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Original Research Article

Assessment of Peripheral Smear and RBC Histograms: A Retrospective Study

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Abstract

Background: Complete blood count (CBC) with red blood cell (RBC) indices and peripheral smear examination are pivotal in diagnosing anemia. Automated RBC histograms offer rapid assessment of erythrocyte size distribution, potentially correlating with morphological abnormalities.

Aim: To evaluate the correlation between peripheral smear findings and RBC histogram patterns in patients with anemia.

Methodology: A retrospective observational study was conducted on 300 adult patients with hemoglobin <12.5 gm/dL at the Department of Pathology, Lord Buddha Koshi Medical College, Saharsa, Bihar, CBCs and RBC histograms were obtained using the Horiba Pentra XLR 80™ analyzer. Peripheral smears were examined microscopically for morphological abnormalities. Anemia was classified by MCV and histogram patterns, and findings were statistically compared using Chi-square tests ($p < 0.05$).

Results: Microcytic hypochromic anemia was the most prevalent type (52% by histogram, 53.7% by smear), followed by normocytic normochromic, dimorphic, and macrocytic anemias; hemolytic anemia was rare. Histogram patterns primarily included broad base (34%) and left shift (33%), correlating with smear morphology. Overall, classification by RBC histograms closely matched peripheral smear findings, with minor variations.

Conclusion: RBC histogram analysis shows strong correlation with peripheral smear findings and can serve as a reliable, rapid adjunct in anemia evaluation. Integrating automated histograms with smear examination enhances diagnostic accuracy and aids clinical decision-making.

Keywords: RBC histogram, peripheral smear, anemia, red cell indices, microcytic hypochromic anemia, automated hematology.

Introduction

The CBC with DLC remains one of the most fundamental and widely used investigations in clinical laboratories all over the world. It provides invaluable information for the diagnosis of a broad spectrum of hematological and systemic conditions, such as anemia, acute and chronic diseases, leukocyte disorders, leukemia, and platelet disorders. During the last 25 years, blood cell analysis has undergone an extraordinary evolution from time-consuming manual methodologies to highly automated systems capable of delivering rapid, accurate, and reproducible results [1]. This evolution has enhanced laboratory productivity and at the same time increased the depth and quality of hematological assessment [2].

Modern automated hematology analyzers provide much more than numerical counts. Graphical outputs, including histograms and scatter plots, are generated with most modern analyzers, and these have come to be useful in a broader interpretation of blood cell data by the clinician and the laboratory professional [3] In these, the RBC histogram has become an important aid in the assessment of erythrocyte morphology and size distribution. The RBC histogram is a graphical representation of the particle size distribution as a plot of cellular frequency against cell volume. It provides information that may not be directly available from numerical values. Deviation or abnormalities in the curve of the histogram can indicate an underlying hematological abnormality, especially when combined with RBC indices, namely MCV, MCH, MCHC, and RDW.

The Horiba Pentra XLR 80™ hematology analyzer utilized in this study operates under the principle of Coulter, which was introduced by Wallace Coulter in 1956 [4]. This is based on an impedance method where cells suspended in an electrolyte solution

pass through a small aperture. Each passing cell displaces a volume of conducting liquid equal to its own volume. As a result, there will be a measurable change in electrical conductance. This thereby generates an electrical pulse whose amplitude will be proportional to the volume of the cell. Analysis of thousands of pulses by the analyzer leads to an accurate count of cells and, further, a distribution profile which is displayed as a histogram [5].

The RBC histogram itself represents the range of red cell volumes and can highlight subtle morphological abnormalities. Normal RBCs have an MCV between 80–100 femtoliters (fl), and the histogram usually spans volumes from 30 fl to 300 fl. Automated analyzers count only cells within the 36–300 fl range [6]. Particles smaller than 30 fl are generally excluded from the RBC count; however, if the histogram starts above the baseline rather than at it, this may indicate the presence of small particles such as platelet clumps, fragmented RBCs, microspherocytes, nucleated RBCs, elliptocytes, bacteria, or malaria parasites [7]. Such deviations provide an early clue to underlying pathology, which can then be further explored through manual examination or additional testing.

RBC histograms are much more than supplementary; they provide pertinent diagnostic information. Deviations from the normal bell-shaped curve can reflect microcytosis, macrocytosis, anisocytosis, or other erythrocyte abnormalities. A leftward shift may indicate microcytic anemia, while a rightward shift may suggest macrocytosis. In conjunction with RBC indices, the pattern of histogram will help in differentiating various conditions like iron deficiency anemia, thalassemia, megaloblastic anemia, and other hematologic disorders. In some acute conditions such as infections and hemolytic crises, transient changes in the RBC histogram may be detectable before standard RBC indices become abnormal [8].

