



“CORRELATION OF PERIPHERAL BLOOD SMEARS FINDINGS WITH CBC HISTOGRAMS IN DIAGNOSIS OF ANEMIA IN 15-49 YEARS AGE GROUP”

Deepti Tiwari¹ Suresh Kumar Sutrar² Jagannath Jatav³ Vibha Misra⁴ Pushpkunjika Sharma⁵ Shambhavi⁶ Nikita Giri⁷ Shweta Paswan⁸

- ¹ Junior Resident, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ² Professor & HOD, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ³ Associate Professor, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ⁴ Junior Resident, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ⁵ Junior Resident, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ⁶ Senior Resident, Department of Pathology, Prasad Institute of Medical Science and hospitals, Lucknow (U.P.).
- ⁷ Junior Resident, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).
- ⁸ Junior Resident, Department of Pathology, Shyam Shah Medical College and associated hospitals, Rewa (M.P.).

***Corresponding Author:** Dr. Deepti Tiwari

*Junior Resident Department of Pathology Shyam Shah Medical College and Associate hospitals Rewa (M.P.).486001 Mob.9140672197

ABSTRACT

Background: Anemia is a common hematological disorder with varied etiology and morphological patterns. Accurate diagnosis is crucial for appropriate management. This study aimed to correlate peripheral blood smear (PBS) findings with CBC histograms to enhance diagnostic accuracy in anemic patients aged 15–49 years.

Methods: A cross-sectional study was conducted on 255 patients presenting with anemia at a tertiary care center. CBC parameters including hemoglobin levels, red blood cell indices (MCV, MCH, MCHC and RDW) and histograms were obtained using an automated hematology analyzer. Peripheral blood smears were reviewed microscopically for red cell morphology including poikilocytosis and these findings were correlated with histogram patterns including flags and categorized into types of anemia (microcytic, normocytic, and macrocytic).

Results: Among the 255 cases, microcytic anemia was the most prevalent with 96 cases (37.6%) followed by macrocytic 91 cases (35.6%) and normocytic 68 cases (26.6%). A significant correlation ($p < 0.01$) was observed between histogram patterns and PBS findings. Microcytic hypochromic anemia showed left-shifted RBC histograms with prominent peaks while macrocytic anemia exhibited right-shifted histograms with broad bases. Normocytic anemia demonstrated normal or

slightly altered histogram curves. Correlation was strongest in microcytic anemia ($r = 0.81$) suggesting a reliable relationship between histogram features and smear morphology.

Conclusion: CBC histograms when interpreted along with peripheral smear findings provide a rapid, cost-effective, and reliable approach for the morphological classification of anemia. This correlation helps the pathologist to diagnose the anemia more confidently and accurately.

Keywords: Anemia, CBC histogram, peripheral blood smear, red cell morphology

INTRODUCTION

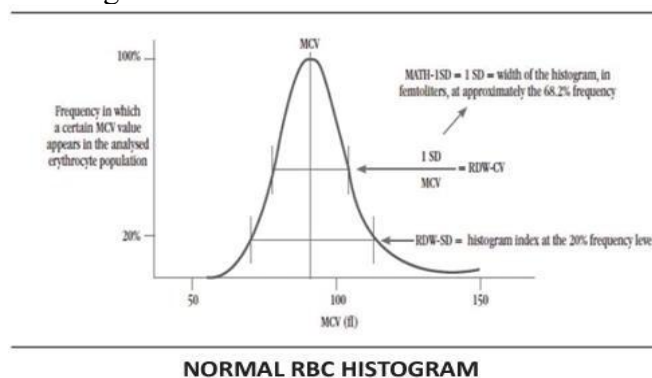
Anemia is a condition in which the number of red blood cells or their oxygen carrying capacity is insufficient to meet the body's physiological requirements which vary by age, sex, altitude, smoking habits and during pregnancy¹.

The use of hematology analyzer is very common in lab practices, on hemogram the analyzer plots the counts as well as the histograms of RBCs, WBCs and platelets series depending on the size of cells. Usually in pathologically affected blood samples the counts which plotted on hemogram are usually false due to changed morphology of cells so it is very important to analyze the histograms findings as histograms are more informative than the counts in pathologically affected blood samples.

Histogram is a graphical representation of numerical data of different cell population in a cell counter. CBC histograms are produced by modern automated hematology analyzers which are routinely used to count blood cells.

RBC Histogram

The RBC histogram is an integral part of automated hematology analysis and is now routinely available on all automated cell counters. This histogram and other associated CBC parameters have been found abnormal in various hematological conditions and may provide major clues in the diagnosis and management of significant red blood cell disorders².



