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Original Research Article

Iron Deficiency Anemia, Comparative Study of CBC, Peripheral Smear, Iron Studies and RDW

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Conflict of interest: Nil

Abstract:

Background: Iron deficiency anaemia (IDA) is the most prevalent nutritional disorder among children worldwide and remains a major public health concern in developing countries. Early diagnosis is essential to prevent impaired physical growth, neurocognitive deficits, and reduced immunity. Although iron profile investigations are considered standard diagnostic tools, they are relatively expensive and may not be readily available in resource-limited settings. Red cell distribution width (RDW), a routinely reported parameter in automated complete blood count (CBC), has emerged as a promising early indicator of iron deficiency.

Objectives: To evaluate the diagnostic utility of RDW in detecting iron deficiency anaemia among children and compare its performance with conventional hematological parameters and iron profile studies.

Methods: A prospective analytical study was conducted over 25 months (June 2011–July 2013) in the Department of Clinical Pathology, Sri Venkateswara Ramnarain Ruia Government General Hospital and Sri Venkateswara Medical College, Tirupati. A total of 159 children clinically suspected of iron deficiency anaemia were enrolled. Complete blood count, peripheral blood smear, red cell indices, RDW, and iron profile investigations including serum ferritin, serum iron, and total iron-binding capacity (TIBC) were analysed. Statistical analysis included Chi-square test, Fisher's exact test, ANOVA, and diagnostic accuracy measures.

Results: Iron deficiency based on iron profile criteria was identified in 130 (82.0%) children. Elevated RDW (>16%) was observed in 81.1% of cases. RDW demonstrated a sensitivity of 89.2%, specificity of 55.2%, positive predictive value of 89.9%, negative predictive value of 53.3%, and an overall diagnostic accuracy of 83.0% for identifying iron deficiency. Low MCV showed significant association with abnormal iron studies ($p<0.001$). Microcytic hypochromic peripheral smear findings were significantly associated with iron deficiency ($p=0.012$).

Conclusion: RDW is a highly sensitive and readily available hematological parameter for the early detection of iron deficiency anaemia in children. When interpreted alongside peripheral smear examination and red cell indices, RDW can serve as an effective screening tool, particularly in resource-constrained healthcare settings.

Keywords: Iron Deficiency Anaemia, Red Cell Distribution Width, RDW, Pediatric Anaemia, Mean Corpuscular Volume, Peripheral Smear, Serum Ferritin.

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Introduction

Iron deficiency anaemia (IDA) is the commonest nutritional deficiency worldwide and remains the leading cause of anaemia among infants and children. The World Health Organization estimates that nearly two billion individuals are anaemic globally, with iron deficiency accounting for approximately half of all cases, particularly in low- and middle-income countries where nutritional deficiencies and recurrent infections frequently coexist. Childhood iron deficiency continues to be an important public health challenge because of its

high prevalence and long-term adverse consequences on health and development [1,2].

Iron plays a fundamental role in haemoglobin synthesis, oxygen transport, cellular metabolism, immune function, and neurological development. During infancy and early childhood, rapid growth substantially increases iron requirements. When dietary intake is inadequate or losses exceed absorption, iron stores become depleted, eventually leading to iron deficiency anaemia. Persistent iron deficiency during early childhood has been associated with impaired cognitive development, poor scholastic performance, behavioural abnormalities, delayed psychomotor development, reduced physical growth, and increased susceptibility to infections [3–5].

The diagnosis of iron deficiency anaemia traditionally relies on a combination of clinical assessment, complete blood count, peripheral blood smear examination, and biochemical markers of iron metabolism. Serum ferritin is regarded as the most reliable indicator of body iron stores under non-inflammatory conditions, while serum iron, transferrin saturation, and total iron-binding capacity (TIBC) provide supportive evidence of iron deficiency. However, these investigations are relatively expensive, influenced by inflammatory states, and may not be readily accessible in many peripheral laboratories and primary healthcare settings [6–8].

Automated haematology analysers routinely generate several red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW). Among these, RDW measures the degree of variation in erythrocyte size (anisocytosis). Because anisocytosis develops early during iron depletion, RDW often becomes abnormal before significant reductions in MCV or haemoglobin occur, making it a potentially useful early screening marker for iron deficiency [9–11].

