Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/kb9mtf54

# COMPARATIVE EVALUATION OF PERIPHERAL BLOOD SMEAR, RBC INDICES, AND RBC HISTOGRAM IN THE DIAGNOSIS OF ANAEMIA IN ADULTS AT A TERTIARY CARE CENTRE

**Dr Nishu Grover[1*], Dr Gunja Dwivedi[2], Dr Shiwangi Chhanwal[3], Dr Minal Jain[4], Dr Jatin Prajapati[5]**

[1,3,4]Post Graduate Student, Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India.
[2]Associate Professor, Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India.
[5]Senior Resident, Department of Community Medicine, World College of Medical Sciences and Research, Jhajjar, Haryana, India

***Corresponding Author:** Dr Nishu Grover
*Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India. (PIN- 313001)
Email ID: grover.nishu9@gmail.com

## ABSTRACT

**Background:** Anaemia is a major global health concern with high prevalence in low- and middle-income countries. Accurate classification of anaemia is essential for appropriate management. Peripheral blood smear (PBS) examination provides detailed morphological information, while automated haematology analyzers offer objective RBC indices and histogram patterns. Limited studies have compared these modalities in adult anaemic populations in India.

**Aim:** To evaluate peripheral blood smear findings in adult anaemia and compare them with RBC indices and RBC histogram patterns for identifying concordance and diagnostic utility.

**Materials and Methods:** This descriptive cross-sectional study included 390 adults with haemoglobin <11 g/dL at a tertiary care centre in Udaipur. Venous blood samples were analyzed using a HORIBA six-part differential haematology analyzer. RBC indices (MCV, MCH, MCHC, RDW) and histogram patterns were recorded. Peripheral blood smears were prepared, Giemsa- stained, and examined for morphological classification. Anaemia was categorized as normocytic normochromic, microcytic hypochromic, macrocytic, dimorphic, or haemolytic. Correlation between PBS morphology, RBC indices, and histogram interpretation was evaluated using Chi- square and t-tests. A p-value <0.05 was considered significant.

**Results:** Females constituted 62.31% of cases. The mean age was 36.87 ± 16.74 years, with most cases occurring in the 18–28 years age group. The most common clinical symptoms were fatigue (69.23%) and shortness of breath (65.38%). Histogram interpretation showed 54.10% normocytic normochromic, 37.69% microcytic hypochromic, 6.15% macrocytic, and 2.05% dimorphic patterns, identical to PBS morphology ($p = 1$). Histogram curve patterns showed a significant association with PBS findings ($p < 0.001$), with normal curves correlating with normocytic anaemia, left- shifted curves with microcytic anaemia, and bimodal curves with dimorphic anaemia. RBC indices also demonstrated expected changes across anaemia types.

**Conclusion:** PBS examination, RBC indices, and histogram interpretation are complementary

diagnostic tools in anaemia evaluation. The strong concordance between histogram patterns and PBS morphology underscores the reliability of automated analysis. Combining both manual and automated methods enhances diagnostic accuracy and should be routinely incorporated into haematological assessment.

**Keywords:** Anaemia, Peripheral Blood Smear, RBC Histogram, RBC Indices, Normocytic Anaemia, Microcytic Anaemia, Automated Haematology Analyzer

## INTRODUCTION

Anaemia is a major global public health burden, particularly in low- and middle-income countries, where it exerts profound effects on individual health, community wellbeing, and national development. The condition affects physical capacity, cognitive performance, immunity, maternal health, fetal growth, and productivity, thereby influencing broad socioeconomic indicators.[1] Its persistence, despite advances in medical science and public health interventions, highlights the need for continued evaluation of diagnostic approaches for proper classification and management.

According to the World Health Organization (WHO), anaemia is defined as a state in which the number of red blood cells (RBCs) or their haemoglobin concentration is insufficient to meet the oxygen demands of tissues.[2] Operationally, anaemia is diagnosed when haemoglobin concentration falls below the reference range established for healthy individuals of similar age, sex, and physiological conditions.[3] Because haemoglobin plays a central role in tissue oxygenation, a decline in its level or RBC mass leads to reduced oxygen-carrying capacity of blood, resulting in multisystemic physiological compromise. This underscores why anaemia is associated with substantial morbidity and, in many settings, increased mortality.

An estimated one-third of the global population is affected by anaemia, amounting to approximately 1.62 billion individuals worldwide [4]. The burden is disproportionately high in developing countries, where nutritional deficiencies, infections, and reproductive health factors are predominant contributors. In India, anaemia continues to be a critical public health challenge, as evidenced by the National Family Health Survey-5 (NFHS-5) conducted in 2021. The survey reported anaemia prevalence of 67% among children aged 6–59 months, 59.2% among adolescent girls aged 15–19 years, 31.1% among adolescent boys, 57% among women aged 15–49 years, and 25% among adult men [5]. The Global Nutrition Report 2023 further emphasizes that more than half (53%) of Indian women of reproductive age remain anaemic, highlighting persistent gaps in prevention, diagnosis, and management [6]. Among the many etiological contributors to anaemia, nutritional deficiency—particularly iron deficiency—emerges as the leading cause globally as well as in India [7]. Given this enormous burden, accurate diagnosis and classification of anaemia are essential for guiding targeted therapy, monitoring response, and planning public health strategies.

