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Original Research Article

A Comparative Study of RBC Histogram Morphology and Indices with Peripheral Smear Evaluation in Anaemia Diagnosis

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Abstract:

Introduction: Anaemia is one of the most frequently encountered clinical conditions in haematology, with diverse aetiologies requiring accurate morphological diagnosis. While peripheral smear (PS) examination remains the cornerstone for classification, advancements in automated haematology analysers have introduced red blood cell (RBC) histograms and indices as valuable supplementary tools. RBC indices provide quantitative data that complement PS findings in anaemia evaluation. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) help classify anaemia as microcytic, macrocytic, or normocytic, aiding in the identification of causes such as iron or vitamin B12 deficiency and chronic diseases. These parameters, along with red cell distribution width (RDW) and histogram patterns, assist in the early detection and differentiation of anaemia subtypes. This study aims to evaluate the correlation between PS morphology, RBC indices, and histogram patterns in patients with anaemia.

Aim: To study the correlation between red blood cell histogram morphology, RBC indices and peripheral smear findings in the diagnosis of anaemia.

Materials and Methods: This cross-sectional study was conducted over a period of three months, from March to May 2025, involving a total of 215 patients at Central Laboratory, Akash Institute of Medical Sciences and Research Centre. EDTA-anticoagulated blood samples were analysed using the Beckman Coulter Unicel DxH 800, an automated 7-part haematology analyser, to obtain RBC histograms and indices. Peripheral blood smears were prepared, stained with Leishman stain, and the microscopic findings were correlated with histogram patterns and RBC indices.

Results: A total of 215 patients with anaemia were included, comprising 61.86% females and 38.14% males. The highest prevalence of anaemia was observed in the 29–38 age group (47.45%), followed by the 18–28 age group (23.25%). Morphologically, microcytic hypochromic anaemia was the most common type (76.74%), followed by normocytic normochromic (17.67%), dimorphic (4.19%), and macrocytic anaemia (1.40%).

Histogram patterns included broad base (37.2%), left shift (33.5%), normal curve (18.6%), bimodal (7.4%), and right shift (3.3%). Microcytic anaemia predominantly showed left shift and broad base curves; normocytic anaemia showed normal and broad base curves; macrocytic anaemia showed right shift; and dimorphic anaemia exhibited varied patterns.

A significant correlation ($p < 0.05$) was observed between histogram patterns and peripheral smear morphology. When combined with red cell indices (MCV, RDW, MCH, MCHC) and histogram analysis, enhanced the accuracy of morphological classification.

Conclusion: Integrating RBC indices and histogram analysis with traditional peripheral smear examination enhances diagnostic accuracy in anaemia evaluation. Automated haematology analysers provide objective, reproducible data that complement morphological findings, allowing for earlier and more precise identification of anaemia subtypes. Given the significant global health burden of anaemia, particularly in resource-limited settings, adopting such combined diagnostic approaches can facilitate timely management and improve patient outcomes.

Introduction

Anemia is a significant global public health concern, affecting an estimated 1.8 billion individuals worldwide, and presents a significant diagnostic challenge due to its diverse aetiologies [6]. In India, anemia affects 57.2% of women and 25.0% of men aged 15–49 years, as per NFHS-5 data. Accurate classification of anemia is critical for effective clinical management and relies heavily on morphological assessment.

Peripheral smear (PS) examination remains the gold standard for red blood cell (RBC) morphology [1,7], aiding in the detection of features like anisopoikilocytosis and hypochromia.

Over the years, blood cell analysis has significantly advanced, transitioning from manual methods to automated systems that offer greater precision and reliability. These advances in laboratory technology have made automated haematology analysers essential, offering complete blood counts along with detailed RBC indices and histograms [2,4,8].

RBC indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) aid in classifying anemia types and identifying underlying causes [9,10,11].

An RBC histogram is a vertical bar chart that represents cell volume distribution. They show characteristic patterns such as left shift, right shift, broad base, or bimodal curves that correlate with specific anemia types [12,13,14]. Despite their diagnostic value, histograms remain under-utilised in many laboratories due to limited awareness and insufficient training in interpretation.