Several investigators have evaluated the diagnostic performance of RDW in paediatric populations. Previous studies have reported that elevated RDW demonstrates high sensitivity for identifying iron deficiency, although specificity varies according to study population, diagnostic criteria, and laboratory cut-off values. When combined with peripheral smear findings and red cell indices, RDW may substantially improve the early recognition of iron deficiency anaemia without increasing laboratory costs [12–15].

Peripheral blood smear examination continues to be an indispensable diagnostic tool in evaluating anaemia. The presence of microcytic hypochromic erythrocytes, anisopoikilocytosis, target cells, and pencil cells strongly suggests iron deficiency. Nevertheless, morphological examination alone cannot reliably distinguish iron deficiency from other causes of microcytic anaemia such as thalassaemia trait or anaemia of chronic disease. Therefore, correlating smear morphology with RDW and biochemical iron parameters enhances diagnostic accuracy [16–18].

Despite advances in laboratory medicine, there remains a need for simple, inexpensive, and widely available screening tools that facilitate early diagnosis of iron deficiency anaemia in children, particularly in resource-limited settings where access to specialised investigations is often restricted. Since RDW is automatically reported as part of every complete blood count without additional cost, its diagnostic value deserves further evaluation in routine clinical practice [19,20].

Accordingly, the present prospective analytical study was undertaken to assess the diagnostic utility of RDW in detecting iron deficiency anaemia among paediatric patients and to compare its performance with conventional haematological parameters, peripheral smear findings, and biochemical iron profile studies.

Materials and Methods

Study Design: This was a hospital-based prospective analytical study.

Study Setting: The study was conducted in the Department of Clinical Pathology, Sri Venkateswara Ramnarain Ruia Government General Hospital (SVRRGGH) and Sri Venkateswara Medical College (SVMC), Tirupati, Andhra Pradesh, India.

Study Duration: June 2011 to July 2013 (25 months).

Study Population: Children presenting with clinical suspicion of iron deficiency anaemia who were referred to the Department of Clinical Pathology during the study period.

Sample Size: A total of 159 consecutive children fulfilling the eligibility criteria were included in the study.

Inclusion Criteria

- Children aged 0–14 years.
- Clinical suspicion of iron deficiency anaemia.
- Availability of complete hematological and iron profile investigations.
- Informed consent obtained from parents or guardians.

Exclusion Criteria

- Children receiving iron supplementation before evaluation.

- Previously diagnosed hematological malignancies.
- Recent blood transfusion.
- Incomplete laboratory investigations.

Study Procedure: After obtaining informed consent, detailed demographic information and clinical history were recorded. Each child underwent thorough clinical examination with particular emphasis on symptoms and signs suggestive of anaemia.

Approximately 3–5 mL of venous blood was collected under aseptic precautions. Laboratory investigations included complete blood count (CBC), peripheral blood smear examination, reticulocyte count, red cell indices (MCV, MCH, MCHC), RDW, serum iron, serum ferritin, and total iron-binding capacity (TIBC).

Peripheral blood smears were stained using Leishman stain and examined independently by experienced pathologists. Morphological findings were categorized as microcytic hypochromic, normocytic normochromic, normocytic hypochromic, macrocytic, or dimorphic anaemia.

Iron deficiency was diagnosed using the following biochemical criteria:

- Serum ferritin <12 ng/mL and/or
- TIBC >389 µg/dL

Children were categorized according to World Health Organization haemoglobin criteria:

- Group I (Moderate anaemia): Hb 7.0–10.0 g/dL
- Group II (Severe anaemia): Hb 5.0–6.9 g/dL
- Group III (Very severe anaemia): Hb <5.0 g/dL

Outcome Measures: The primary outcome was the diagnostic performance of RDW in identifying iron deficiency anaemia.

Secondary Outcomes Included:

- Association between RDW and severity of anaemia.
- Correlation of RDW with iron profile parameters.
- Relationship between peripheral smear morphology and iron deficiency.
- Diagnostic performance of MCV and peripheral smear compared with iron profile studies.

Statistical Analysis: Data were entered into Microsoft Excel and analysed using SPSS software (Version 20.0; IBM Corp., Armonk, NY, USA).

Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were summarized as frequencies and percentages.

Comparisons between categorical variables were performed using the Chi-square test or Fisher's exact test, as appropriate. Differences in continuous variables among severity groups were analysed using one-way analysis of variance (ANOVA).

Diagnostic validity of RDW and peripheral smear was assessed by calculating:

- Sensitivity
- Specificity
- Positive Predictive Value (PPV)
- Negative Predictive Value (NPV)
- Diagnostic Accuracy

Agreement between RDW and iron profile was evaluated using the Kappa statistic.

A p-value <0.05 was considered statistically significant.

Ethical Considerations: The study protocol was approved by the Institutional Ethics Committee of Sri Venkateswara Medical College, Tirupati. Written informed consent was obtained from parents or legal guardians before enrolment. Confidentiality of patient information was maintained throughout the study in accordance with the ethical principles of the Declaration of Helsinki.

Results

Study Population: A total of 159 children with clinically suspected iron deficiency anaemia were included in the study. The majority belonged to the 0–4-year age group (41.5%), followed by 9–14 years (33.3%) and 5–8 years (25.2%) (Table 1, Figure 1). The study population comprised 79 (49.7%) males and 80 (50.3%) females, with no significant association between age and gender (χ^2=0.86, p=0.64) (Figure 2).

Table 1: Demographic characteristics of study participants (n=159)

Variable	n (%)
Age group	
0–4 years	66 (41.5)
5–8 years	40 (25.2)
9–14 years	53 (33.3)
Gender	
Male	79 (49.7)
Female	80 (50.3)

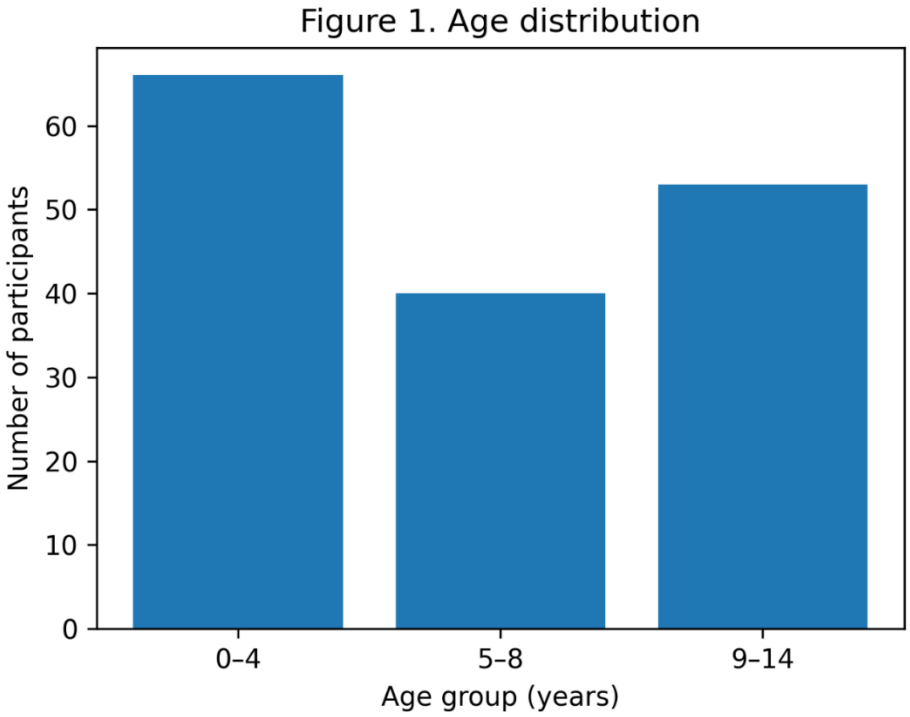

Figure 1: Age distribution of study participants.