Historically, the peripheral blood smear (PBS) has served as one of the most important diagnostic tools for evaluating anaemia and other haematological disorders. Its value lies in the direct visualization of red cell morphology, including cell size, shape, color, anisocytosis, poikilocytosis, and the presence of abnormal cells. Regular examination of blood smears has significantly contributed to understanding various haematological abnormalities and their underlying mechanisms. Until a few decades ago, haematology laboratories relied predominantly on manual techniques such as the cyanmethaemoglobin method for haemoglobin estimation and hand-prepared reagents, which were labor-intensive and time-consuming. This scenario changed dramatically with the introduction and gradual evolution of automated haematology analyzers.

Modern automated haematology analyzers have transformed haematology practice, offering rapid, standardized, and highly precise quantitative measurements. These analyzers employ technologies such as electrical impedance, conductivity, cytochaemical staining, optical light scatter, and flow

cytometry principles to measure red cell parameters with high accuracy. Automation reduces interobserver variability, minimizes manual errors, and enhances laboratory efficiency. Among the various parameters generated by these analyzers, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) are particularly crucial for the morphological interpretation of anaemia [8].

A valuable adjunct to RBC indices is the RBC histogram, which graphically represents the frequency distribution of RBCs based on cell size. The histogram is now a routine output on virtually all automated haematology counters and has become indispensable in evaluating red cell disorders. Interpreting RBC histograms requires familiarity with the normal distribution curve and an understanding of deviations in pathological states. The normal RDW curve is typically bell- shaped, peaking between 80 and 100 fL. A narrow curve indicates homogeneity in cell size, whereas a broad curve suggests heterogeneity in RBC populations. In macrocytic anaemia, particularly megaloblastic anaemia, the histogram curve shifts to the right due to larger cell size, while microcytic anaemia produces a left shift [9]. A dimorphic pattern—seen in conditions such as sideroblastic anaemia, dual deficiency anaemia, or post-transfusion states—may present with two or more peaks on the histogram, reflecting dual cell populations. In such cases, RDW often serves as a more sensitive indicator of anisocytosis than MCV [10]. An increased RDW is associated with the presence of both small and large cells, including immature RBCs, thereby providing important diagnostic clues.

The combined use of automated haematology analyzers and PBS examination has proven invaluable in the comprehensive assessment of anaemia. Automated systems offer objective, reproducible, and rapid measurements, whereas PBS provides qualitative morphological details that cannot be captured numerically. RBC histogram patterns bridge the gap between these two approaches by visually representing quantitative data and aiding in the interpretation of morphological abnormalities, especially in mixed or evolving anaemias [11]. Many modern analyzers incorporate sophisticated combinations of impedance, optical scatter, and staining technologies to provide detailed cellular information. Nevertheless, PBS examination continues to be regarded as the gold standard in diagnosing morphological red cell abnormalities, with histogram data serving as an important adjunct [12].

While automation has greatly enhanced diagnostic efficiency, manual examination of blood smears remains critical for quality control and for identifying subtle morphological features that instruments may overlook. The classification of anaemia relies on the integration of haemoglobin, haematocrit, MCV, RDW, MCH, and MCHC values with morphological findings on PBS. RBC histograms support this classification by visually highlighting deviations in cell size distribution that correspond to various types of anaemia. The use of histograms in conjunction with RBC indices increases diagnostic confidence and reduces misclassification [13]. However, PBS interpretation is time-consuming and subject to interobserver variability, whereas reliance solely on automated parameters may overlook morphological nuances. Thus, a combined interpretative approach improves diagnostic accuracy and reliability.

Despite the significant advances in haematology automation, few studies have comprehensively compared peripheral smear morphology with RBC indices and RBC histogram patterns in adult anaemic patients, particularly in the Indian setting [10]. Calibration of automated instruments still depends on manual techniques, reaffirming the continued need for strong technical skills among laboratory personnel. This highlights a gap in existing literature regarding the extent of correlation between manual and automated methods in routine diagnostic haematology.

Given these gaps, the present study was conducted in the Department of Pathology, RNT Medical College, Udaipur, to analyze peripheral blood smear findings in adults with anaemia and compare thaem with RBC indices and RBC histogram patterns. By evaluating the degree of concordance between these diagnostic modalities, the study aims to strengthen evidence for an integrated approach in the morphological and quantitative assessment of anaemia.

## MATERIALS AND METHODS

**Study Design:** This was a descriptive cross-sectional study.
**Study Setting:** The study was conducted in the Haematology Section of the Central Laboratory, Department of Pathology, R.N.T. Medical College and its associated group of hospitals, Udaipur, Rajasthan. The laboratory is equipped with advanced diagnostic facilities and caters to a diverse patient population, enabling comprehensive evaluation of haematological parameters in anaemic adults.