Causes of deviation from normal RBC histogram³:

S.N.	Deviation in histograms	Description	Examples
1.	Shift to left	Indicates microcytosis	Iron deficiency anemia
2.	Shift to right	Indicates macrocytosis	<ul style="list-style-type: none"> Megaloblastic anemia Macrocytic anemia
3.	RL flag	Lower discriminator exceeds preset height by greater than 10% (RBC histogram not starting from baseline).	<ul style="list-style-type: none"> Giant platelets Microerythrocytes Fragmented RBCs
4.	RU flag	Upper discriminator exceeds preset height by greater than 5% (RBC histogram not ending at baseline).	<ul style="list-style-type: none"> Cold agglutination Rouleaux formation

5.	Multiple peaks	More than one peak is seen , indicates two or more populations of RBCs.	<ul style="list-style-type: none">• Post transfusion• Extreme leucocytosis• Cold agglutinin disease• Dimorphic anemia
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AIM AND OBJECTIVES:

AIM:

To correlate RBC histogram abnormalities with peripheral blood smear findings in diagnosis of anemia.

OBJECTIVES: -

1. To determine sensitivity, specificity, Positive predictive value, Negative predictive value of RBC histogram in diagnosis of anemia.
2. To study automated histograms pattern in various anemia

MATERIAL & METHOD

Study Design and Setting:

This is a cross-sectional study conducted in the Department of Pathology at Shyam Shah Medical College and associated hospitals Rewa (M.P.), over a period of 12 months.

INCLUSION CRITERIA –

1. All cases of anemia diagnosed in age group 15-49 years.
2. Hb <11gm /dl for pregnant females.
3. Hb <12gm/dl for non-pregnant females.
4. Hb <13 gm/dl for males.

EXCLUSION CRITERIA –

1. Age group other than 15-49 years.
2. Patients suffering from hematological and other malignancies.
3. Inadequate quantity of sample for automated hematology analyzer.

Sample Size

- A total of 255 anemic patients meeting the inclusion criteria were selected using consecutive sampling technique.

PROCEDURE PLANNED –

Each blood sample was processed on Zybion hematology analyzer to obtain the particle volume distribution histogram and its PBS was made and the findings of PBS were compared with that of histogram.

Statistical Analysis

Diagnostic accuracy was calculated by using formulas.

RESULTS

The study included 255 anemic patients in which higher prevalence among females with 169 out of 255 cases (66.28%) compared to 86 out of 255 cases (33.72%) in males. In the younger age groups (15-19 and 20-24 years) the number of females with anemia is higher than that of males with 15 and 35 females respectively compared to 12 and 11 males.

The categorization of anemia based on RBC indices (MCV, MCH, MCHC and RDW) reveals the following distribution: 37.6% (n=96) of cases are microcytic hypochromic, 35.6% (n=91) are macrocytic, and 26.6% (n=68) are normocytic normochromic.

Table 1 Microcytic hypochromic anemia table for calculating diagnostic accuracy of histograms in comparison to peripheral smear: -

Microcytic	Peripheral smear	Peripheral smear
	Positive	Negative
Histogram (Positive)	65 (TP)	05 (FP)
Histogram (Negative)	07 (FN)	178 (TN)

In total, 65 cases were true positives, accurately identified as microcytic hypochromic anemia by both histogram and peripheral smear. 5 cases were false positives, identified only by the histogram. 7 cases were false negatives, missed by the histogram but detected on smear. 178 cases were true negatives, correctly identified as not having the condition by both methods.

Sensitivity : $TP/TP+FN \times 100 = 65/65+7 \times 100 = 90.2\%$

Specificity : $TN/TN+FP \times 100 = 178/178+5 \times 100 = 97.2\%$

Positive predictive value: $TP/TP+FP = 0.92$

Negative predictive value : $TN/TN+FN = 0.96$

Table 2 Macrocytic anemia table for calculating diagnostic accuracy of histograms in comparison to peripheral smear: -

Macrocytic	Peripheral smear	Peripheral smear
	Positive	Negative
Histogram (Positive)	55 (TP)	05 (FP)
Histogram (Negative)	06 (FN)	189 (TN)

Out of the total cases, 55 were true positives, correctly identified as macrocytic anemia by both the histogram and peripheral smear. 5 cases were false positives, where the histogram indicated macrocytic anemia, but the smear did not confirm it. 6 cases were false negatives, missed by the histogram but identified by the smear. Lastly, 189 cases were true negatives, correctly identified by both methods as not having macrocytic anemia.