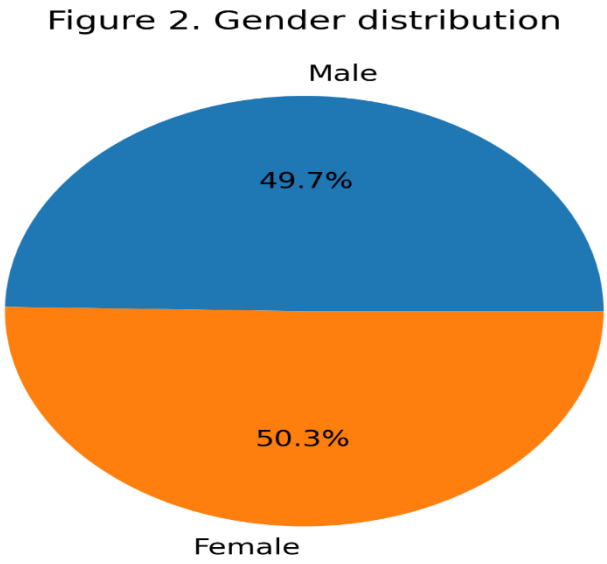

Figure 2: Gender distribution of study participants.

Severity of Anaemia: Based on haemoglobin concentration, 76 (47.8%) children had moderate anaemia, 41 (25.8%) had severe anaemia, and 42 (26.4%) had very severe anaemia. Overall, more than half (52.2%) presented with haemoglobin levels below 7 g/dL (Table 2, Figure 3).

Table 2: Severity of anaemia among study participants

Severity	Hb (g/dL)	n (%)
Moderate	7–10	76 (47.8)
Severe	5–6.9	41 (25.8)
Very severe	<5	42 (26.4)

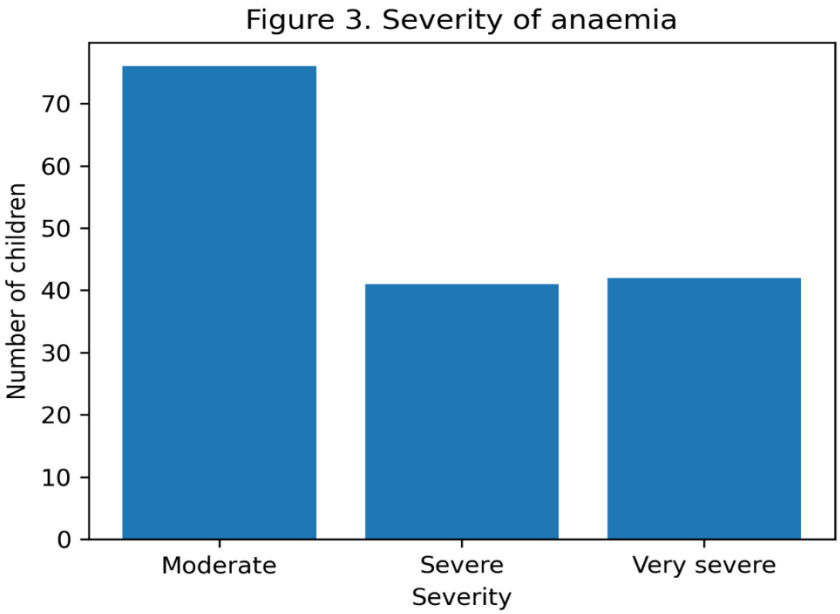

Figure 3: Distribution of children according to severity of anaemia.

Peripheral Blood Smear Findings: Peripheral blood smear examination demonstrated microcytic hypochromic anaemia as the predominant morphological pattern, observed in 103 (64.8%) children. This pattern showed a significant association with increasing severity of anaemia (χ^2=20.25, p=0.0025) (Table 3, Figure 4).

Table 3: Peripheral smear morphology

Peripheral smear finding	n (%)
Microcytic hypochromic	103 (64.8)
Normocytic hypochromic	22 (13.8)
Macrocytic	21 (13.2)
Normocytic normochromic	7 (4.4)
Dimorphic	6 (3.8)