**Study Period:** The study was carried out over a period of 1 year from July 2024 to June 2025.
**Study Population:** The study population included adult patients (>18 years) with haemoglobin levels <11 g/dL attending the hematology section of R.N.T. Medical College and associated hospitals during the study period.

### Inclusion Criteria

- Adults aged >18 years
- Male and female patients with haemoglobin <11 g/dL

### Exclusion Criteria

- Individuals with normal haemoglobin values (Adult male: 14–18 g/dL; Adult female: 12– 16 g/dL)
- Known haematological malignancies
- Patients on treatment or those who had received blood transfusion therapy
- Inadequate or clotted samples

**Sample Size:** The sample size was calculated assuming a categorical variable and using the formula for prevalence estimation. With a two-tailed Z value of 1.96 at 5% α-error, an expected anaemia prevalence (P) of 52% as reported by Jain et al. [14], and 10% relative precision (5.2%), a total of 390 anaemic adult patients were included.

**Sampling Technique:** A consecutive sampling technique was used, wherein all eligible anaemic adult patients meeting inclusion criteria during the study period were included.

**Study Procedure:** Blood samples were processed through automated hematology analysis, followed by peripheral smear preparation and staining. RBC indices, histogram patterns, and microscopic morphology were evaluated and then correlated for final classification of anaemia.

**Clinical Evaluation:** Relevant clinical history was obtained prior to blood collection. Symptoms such as fatigue, weakness, pallor, breathlessness, palpitations, dizziness, nutritional status, and history of chronic illness were documented. Demographic and clinical details were systematically recorded to correlate clinical presentation with laboratory findings.

**Sample Collection:** Two milliliters of venous blood were collected aseptically into EDTA vials. Samples were mixed gently by inversion to prevent clotting and ensure uniform distribution of cellular elements.
**Automated Haematology Analysis:** All samples were analyzed using the HORIBA automated haematology analyzer (Model: 103YAXH03282), a six-part differential counter. Haemoglobin, RBC

count, haematocrit, MCV, MCH, MCHC, RDW, and RBC histograms were obtained. The analyzer provided quantitative measurements and graphical representation of RBC size distribution.

**Peripheral Blood Smear Examination:** Peripheral smears were prepared from EDTA blood using standardized techniques. Smears were air-dried, fixed with methanol, and stained using Giemsa stain.

**Giemsa Staining Procedure**

1. Air-dry the smear and fix in methanol for 5–10 minutes.
2. Stain in 1:9 diluted Giemsa stain (buffered to pH 6.8) for 20–30 minutes.
3. Rinse gently in pH 6.8 buffered water for 3 minutes.
4. Allow slides to air-dry in an upright position without blotting.

The stained smears were examined under a microscope by experienced personnel to assess RBC morphology, anisocytosis, poikilocytosis, haemolytic features, and any abnormal cells.

**RBC Histogram Evaluation: The** RBC histograms generated by the haematology analyzer were studied for

• Position: normal, left shift, or right shift
• Shape: normal Gaussian curve, broad-based curve, bimodal peak, or curves shifted left/ right

**Operational Definitions**

**Classification of Anaemia:** Anaemia was categorized separately by

**Automated Analyzer (RBC Indices)**

• Normocytic normochromic
• Microcytic hypochromic
• Macrocytic
• Dimorphic

**Peripheral Blood Smear Morphology**

• Normocytic normochromic anaemia
• Microcytic hypochromic anaemia
• Macrocytic anaemia
• Dimorphic anaemia
• Haemolytic anaemia

The final morphological diagnosis was based on PBS findings. Correlation with RBC indices and histogram patterns was performed for diagnostic comparison.

**Statistical Analysis:** All data were entered into Microsoft Excel 2016 and subsequently analyzed using SPSS version 25. Qualitative (categorical) variables were expressed as frequencies and percentages, and associations between categorical variables were evaluated using the Chi-square test. Quantitative variables were summarized as mean ± standard deviation (SD). Group comparisons for continuous variables were performed using the independent samples t-test. A p- value $<0.05$ was considered statistically significant. This combined descriptive and inferential statistical approach ensured rigorous evaluation of both categorical and continuous parameters.

**Ethical Considerations:** The study was conducted after obtaining approval from the Institutional Ethics Committee of R.N.T. Medical College, Udaipur (IEC Approval Number: RNT/ACAD./IEC/ 2024/236 dated 04.04.2024). Written informed consent was obtained from all participants prior to blood collection. Patient confidentiality was strictly maintained, and all procedures adhered to ethical standards of biomedical research involving human participants.

## RESULTS

A total of 390 adult patients with anaemia were included in the study. All samples were analyzed using an automated haematology analyzer and peripheral blood smear (PBS), followed by correlation with RBC histogram patterns.