These RBC indices and histogram patterns, along with PS examination assist in the early detection and differentiation of anemia subtypes. This study aims to evaluate the correlation between PS morphology, RBC indices, and histogram patterns in patients with anemia.

Aim:

To study the correlation between red blood cell histogram morphology, RBC indices and peripheral smear findings in the diagnosis of anaemia.

Materials and Methods

This cross-sectional study was conducted over three months, from March to May 2025 in the Central Laboratory of a tertiary care center. A total of 215 patients diagnosed with anemia, as per WHO reference ranges [15], were included.

All patients above 18 years of age who were diagnosed with anaemia based on WHO reference ranges, using both automated analyser values and peripheral smear (PS) findings, were included in the study. EDTA samples that were clotted, haemolysed, diluted, or insufficient in volume were excluded. Patients with leukocytosis, leukemoid reactions, or leukemia, as well as anaemic patients who had received a blood transfusion, were also excluded from the study.

3 mL of venous blood was collected in EDTA-anticoagulated vacutainers and processed within one hour. Samples were analyzed using the Beckman Coulter Unicel DxH 800, a fully automated 7-part hematology analyzer, to obtain CBC, RBC indices (MCV, MCH, MCHC, RDW), and RBC histogram patterns. Peripheral blood smears (PBS) were prepared and stained with Leishman stain.

RBC morphology was evaluated microscopically to classify anemia types (microcytic, normocytic, macrocytic, dimorphic). PS findings were correlated with histogram patterns and RBC indices to evaluate and diagnose different anemia subtypes.

Statistical Analysis:

Data was entered into Microsoft Excel and analyzed using SPSS version 27. Pearson's chi-square test was applied for categorical data, with $p < 0.05$ considered statistically significant. Results were presented through tables and charts.

Results:

1. **Demographic Characteristics**

A total of 215 patients diagnosed with anaemia were included in the study, comprising 61.86% females and 38.14% males. Anaemia was most prevalent in the 29–38 years age group (47.45%), followed by the 18–28 years age group (23.25%), indicating a higher burden of anaemia among young and middle-aged adults. This age-wise distribution reflects the demographic pattern commonly observed in regions with high nutritional deficiency and reproductive-age female predominance.