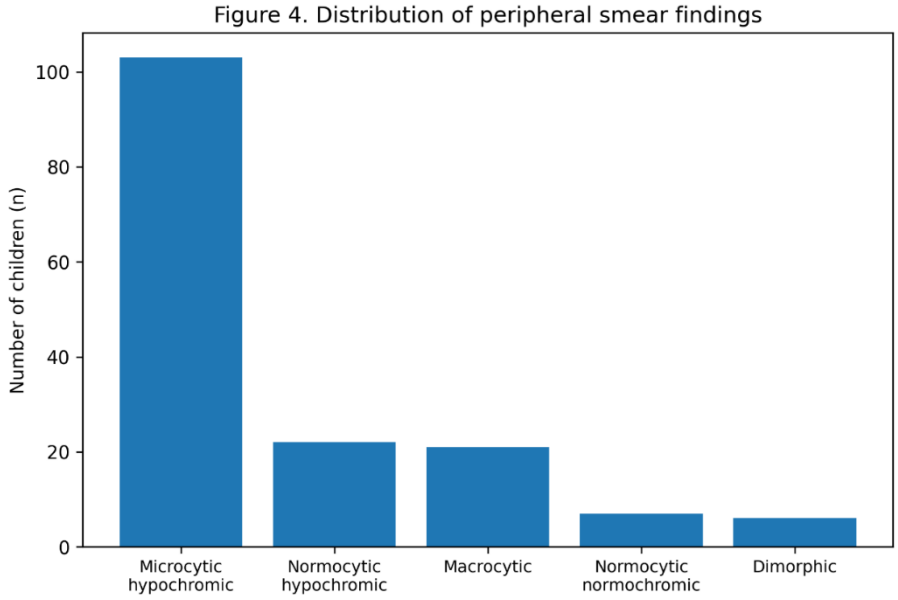

Figure 4: Distribution of peripheral smear findings.

Red Cell Indices: Among the study population, 54.1% had MCV values between 60–75 fL, while 90.6% had low MCH (<27 pg). Elevated RDW (>16%) was observed in 129 (81.1%) children (Table 4, Figure 5).

Table 4: Distribution of red cell indices

Parameter	Finding	n (%)
MCV	<80 fL	151 (95.0)
MCH	<27 pg	144 (90.6)
RDW-CV	>16%	129 (81.1)

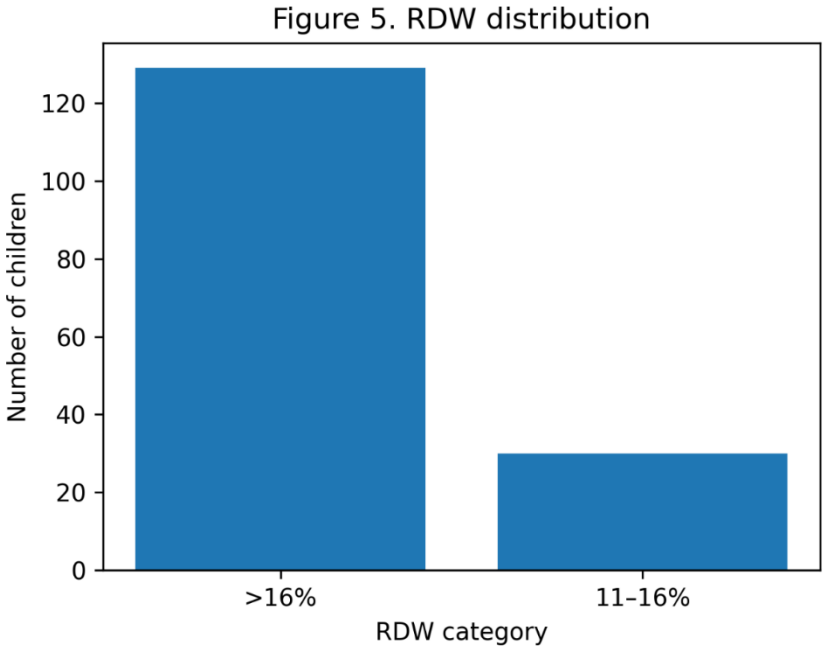

Figure 5: Distribution of RDW among study participants.

Iron Profile: Based on the predefined biochemical criteria (serum ferritin <12 ng/mL and/or TIBC >389 µg/dL), 130 (82.0%) children were diagnosed with iron deficiency, whereas 29 (18.0%) had normal iron studies (Table 5, Figure 6).