Females constituted 62.31% of the study population, while males accounted for 37.69%. The mean age of the population was 36.87 ± 16.74 years. The highest proportion of cases belonged to the 18–28 years age group.(Table 1)

**Table 1: Demographic characteristics of study population (Gender and Age Distribution)**

| Parameter | Category | n (%) |
|---|---|---|
| Gender | Female | 243 (62.31) |
| | Male | 147 (37.69) |
| Age (years) | 18–28 | 168 (43.08) |
| | 29–38 | 78 (20.00) |
| | 39–48 | 47 (12.05) |
| | 49–58 | 42 (10.77) |
| | 59–68 | 26 (6.67) |
| | 69–78 | 23 (5.90) |
| | 79–88 | 6 (1.54) |

The most frequent symptom was fatigue/weakness (69.23%), followed by shortness of breath (65.38%). Dizziness and palpitations were less common. Normal histogram curves were most common, followed by left-shifted curves. Histogram interpretation showed the majority of cases to be normocytic normochromic or microcytic hypochromic.(Table 2)

**Table 2: Distribution of histogram curve patterns and histogram interpretation**

| Parameter | Category | n (%) |
|---|---|---|
| Histogram Curve | Normal | 167 (42.82) |
| | Left shift | 157 (40.25) |
| | Broad base | 31 (7.95) |
| | Right shift | 27 (6.92) |
| | Double peak | 8 (2.05) |
| | Short peak | 1 (0.26) |
| Histogram Interpretation | Normocytic normochromic | 211 (54.10) |
| | Microcytic hypochromic | 147 (37.69) |
| | Macrocytic | 24 (6.15) |
| | Dimorphic | 8 (2.05) |

PBS examination revealed patterns identical in proportion to histogram interpretation, with normocytic normochromic anaemia being the most common type, followed by microcytic hypochromic anaemia. There was no significant gender difference in PBS patterns (p = 0.63).(Table 3)

**Table 3. Peripheral blood smear findings and comparison with gender**

| PBS Pattern | Female n (%) | Male n (%) | Total n (%) | p value |
|---|---|---|---|---|
| Normocytic normochromic | 132 (54.32) | 79 (53.74) | 211 (54.10) | 0.63 |
| Microcytic hypochromic | 94 (38.68) | 53 (36.05) | 147 (37.69) | |
| Macrocytic | 12 (4.94) | 12 (8.16) | 24 (6.15) | |
| Dimorphic | 5 (2.06) | 3 (2.04) | 8 (2.05) | |

Histogram interpretation showed perfect concordance with PBS findings (p = 1.0). Histogram curve patterns showed a significant association with PBS morphology (p < 0.001).(Table 4)

**Table 4: Comparison of PBS findings with histogram interpretation and histogram curve**

| Category | Dimorphic | Macrocytic | Microcytic Hypochromic | Normocytic Normochromic | p-value |
|---|---|---|---|---|---|
| Histogram Interpretation (n) | 8 | 24 | 147 | 211 | 1.0 |
| Histogram Curve (n) | Double peak: 8 | Right shift/Broad base: 24 | Left shift: 139 | Normal: 159 | **<0.001*** |

*highly significant at p<0.001

MCV, MCH, and RDW-CV values were reviewed to characterize anaemia patterns. Microcytic cases had reduced MCV and MCH with elevated RDW-CV. Macrocytic cases showed high MCV with markedly increased RDW-CV. Dimorphic anaemia showed dual-range MCV and significantly raised RDW-CV.(Table 5)

**Table 5. Haematological indices across anaemia types**

| Type of Anaemia | n (%) | MCV (fL) | MCH (pg) | RDW-CV (%) |
|---|---|---|---|---|
| Microcytic hypochromic | 147 (37.69) | 50–80 | 10–40 | 10–40 |
| Normocytic normochromic | 211 (54.10) | 65–105 | 20–35 | 10–30 |
| Macrocytic | 24 (6.15) | 95–110 | 35–45 | 50–70 |
| Dimorphic | 8 (2.05) | 65–75 | 20–30 | 60–80 |