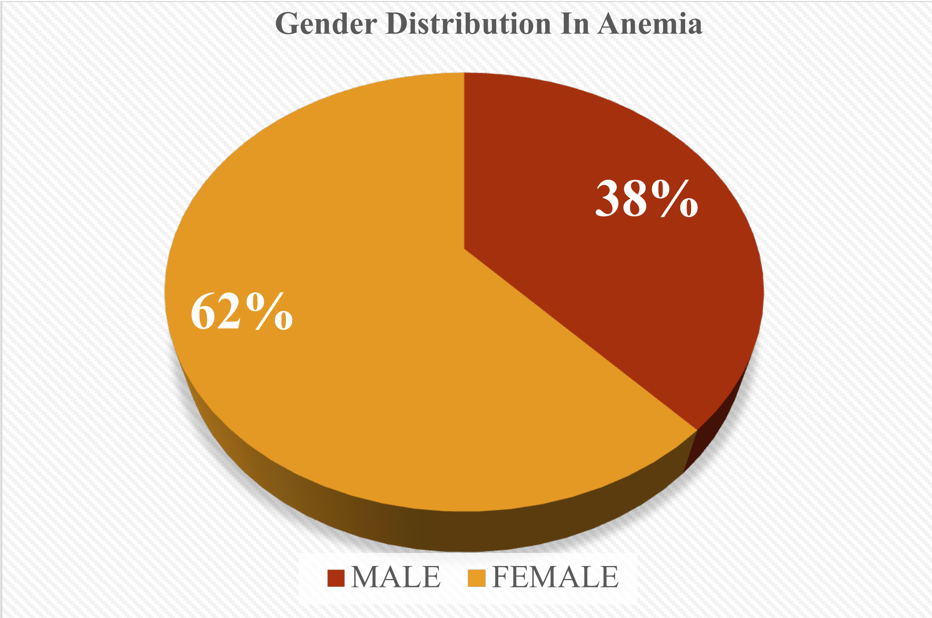

Figure 1: Gender Distribution in Anemia

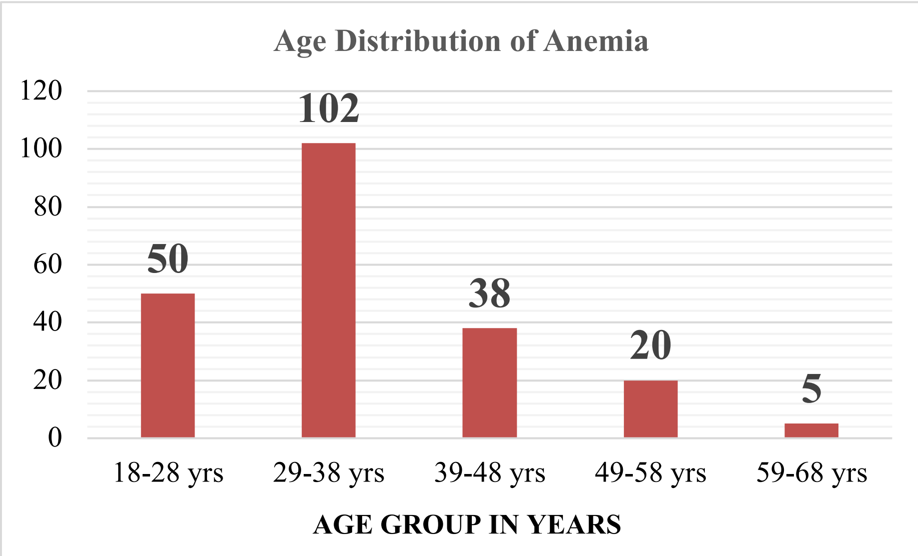

Figure 2: Age Distribution of Anemia

2. **Peripheral Smear Morphological Patterns:** Peripheral smear examination revealed that microcytic hypochromic anaemia (MHA) was the most common morphological subtype, accounting for 76.74% (165/215) of cases. Normocytic normochromic anaemia (NNA) constituted 17.67% (38/215), while dimorphic anaemia accounted for 4.19% (9/215). Macrocytic anaemia was the least frequent subtype, observed in 1.40% (3/215) of cases.

Table 1: Types based on PS morphology

Types	Number of Cases	Percentage (%)
Microcytic hypochromic anemia	165	76.74
Normocytic normochromic anemia	38	17.67
Dimorphic anemia	09	04.19
Macrocytic anemia	03	01.40

3. **RBC Histogram Pattern Distribution:** Analysis of RBC histogram patterns demonstrated that broad-base curves were the most common feature, occurring in 37.21% of cases, followed by left-shifted curves in 33.49%. A normal Gaussian curve was observed in 18.61%, whereas bimodal patterns were seen in 7.44%, corresponding to dimorphic states. Right-shifted curves,

associated with macrocytosis, accounted for 3.25% of cases.

Table 2: RBC histogram patterns in this study

Types	Number of Cases	Percentage (%)
Broad base curve	80	37.21
Left shift	72	33.49
Normal curve	40	18.61
Bimodal curve	16	07.44
Right shift	07	03.25

Correlation analysis revealed distinct and predictable associations between histogram morphology and peripheral smear findings:

Microcytic hypochromic anaemia showed predominantly left-shifted and broad-base histograms along with increased RDW, reflecting microcytosis and anisocytosis [16].

Normocytic normochromic anaemia corresponded mainly with normal and broad-base curves.

Dimorphic anaemia consistently demonstrated bimodal peaks, indicating dual RBC populations [17].

Macrocytic anaemia exhibited right-shifted curves with elevated MCV values [18].

These correlations were found to be statistically significant ($\chi^2 = 197.86$, $p < 0.001$), confirming a strong association between automated histogram interpretation and manual smear morphology.