Table 5: Iron profile findings

Iron profile	n (%)
Iron deficiency	130 (82.0)
Normal iron status	29 (18.0)

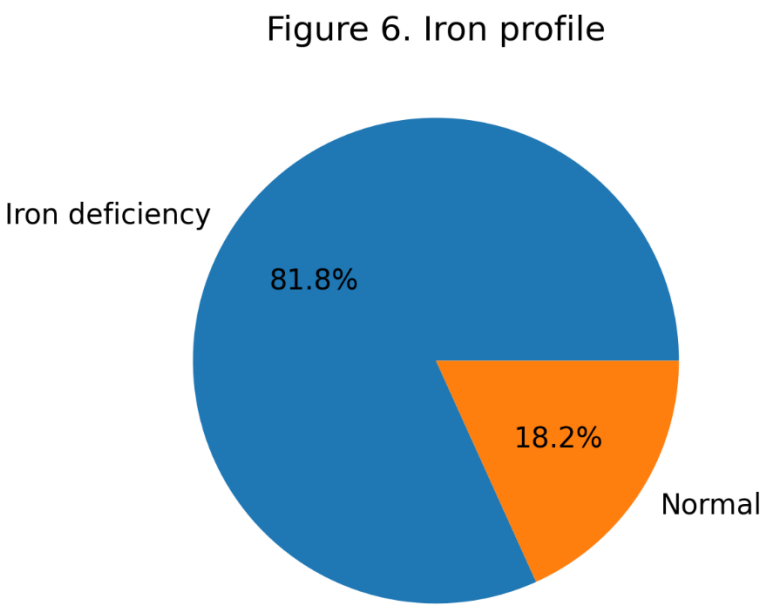

Figure 6: Distribution of iron profile findings.

Association Between MCV and Iron Deficiency: Among children with MCV <80 fL, 128 of 151 (84.8%) had biochemical evidence of iron deficiency. In contrast, only 2 of 8 (25.0%) children with MCV ≥80 fL had abnormal iron studies. This association was highly significant (Fisher's exact test, p<0.001) (Table 6).

Table 6. Association between MCV and iron profile

MCV	Iron deficiency	Normal iron status
<80 fL	128 (84.8%)	23 (15.2%)
≥80 fL	2 (25.0%)	6 (75.0%)

Association Between Peripheral Smear Morphology and Iron Deficiency: Peripheral smear findings were compared with biochemical iron studies to evaluate their diagnostic utility. Among the 103 children demonstrating microcytic hypochromic red blood cells, 90 (87.4%) had biochemical evidence of iron deficiency, whereas only 13 (12.6%) had normal iron studies. In comparison, among the 56 children without microcytic hypochromic morphology, 40 (71.4%) had iron deficiency and 16 (28.6%) had normal iron status. This association was statistically significant (χ^2=6.18, p=0.012) (Table 7, Figure 7).

Table 7: Association between peripheral smear morphology and iron profile

Peripheral smear morphology	Iron deficiency n (%)	Normal iron status n (%)	Total
Microcytic hypochromic present	90 (87.4)	13 (12.6)	103
Microcytic hypochromic absent	40 (71.4)	16 (28.6)	56

χ^2 = 6.18; p = 0.012

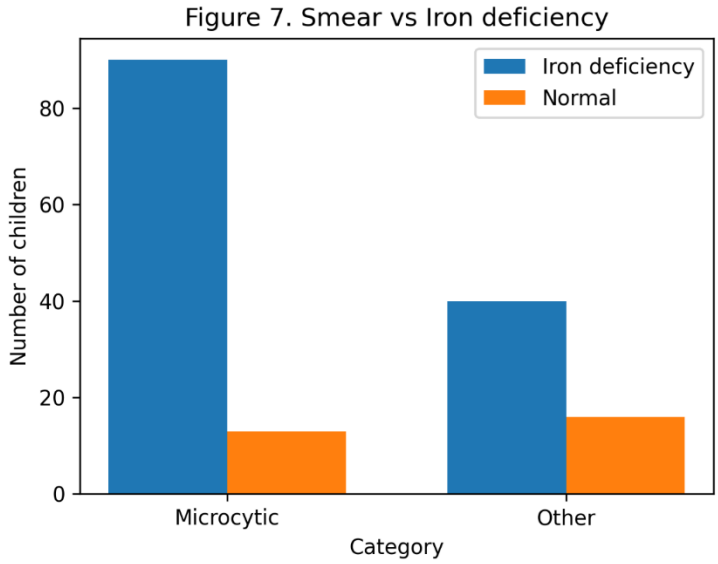

Figure 7: Association between peripheral smear morphology and biochemical iron deficiency.