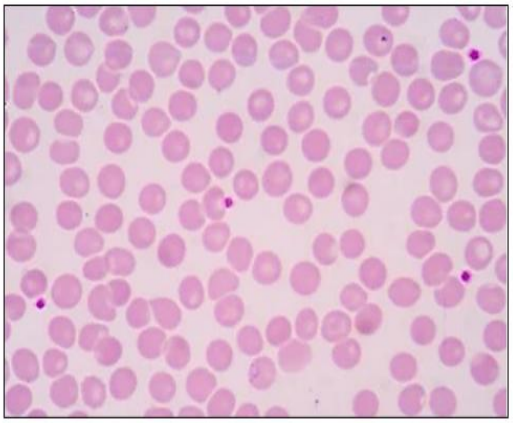

**Figure 1: PBS showing normal size and shape RBCs suggestive of Normocytic Normochromic anaemia (Giemsa stain-400x)**

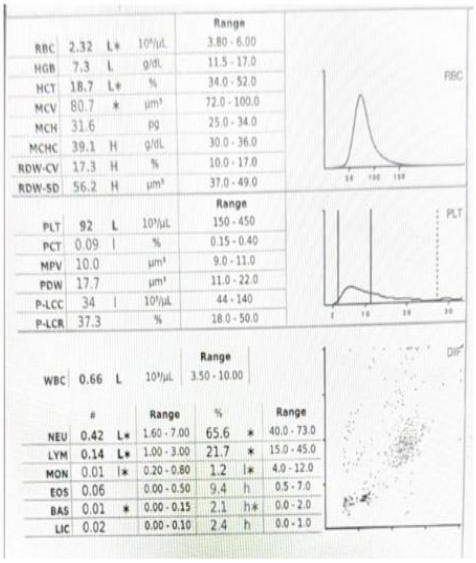


**Figure 2: Histogram showing reduced hemoglobin with normal peak suggestive of Normocytic Normochromic anaemia**

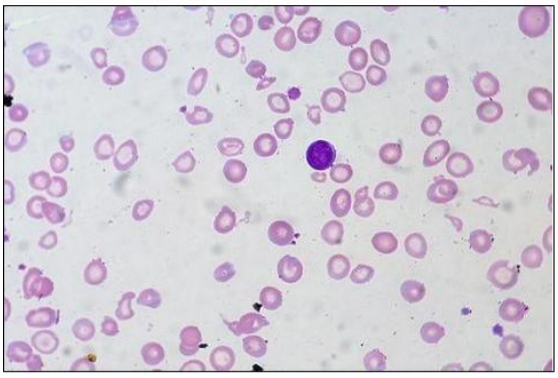

**Figure 3: PBS showing anisopoikilocytosis and pencil cells suggestive of Microcytic Hypochromic anaemia (Giemsa stain-400x)**