Table 3: Correlation of PS findings and Histogram patterns

Types	Normal Curve	Left Shift	Right Shift	Broad Base Curve	Bimodal Curve	Total
Microcytic hypochromic anemia	35 (16.28%)	75 (34.88%)	-	35 (16.28%)	20 (9.30%)	165
Normocytic normochromic anemia	22 (10.23%)	-	-	16 (7.44%)	-	38
Dimorphic anemia	01 (0.46%)	-	3 (1.39%)	01 (0.46%)	04 (1.86%)	09
Macrocytic anemia	-	-	03 (1.39%)	-	-	03
Total	58	75	06	52	24	215

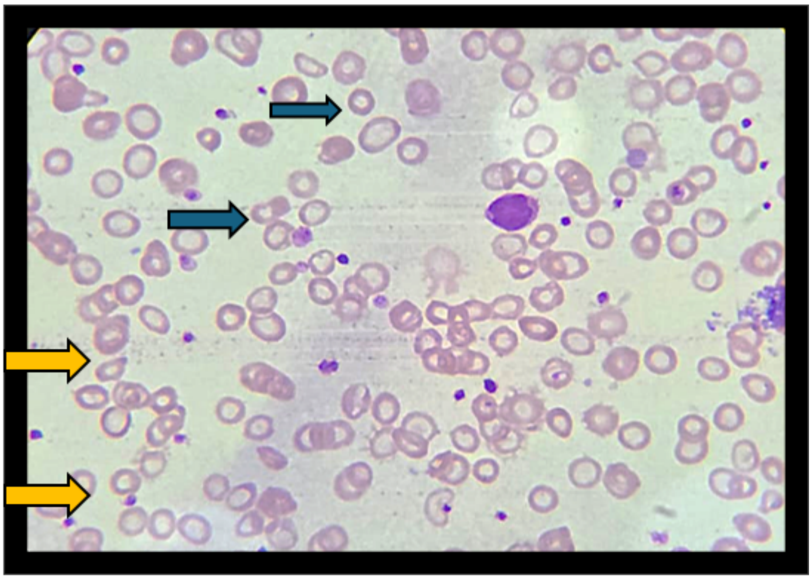

Figure 1a: Microcytic Hypochromic Anemia (MHA).

Shows microcytes and tear drop cells

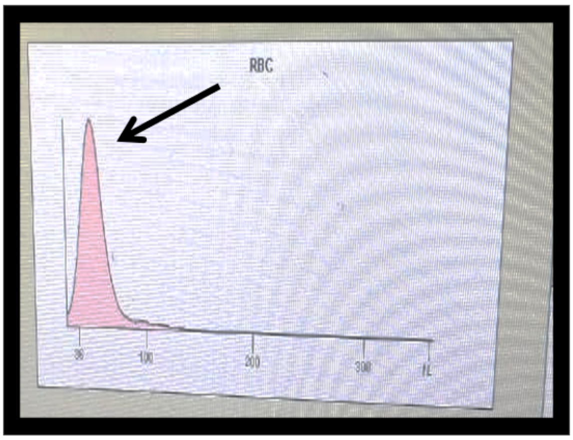

Figure 1b: Shift to left seen

Figure 2a: Normocytic Normochromic Anemia (NNA)

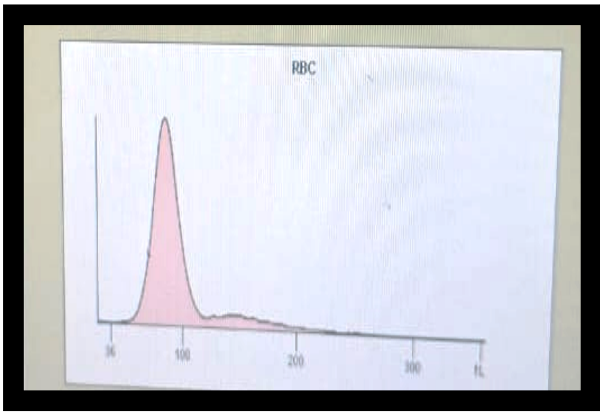

Figure 2b: Normal symmetrical bell-shaped curve seen

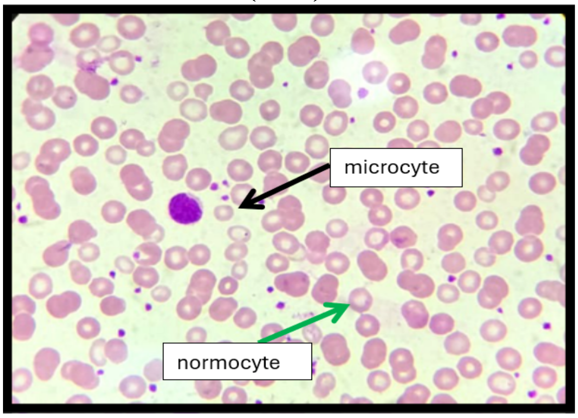

Figure 3a: Dimorphic Anaemia.