Sensitivity : $TP/TP+FN \times 100 = 90.1\%$

Specificity : $TN/TN+FP \times 100 = 97.4\%$

Positive predictive value: $TP/TP+FP = 0.92$

Negative predictive value : $TN/TN+FN = 0.96$

Table 3 : Normocytic Normochromic anemia table for calculating diagnostic accuracy of histograms in comparison to peripheral smear:

Normocytic	Peripheral smear	Peripheral smear
	Positive	Negative
Histogram (Positive)	39 (TP)	6 (FP)
Histogram (Negative)	4 (FN)	206 (TN)

A total of 39 cases were true positives, correctly identified as anemic by both histogram and peripheral smear. 6 cases were false positives identified by histogram only. 4 cases were false negatives, missed by the histogram but detected by smear. 206 cases were true negatives correctly identified by both methods as not having normocytic anemia.

Sensitivity : $TP/TP+FN \times 100 = 90.6\%$

Specificity : $TN/TN+FP \times 100 = 97.2\%$

Positive predictive value: $TP/TP+FP = 0.87$

Negative predictive value : $TN/TN+FN = 0.98$

Discussion

The correlation between peripheral blood smear findings and CBC histograms is essential for accurately diagnosing anemia in the 15–49 years age group. Combining these tools enhances diagnostic accuracy, particularly relevant in reproductive-age females, where anemia is common due to menstrual loss, pregnancy, and nutritional deficiencies.

In gender-wise comparison, **Phukan et al. (2022)**⁴ reported a female predominance (59.8%), as did **Sumira Abbas et al. (2024)**⁵, although with near-equal gender distribution. The present study also observed female predominance (66.28%), likely due to anemia's higher prevalence in reproductive-age women. Age-wise distribution shows females are more affected, especially in the 20–29 year range. Comparatively, **Aditya Singh et al. (2022)**⁶ observed high anemia prevalence among rural men aged 15–19 and 50–54 years, but the current study highlights a female-dominated distribution in younger age groups, linked to reproductive factors.

Morphological classification shows microcytic hypochromic anemia as most prevalent in several studies: **Sandhya V et al. (2017)**⁷ at 46%, **Shifa et al. (2019)**⁸ at 68%, and **Avni Patel et al. (2022)**⁹ at 50.4%. The present study also found a high percentage (37.6%), followed by macrocytic (35.6%) and normocytic (26.6%) anemia, indicating predominant iron and vitamin B12/folate deficiencies.

Regarding efficacy of histograms, **Sarita et al. (2018)**¹⁰ found high sensitivity (93.9%) but lower specificity (64.2%) for microcytic anemia. **Alina Mariam Varghese et al. (2023)**¹¹ reported 66.44% sensitivity and 97.59% specificity. The present study showed 90.2% sensitivity and 77.2% specificity. This indicates high effectiveness in detecting true positives but suggests a need for smear confirmation in negative cases.

For macrocytic anemia, **Sarita et al. (2018)**¹⁰ reported 93.3% sensitivity and 98.7% specificity, while **Varghese et al. (2023)**¹¹ noted perfect sensitivity (100%) and 90.48% specificity. The current study showed strong diagnostic performance with 90.1% sensitivity, 83.3% specificity indicating reliability for positive results but suggesting confirmatory testing for negative cases.

For normocytic anemia, **Varghese et al. (2023)**¹¹ noted 82.56% sensitivity and 68.55% specificity while current study showed 90.6% sensitivity, 97.2% specificity.

Conclusion

The study concludes that CBC histograms, with high sensitivity and positive predictive value are effective screening tools for anemias. When combined with peripheral smears, they offer a reliable diagnostic approach, especially in reproductive-age patients.

Acknowledgement: -

We sincerely thank the department of Pathology, Shyam Shah Medical College Rewa MP for providing facility and granting permission to carry out the work.

Orchid ID:

Deepti Tiwari - <https://orcid.org/0009-0007-1903-0372>

S.K. Sutrar - <https://orcid.org/0000-0002-1892-2770>

Jagannath Jatav - <https://orcid.org/0000-0003-1341-7211>

Vibha Misra - <https://orcid.org/0009-0000-5533-2510>

Pushpkunjika Sharma - <https://orcid.org/0009-0000-7234-9728>

Shambhavi - <https://orcid.org/0000-0002-0317-0991>

Nikita Giri - <https://orcid.org/0009-0003-7760-5083>

Shweta Paswan - <https://orcid.org/0009-0000-0208-7478>

Work Attributed to: Shyam Shah Medical College, Rewa M.P. India

Author's Contribution:

ST- Definition of intellectual content, Literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and

submission of article; **SKS**- Concept, design, clinical protocol, manuscript preparation, Design of study, statistical Analysis and Interpretation; **JJ**- Coordination and Manuscript revision

Conflict of interest: No! Conflict of interest is found elsewhere considering this work.

Source of Funding: There was no financial support concerning this work.

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