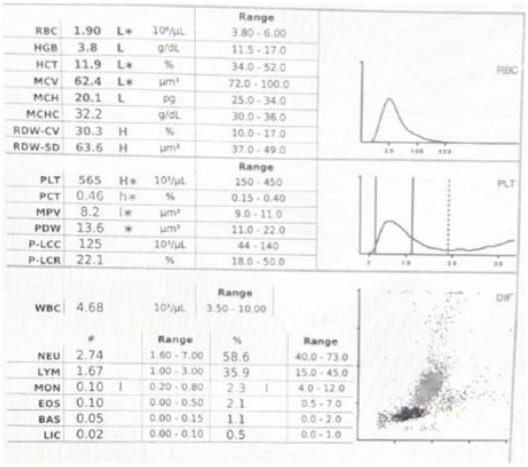


**Figure 4: Histogram showing reduced hemoglobin with left shift suggestive of Microcytic Hypochromic anaemia**

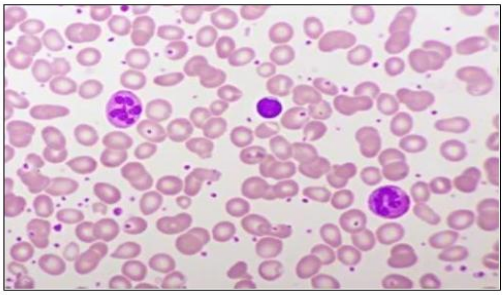

**Figure 5: PBS showing macrocytes and hypersegmented neutrophils suggestive of Macrocytic anaemia (Giemsa stain-400x)**

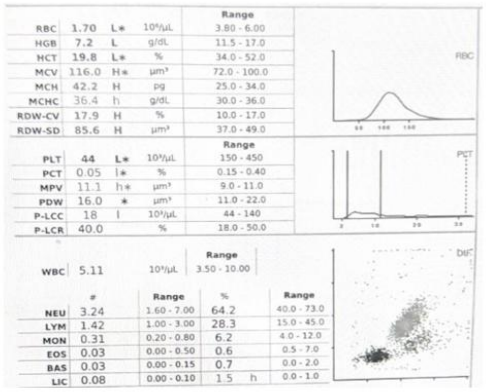


**Figure 6: Histogram showing reduced haemoglobin with right shift suggestive of Macrocytic Hypochromic anaemia**

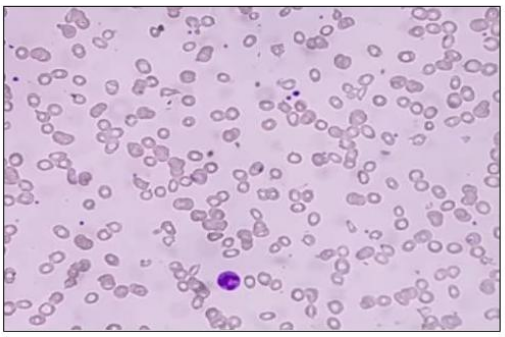

**Figure 7: PBS showing dimorphic population of red cells suggestive of dimorphic anaemia (Giemsa stain-400x)**

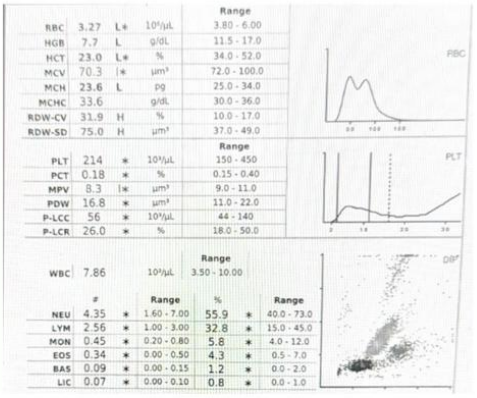


**Figure 8: Histogram showing reduced haemoglobin with bimodal peak suggestive of Dimorphic anaemia**

## DISCUSSION

Anaemia remains one of the most prevalent haematological disorders worldwide, contributing significantly to morbidity across adult populations. It is characterized by a reduced haemoglobin concentration or red blood cell (RBC) mass, ultimately impairing oxygen delivery to tissues and resulting in manifestations that range from mild fatigue to severe systemic compromise, depending on the degree and etiology of anaemia.[1-3] Accurate classification of anaemia is essential for guiding appropriate clinical management. The peripheral blood smear (PBS) continues to be a cornerstone diagnostic tool as it allows direct visualization of RBC morphology, including variations in size, shape, and staining patterns. This makes PBS invaluable for identifying morphologic categories such as microcytic hypochromic anaemia, commonly associated with iron deficiency; macrocytic anaemia, often seen in vitamin B12 or folate deficiency; and dimorphic anaemia, which may reflect dual nutritional deficiencies, sideroblastic anaemia, or post-transfusion states.[4-6] In recent decades, automated haematology analyzers have introduced RBC histogram analysis, offering graphical insights into red cell size distribution. Typical patterns—such as left shift in microcytic anaemia, right shift in macrocytosis, and bimodal peaks in dimorphic anaemia— provide additional objective data that complement the morphological interpretation of PBS.[7-9] The present study aimed to comparatively evaluate PBS findings with RBC indices and histogram interpretation in adult anaemic patients.

In this study of 390 anaemic adults, females accounted for 62.31% of cases, reflecting a clear gender disparity. This finding aligns with previous research demonstrating a consistent female predominance in anaemia prevalence. While Bhargava et al.[15] reported only slight female predominance (53%), several studies observed substantially higher proportions of anaemic females, including Meena et al.[16] (61%) and Gupta et al.[17] (69%). These trends have generally been attributed to menstrual blood loss, increased nutritional demands during pregnancy, and inadequate dietary iron intake in women. Thus, the predominance of female patients in the present study is consistent with broader epidemiological findings and highlights the gender-specific risk factors contributing to anaemia.

Age distribution analysis revealed that the highest proportion of patients belonged to the 18–28 years age group (43.08%), followed by those aged 29–38 years (20%). These findings are comparable to the age patterns described in previous research. Bhargava et al.[15] documented the highest anaemia burden between 31–40 years, whereas Agarwal et al.[18] and Meena et al.[16] reported peaks in the 20–30 years group. Across studies, anaemia consistently appears more common in early and middle adulthood, likely reflecting nutritional deficiencies, reproductive factors, and lifestyle influences, with a decline in older age groups. Gender-wise age analysis in our study showed that anaemia manifested earlier and more frequently in young adult females, a trend also noted by Gupta et al.[17] and Patel et al.[19], whereas males often showed higher representation in older age categories. This pattern supports the understanding that anaemia in females is influenced heavily by reproductive physiology, while in males it tends to appear in the later decades due to chronic diseases and nutritional factors.

Clinical symptoms in the present study were dominated by fatigue and generalized weakness (69.23%), followed by shortness of breath (65.38%), dizziness (20%), and palpitations (10%). These findings closely mirror those of Kumar et al.[20], who identified easy fatigability as the most common symptom (76.2%), and Sharma et al.[21], who reported fatigue in more than 85% of their anaemic patients. In contrast, Völzke et al.[22], reporting from a general population study, found substantially lower prevalence of fatigue and dyspnea, underscoring the difference between community-based and hospital-based cohorts. The predominance of fatigue and dyspnea in symptomatic anaemic adults underscores the importance of early detection and evaluation, particularly in high-risk groups.

In our study, normal RBC histogram curves (42.82%) and left-shifted curves (40.25%) were the most frequently observed patterns. These results closely resemble the findings of Agarwal et al.[18], who documented near-identical distributions. However, several other studies—such as those by Sinha et

al.[23], Shrivastava et al.[24], Rao et al.[25], Chavda J et al.[10], and Sandhya et al.[26]— reported a predominance of left shifts over normal curves, suggesting a higher proportion of microcytic anaemia in their populations. Thus, while our findings demonstrate a balance between normal and left-shifted curves, some variation is evident across different populations, possibly reflecting differences in nutritional deficiencies and underlying etiological factors.