Both microcytes and normocytes seen.

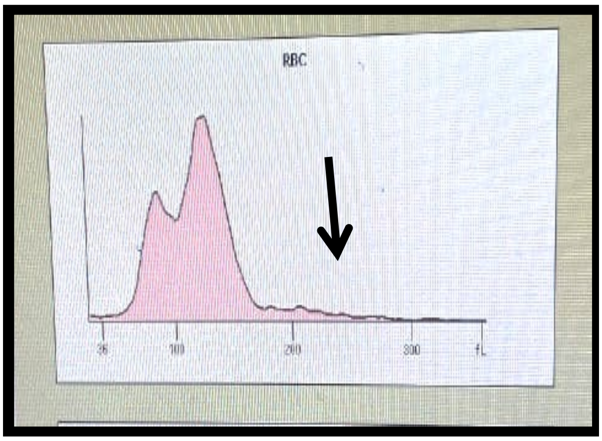

Figure 3b: Bimodal peak due to dual population of cells

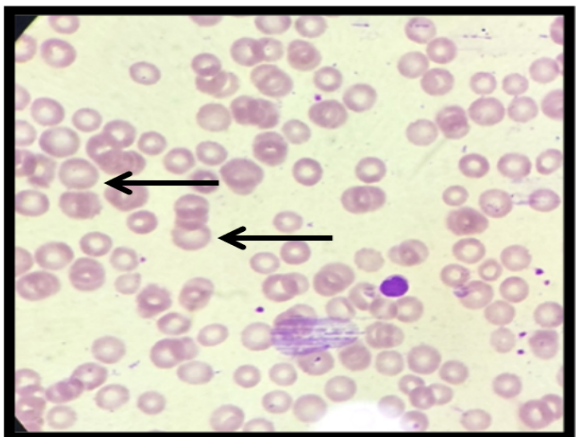

Figure 4a: Macrocytic Anaemia. Macrocytes seen

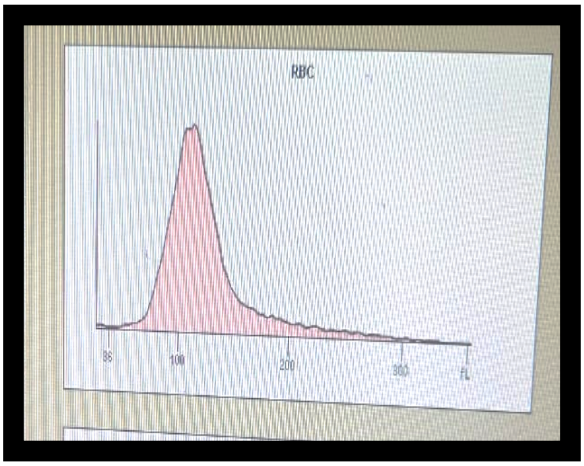

Figure 4b: Shift to right seen

Discussion

Anaemia is a clinical condition characterized by a reduced number of red blood cells or decreased haemoglobin concentration, resulting in diminished oxygen-carrying capacity to tissues. Interpretation of RBC morphology has traditionally relied on peripheral smear examination, which remains the diagnostic cornerstone for classifying anaemia [1]. RBC histograms, generated through the Coulter principle, provide an automated graphical representation of RBC size distribution, where the X-axis reflects cell volume and the Y-axis denotes cell count [2]. Under normal conditions, the RBC histogram forms a symmetrical bell-shaped (Gaussian) curve with an MCV range of 80–100 fL [3].

In the present study involving 215 anaemic patients, microcytic hypochromic anaemia (MHA) emerged as the most frequent subtype (76.74%). These cases demonstrated low MCV and MCH values and were predominantly associated with left-shifted (34.88%) or broad-base (16.28%) histogram curves, accompanied by elevated RDW—findings that reflect microcytosis and anisocytosis. These results align with the work of Sandhya et al. [24], BSS Rao et al. [25], and Chavda et al. [19], who similarly reported MHA as the leading morphological pattern in their cohorts.

