iron. In the present study, TSAT and TIBC outperformed serum ferritin in detecting IDA. Serum ferritin was beneficial in detecting IDA in 60% of individuals.

TSAT demonstrated the highest diagnostic accuracy, whereas serum iron showed the highest sensitivity. RDW >13 % in a full haemogram in the presence of low haemoglobin should raise suspicion of IDA.

Authors' contribution: All authors contributed substantially to the conception and design of the study, data collection, analysis and interpretation of data, drafting and critical revision of the manuscript for important intellectual content. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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