Association Between RDW and Iron Deficiency: Elevated RDW (>16%) showed a strong association with biochemical evidence of iron deficiency. Of the 129 children with increased RDW, 116 (90.0%) had abnormal iron studies, whereas only 13 (10.0%) had normal iron parameters. Conversely, among children with normal RDW, iron deficiency was identified in 14 (46.7%), while 16 (53.3%) had normal iron studies. This association was highly statistically significant (χ^2=30.5, p<0.001) (Table 8, Figure 8).

Table 8: Association between RDW and iron profile

RDW-CV	Iron deficiency n (%)	Normal iron status n (%)	Total
>16%	116 (90.0)	13 (10.0)	129
11–16%	14 (46.7)	16 (53.3)	30

χ^2 = 30.5; p <0.001

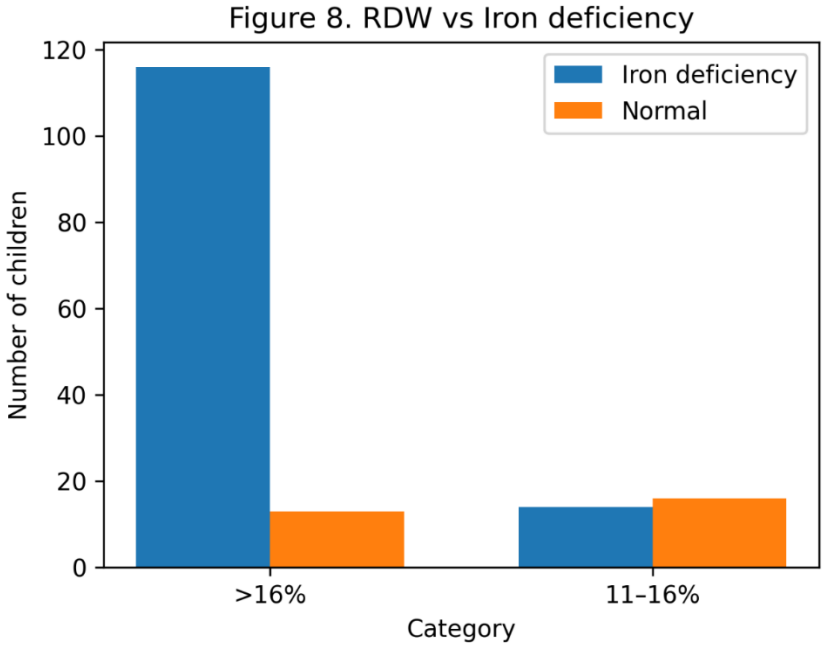

Figure 8: Association between RDW and biochemical iron deficiency.

Diagnostic Performance of RDW for Detecting Iron Deficiency: Using biochemical iron studies as the reference standard, RDW demonstrated excellent sensitivity for detecting iron deficiency

anemia. The sensitivity was 89.2%, specificity 55.2%, positive predictive value 89.9%, negative predictive value 53.3%, and overall diagnostic accuracy 83.0%. The kappa coefficient of 0.44 indicated moderate agreement between RDW and biochemical iron studies (Table 9).

Table 9: Diagnostic performance of RDW for detection of iron deficiency

Parameter	Value (%)
Sensitivity	89.2
Specificity	55.2
Positive Predictive Value	89.9
Negative Predictive Value	53.3
Diagnostic Accuracy	83.0
False Positive Rate	44.8
False Negative Rate	10.8
Kappa coefficient	0.44 (Moderate agreement)

Iron Profile According to Severity of Anaemia: Mean serum iron levels decreased significantly with increasing severity of anemia ($p<0.001$), while serum ferritin demonstrated significant variability, particularly among children with very severe anemia, likely reflecting its role as an acute-phase reactant. Mean TIBC remained elevated across all severity groups but did not differ significantly ($p=0.47$) (Table 10).