Normocytic normochromic anaemia (NNA), accounting for 17.67% of cases, showed largely normal Gaussian curves (10.23%) or broad-base patterns (7.44%), consistent with preserved RBC size uniformity. Comparable distributions of NNA were reported by Sandhya et al. [24] and Chavda et al. [19], supporting the reliability of histogram interpretation in normocytic states.

Dimorphic anaemia, found in 4.19% of cases, presented characteristically with bimodal histogram curves (1.86%), indicating two distinct red cell populations. This pattern strongly correlates with mixed nutritional deficiencies or recovery phases and parallels findings from BSS Rao et al. [25] and Chavda et al. [19]. Meena et al. [21], who reported a higher proportion of dimorphic anaemia, also emphasized the diagnostic significance of bimodal peaks in identifying dual RBC populations.

Macrocytic anaemia was the least common subtype (1.40%) and consistently showed right-shifted histogram curves, corresponding to increased MCV values. These findings mirror those of Meena et al. [21] and Agarwal et al. [22], who also observed a strong association between macrocytosis and right-shifted histogram patterns.

Comparison of histogram patterns across studies further reinforces the diagnostic utility of RBC histograms. The proportion of broad-base curves in our study (37.21%) closely matches those reported by BSS Rao et al.25 (37.72%), Chavda et al. [19] (38%), and Meena et al.21 (38%). Left-shifted curves (33.49%) in our cohort are similar to patterns observed by Sandhya et al.24 (30%) and BSS Rao et al. [25] (29%). The prevalence of bimodal curves (7.44%) aligns with findings from BSS Rao et al. [25] (7.27%) and Banga et al. [23] (7.33%). These parallels across independent studies highlight the reproducibility and diagnostic consistency of histogram patterns in anaemia evaluation.

Overall, the findings of the present study strongly support the work of Meena et al. [21], Banga et al. [23], and Agarwal et al. [22], all of whom demonstrated a robust correlation between RBC indices, histogram morphology, and peripheral smear features. The strong statistical significance observed in our study (χ^2 = 197.86, $p < 0.001$) underscores the value of integrating automated RBC histograms with traditional smear examination. This combined diagnostic approach enhances early recognition of anaemia subtypes, facilitates timely initiation of treatment, and provides clearer guidance for further investigations.

Table 4: Comparison of peripheral smear findings in various studies

Types	MHA (%)	NNA (%)	Dimorphic anemia (%)	Macrocytic anemia (%)
Sandhya et al	**61**	**17**	**15**	**3**
BSS Rao et al	**63.63**	**19.4**	**12.7**	**2.2**
Chavda J et al	**65**	**17.4**	**14**	**3.6**
Meena H et al	**24.4**	**6.4**	**49.5**	**13.8**
Banga V et al	**60**	**21.33**	**12.67**	**6**
Agarwal A et al	**36**	**40**	**17**	**7**
Present study	**76.74**	**17.67**	**4.19**	**1.40**

Table 5: Comparison of RBC histogram pattern in various studies

Types	Broad base curve (%)	Left shift (%)	Normal curve (%)	Bimodal curve (%)	Right shift (%)
Sandhya et al	40	30	15	04	06
BSS Rao et al	37.72	29	17.7	7.27	5.45
Chavda J et al	38	27	19	3	7
Meena H et al	38	27	7	14	14
Banga V et al	4.33	57.67	22.67	7.33	7.33
Agarwal A et al	0	36	41	16	7
Present study	**37.21**	**33.49**	**18.61**	**7.44**	**3.25**

Conclusion

Integrating RBC indices and histogram analysis with peripheral smear examination enhances diagnostic accuracy for anemia. Automated hematology analyzers provide objective and reproducible data that support morphological findings. It enables earlier and more precise identification of anemia subtypes. Histogram acts as a screening tool and is valuable in resource-limited settings with higher anemia burden. A combined diagnostic approach improves timely management and enhances patient outcomes and treatment.

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