Histogram interpretation in the present study revealed normocytic normochromic anaemia as the most common type (54.10%), followed by microcytic hypochromic anaemia (37.69%), macrocytic anaemia (6.15%), and dimorphic anaemia (2.05%). This distribution contrasts sharply with studies such as Meena et al.[16], who reported dimorphic anaemia as the predominant pattern, but aligns well with the findings of Jayant et al.[27], who observed a predominance of microcytic and normocytic anaemia. Differences across studies likely reflect variations in dietary habits, prevalence of nutritional deficiencies, and regional health disparities.

PBS examination findings in our study mirrored the histogram interpretation results, with normocytic normochromic morphology most frequent, followed by microcytic hypochromic patterns. In contrast, studies by Patel et al.[19], Meena et al.[16], and Patidar et al.[28] reported microcytic hypochromic anaemia as the most common subtype, with dimorphic anaemia also more frequently observed in their cohorts. Thyagaraju et al.[29], however, demonstrated a trend similar to ours, with normocytic normochromic anaemia commonly associated with normal histogram curves and microcytic hypochromic anaemia exhibiting left-shifted histograms. These variances across studies highlight the geographical and population-specific differences influencing anaemia phenotypes.

Gender-wise comparison of PBS results in our study showed almost identical distributions between males and females, with no statistically significant difference. However, previous studies such as those by Goti et al.[30] and Gupta et al.[17] reported a greater burden of microcytic hypochromic anaemia among females, likely due to iron deficiency. Our finding of normocytic normochromic anaemia predominance differs from these reports, again pointing toward population-level variation in anaemia etiology.

A key finding of the present study was the complete concordance between histogram interpretation and PBS morphology ($p = 1$). This contrasts with several earlier studies— including those by Patel et al.[19] and Sinha et al.[23]—that reported differences in classification between the two methods, suggesting that automated parameters may overestimate certain categories (such as microcytic anaemia) or underestimate others (such as dimorphic or haemolytic anaemia). In our cohort, however, both modalities demonstrated identical classification across all anaemia types, highlighting the reliability of automated histogram interpretation when used alongside PBS.
The correlation between histogram curves and PBS morphology was statistically significant ($p < 0.001$), demonstrating that specific histogram patterns were closely associated with corresponding red cell morphologies. Normal histograms strongly correlated with normocytic normochromic anaemia, left-shifted curves with microcytic hypochromic anaemia, double peaks with dimorphic patterns, and right-shifted or broad-based curves with macrocytic anaemia. These findings are consistent with those of Meena et al.[16] and Thyagaraju et al.[29], and partially comparable with the large-scale analysis by Patel et al.[19], though the relative distributions differed across studies.

Overall, the present study highlights the value of combining PBS with automated RBC indices and histogram interpretation for comprehensive anaemia evaluation. While PBS provides detailed qualitative assessment, histograms offer objective, reproducible graphical data. The strong concordance observed in our study emphasizes the complementary roles of these modalities.
Differences seen when comparing our data with other studies likely reflect underlying variations in nutritional deficiencies, socioeconomic profiles, and regional disease patterns. Nonetheless, the

consistent association between histogram patterns and PBS morphology supports the combined use of both techniques in the routine diagnostic evaluation of anaemia.

## CONCLUSION

The present study demonstrated that peripheral blood smear examination, RBC indices, and RBC histogram analysis are highly complementary tools in the diagnostic evaluation of anaemia.
Normocytic normochromic anaemia emerged as the most common pattern, followed by microcytic hypochromic anaemia. A key finding was the complete concordance between histogram interpretation and PBS morphology, indicating that automated histogram patterns are reliable indicators of underlying red cell morphology. The significant association between histogram curve patterns and PBS findings further confirms the diagnostic value of histograms, particularly in identifying microcytic, macrocytic, and dimorphic anaemia. While PBS remains indispensable for identifying subtle morphological abnormalities, automated RBC parameters enhance objectivity, reproducibility, and efficiency. Together, these modalities provide a comprehensive and accurate approach to anaemia classification. Integrating both automated and manual techniques in routine haematological evaluation can improve diagnostic precision and guide appropriate clinical management, especially in resource-limited settings.