Table 10: Iron profile according to severity of anaemia

Severity of anaemia	TIBC (µg/dL) Mean±SD	Serum iron (µg/dL) Mean±SD	Serum ferritin (ng/mL) Mean±SD
Moderate (Hb 7–10 g/dL)	422.0 ±49.1	40.3 ±13.6	40.8 ±79.9
Severe (Hb 5–6.9 g/dL)	435.0 ±54.7	30.4 ±12.2	38.4 ±105.3
Very severe (Hb <5 g/dL)	423.9 ±66.6	30.2 ±20.5	108.4 ±183.9
p-value	**0.47**	**<0.001**	**<0.001**

Summary of Key Findings: Overall, 82.0% of clinically suspected children had biochemical evidence of iron deficiency. Elevated RDW and microcytic hypochromic peripheral smear morphology were both significantly associated with iron deficiency, with RDW demonstrating high sensitivity (89.2%) and good diagnostic accuracy (83.0%). These findings support the use of RDW as a simple and cost-effective screening marker for pediatric iron deficiency anemia, particularly in resource-limited settings.

Discussion

Iron deficiency anemia (IDA) remains the leading cause of nutritional anemia among children worldwide and continues to be an important public health problem in developing countries. Early diagnosis is essential because prolonged iron deficiency adversely affects growth, neurodevelopment, cognitive performance, immune function, and overall quality of life. [21]

In the present study, the highest proportion of children belonged to the 0–4-year age group (41.5%), with an almost equal distribution between males and females. These findings are comparable to those reported by Presenjit Raut et al., Roosy Aulakh et al., and Vishwanath et al., who also observed that preschool children constitute the most vulnerable age group owing to rapid growth, increased iron requirements, and inadequate dietary intake. [22-24] The absence of a significant gender difference in our study is also consistent with community-based pediatric studies conducted in India. [25]

More than half of the enrolled children presented with hemoglobin concentrations below 7 g/dL, indicating that severe anemia remains common among children attending tertiary-care hospitals. Similar observations have been reported from other hospital-based studies where delayed healthcare seeking, poor socioeconomic status, nutritional deficiencies, and recurrent infections contributed to late presentation. [26]

Peripheral smear examination demonstrated microcytic hypochromic anemia in 64.8% of cases, making it the predominant morphological pattern. This finding is in agreement with previous studies showing that iron deficiency characteristically produces microcytic hypochromic erythrocytes because of impaired hemoglobin synthesis. [23,27] Although peripheral smear remains an inexpensive and useful initial investigation, similar morphological changes may also occur in thalassemia trait and anemia of chronic disease, limiting its specificity when used alone. [28]

Low MCV also demonstrated a significant association with abnormal iron studies; however,

RDW appears to increase earlier during iron-deficient erythropoiesis before appreciable reductions in MCV occur. Consequently, simultaneous interpretation of RDW, MCV, and peripheral smear findings enhances diagnostic confidence and improves early detection of iron deficiency, particularly in resource-constrained healthcare settings where biochemical iron studies are not routinely available. [28-30]

The most important observation of the present study was the excellent diagnostic performance of red cell distribution width (RDW). Elevated RDW (>16%) was observed in 81.1% of children and demonstrated a strong association with biochemical evidence of iron deficiency. RDW showed a sensitivity of 89.2%, specificity of 55.2%, positive predictive value of 89.9%, negative predictive value of 53.3%, and overall diagnostic accuracy of 83.0%. These findings indicate that RDW is a highly sensitive screening marker for iron deficiency and are comparable with previous reports, although differences in specificity among studies may reflect variations in study populations, laboratory methods, and diagnostic criteria. [30]

Serum iron levels declined significantly with increasing severity of anemia, whereas serum ferritin showed marked variability, especially among children with very severe anemia. Because ferritin is an acute-phase reactant, elevated values may occur in the presence of infection, inflammation, or hemolysis despite underlying iron deficiency. Therefore, ferritin should always be interpreted alongside clinical findings and complementary laboratory parameters. [21,29]

Overall, the findings of the present study support the routine use of RDW as a simple, inexpensive, and readily available screening parameter in the evaluation of pediatric anemia. When interpreted together with MCV and peripheral smear examination, RDW can facilitate early identification of iron deficiency and help prioritize children who require confirmatory biochemical testing and prompt therapeutic intervention.

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