**Declarations**
Funding: None Acknowledgements: None
Conflict of Interest: The authors declare no conflict of interest.

## REFERENCES

1. Shetty A, Saha A, Komala HN. Morphological types of anaemia integrated with distribution of red cell and platelet indices: Study in a rural tertiary healthcare centre. Indian J Pathol Oncol. 2021;8(1):45-9.
2. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva: World Health Organization; 2011 [cited 2025 Sep 9]. Report No.: WHO/NMH/NHD/MNM/11.1. Available from: https://apps.who.int/iris/handle/10665/85839
3. Mehta AB. Anaemias. In: Kawthalkar SM, editor. Essentials of clinical pathology. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 2019. p. 85-102.
4. Hafizl F, Mahajan S, Koul K. Study of morphological patterns of anemia in adults. IOSR J Dent Med Sci. 2019;18(11):46-50.
5. International Institute for Population Sciences (IIPS) and ICF. National Family Health Survey (NFHS-5), India, 2019–21. Mumbai: IIPS; 2021 [cited 2025 Sep 9]. Available from: http://rchiips.org/nfhs/NFHS-5Reports.shtml
6. Development Initiatives. India country nutrition profile. In: Global Nutrition Report 2023. Bristol (UK): Development Initiatives; 2023 [cited 2025 Sep 9]. Available from: https:// globalnutritionreport.org
7. Revathi SR, Khan DM, Naseem N, Selvi T, Chandana A. Study of anemia pattern and its correlation with hematological parameters in a tertiary care centre. Indian J Pathol Oncol. 2018;5(4):642-5.
8. Bessman JD, Gilmer PR, Gardner FH. Improved classification of anemias by MCV and RDW. Am J Clin Pathol. 1983;80(3):322-6.
9. Gulati GL, Hyun BH. The automated CBC: a current perspective. Hematol Oncol Clin North Am. 1994;8(4):593-603.
10. Chavda J, Goswami P, Goswami A. RBC histogram as diagnostic tool in anemias. IOSR J Dent Med Sci. 2015;14(10):19-22.
11. Singla S, Bedi S, Joshi K. Comparative study of anemia cases based on peripheral blood smears and cell counter generated red cell indices. Int Med J. 2017;4(1):44-8.

12. Korgaonker KA, Shashidhar MR. Utility of automated cell counter histograms in reporting peripheral smears in anemia. Int J Clin Diagn Pathol. 2019;2:230-9.
13. Garg M, Gitika, Sangwan K. Comparison of automated analyzer generated red blood cell parameters and histogram with peripheral smear in the diagnosis of anaemia. Int J Contemp Med Res. 2019;6(8):H1–6.
14. Jain A, Ayyagari A, Prakash R. Prevalence of anemia in women of reproductive age group attending a tertiary care hospital of Uttar Pradesh. J Evol Med Dent Sci. 2019;8(7):425–9.
15. Bhargava R, Varalakshmi K. Comparison of automated analyzer produced red blood cell parameters and peripheral smear histogram in anaemia diagnosis. MedPulse Int J Pathol. 2020;15(3):44–7.
16. Meena H, Pruthviraj CS, Gupta R, Guru A, Sutrakar SK. Correlation of automated cell counter RBC histograms and peripheral smear in anemia typing. Asian J Med Sci. 2023;14(2):128–33.
17. Gupta V, Gautam R, Devdhar S, Chaudhary A. Relevance of peripheral blood smear examination in diagnosis of anemia in an era of automation. Int J Acad Med Pharm. 2023;5(4):987-91.
18. Agarwal A, Goel N, Goel S. Comparative study of automated cell counter histogram and peripheral blood smear in diagnosis of anemia. Indian J Appl Res. 2023;13(3).
19. Patel A, Shah I, Goswami H, Shah V. Correlation of peripheral smear with RBC and WBC histogram in the diagnosis of anemia. Int J Sci Res. 2022;11(9):18–21.
20. Kumar R, Singh A, Meena LK, Meena R, Meena VK. Clinical profile of anemia among adults in a tertiary care hospital. J Evol Med Dent Sci. 2022;11(18):1456–60.
21. Sharma A, Sharma A, Shukla J, Parmar P, Choudhary S. Prevalence and clinical profile of anemia in the elderly: a hospital-based study. J Clin Diagn Res. 2019;13(2):OC01–4.
22. Völzke H, Ittermann T, Douros A, Bahls M, Markus MRP, Schipf S, et al. Symptoms and prevalence of anemia in the general population: findings from the SHIP study. J Clin Med. 2023;12(5):1012.
23. Sinha R, Dhotre S, Goswami HM. Interpretation of RBC histograms and their correlation with peripheral smear findings in patients of anemia. Int J Contemp Pathol. 2020;6(1):213– 8.
24. Shrivastav A, Shah N, Goyal S, Shah CK. RBC histogram: utility in diagnosis of various anemia. Int J Clin Diagn Pathol. 2019;2(1):14-7.
25. Rao P, Nandita M. Automated red blood cell analysis compared with routine RBC morphology by smear review. Blood. 2011;56:34–9.
26. Sandhya I, Muhasin TP. Study of RBC histogram in various anemias. J Evol Med Dent Sci. 2014;3(74):15521-34.
27. Jayant S, Mishra V, Gaur R, Jain A. Comparative study of automated cell counter histogram and peripheral blood smear in diagnosis of anaemia at a tertiary care hospital. Int J Sci Res. 2023;13(4):63–6.
28. Patidar H, Agrawal U, Rawat S, Rohit N. Peripheral blood smear and RBC histogram in anemia: a two-year observational study. Sci Med J. 2023;19(2):94–100.
29. Thyagaraju B, Manigatta Doddagowda S. A comparative study of peripheral smear findings with RBC indices and automated analyzer generated RBC histograms in the diagnosis of anemias. J Health Allied Sci NU. 2024;14(1):31–6.
30. Goti R, Shah PH, Raval J. Comparison between automated hematology analyzer and peripheral blood smear examination for assessment of morphological types of anemia. GAIMS J Med Sci. 2024;4(1):188-91.