Peripheral smear examination is a direct visualization of erythrocyte morphology that complements automated histogram analysis. Smears can reveal anis poikilocytosis, target cells, spherocytes, or fragmented cells, which may not be clearly reflected in the automated count alone. In conjunction with the RBC histogram, these findings provide a more complete profile of a patient's hematological status. Retrospective studies examining the correlation between peripheral smear findings and RBC histogram abnormalities play a major role in validating automated parameters, identifying patterns of abnormality, and enhancing the predictive value of routine hematology testing.

Understanding the correlation of RBC histogram abnormalities with peripheral smear findings is growing in importance, as reliance upon automated hematology analyzers increases. While these automated counts provide speed, reproducibility, and economy, manual examination of smears remains the gold standard for morphologic assessment. Data from both sources can be analyzed retrospectively to determine how reliably RBC histograms reflect specific morphologic changes. Such studies identify limitations in automated systems and permit the optimization of laboratory protocols to help ensure more accurate and timely diagnostic information for clinical use.

With the increasing dependency on automated hematology analyzers, the relationship between RBC histogram abnormalities and peripheral smear findings has gained importance. While automated counts provide speed and reproducibility, manual smear examination remains the gold standard for morphological assessment. Data from both sources were analyzed retrospectively in an attempt to determine the degree to which RBC histograms can predict specific morphologic abnormalities. In the present study, data from 300 patients examined for complete blood count on the Horiba Pentra XLR 80™ analyzer were analyzed retrospectively. Peripheral smears of the subjects were reviewed for morphological abnormalities; simultaneously, the respective RBC histograms were examined for deviation from the normal curve. Such a review not only reinforces the utility of automated systems but helps in identifying limitations and discrepancies that may provide valuable feedback to the laboratory for improving diagnostic protocols and patient care outcomes.

Materials and Methods

Study Design: This study was a retrospective observational analysis conducted to evaluate the correlation between peripheral smear findings and RBC histograms in patients with anemia.

Study Area: The study was conducted in the Department of Pathology, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.

Study Duration: The study was carried out over a period of one year.

Sample Size: A total of 300 patient samples were included in the study.

Study Population: The study population consisted of adult patients presenting with hemoglobin levels <12.5 gm/dL.

Inclusion Criteria

- Adult patients (age ≥18 years) with hemoglobin <12.5 gm/dL.
- Patients whose blood samples were sent to the Central Hematology Laboratory for routine evaluation.

Exclusion Criteria

- Patients with known malignancies.
- Samples with incomplete clinical data or improperly collected/handled blood specimens.

Data Collection: Data for the study were obtained from the Central Hematology Laboratory. Blood samples were collected in EDTA vacutainers, accompanied by a duly filled request form from the clinician.

Procedure: All blood samples included in the study were collected in EDTA vacutainers and registered in the Laboratory Information System upon receipt. Each sample was first analyzed using the Horiba Pentra XLR 80™ automated hematology analyzer to obtain complete blood counts and RBC histograms. Peripheral blood smears were then prepared from the same samples and stained using Giemsa stain. After proper staining, microscopic examination of the smears was performed to assess red blood cell morphology, including evaluation of size, shape, and chromasia. The histogram patterns generated by the analyzer were simultaneously reviewed, with specific attention to the position of the curve (normal, left shift, or right shift) and the nature of its shape, including normal Gaussian bell curve, broadened base, bimodal peak, skewed curve, or U-shaped pattern. These histogram findings were correlated with RBC indices and peripheral smear morphology. Anemia was categorized based on MCV values into normocytic normochromic, microcytic hypochromic, and macrocytic normochromic types. Histogram interpretations were matched with smear findings, identifying patterns such as dimorphic anemia or hemolytic anemia where relevant. All results were recorded and compiled for comparative analysis.

Statistical Analysis: Statistical analysis was performed using appropriate software such as SPSS (version 25.0) or an equivalent statistical package. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used to summarize demographic variables and hematological parameters. The correlation between peripheral smear findings and RBC histogram patterns was assessed using the Chi-square test or Fisher's exact test, depending on the distribution and adequacy of expected cell counts. Additional correlation coefficients were calculated where continuous variables required further comparison. A p-value of less than 0.05 was considered statistically significant for all analyses. The statistical results helped determine the strength of association between automated histogram interpretations and manual peripheral smear evaluations.

Result

Table 1 presents the distribution of anemia types based on red cell indices and histogram analysis among 300 patients. Microcytic hypochromic anemia was the most common type, observed in 156 patients (52%), followed by dimorphic anemia in 40 patients (13.3%) and macrocytic anemia in 38 patients (12.7%). Normocytic normochromic anemia was identified in 60 patients (20%), while hemolytic anemia was the least frequent, seen in 6 patients (2%). Overall, the majority of cases were microcytic hypochromic, consistent with common patterns of anemia in this population.

Table 1: Distribution of Anemia Cases on Red Cell Indices and Histograms

Diagnosis	No. of cases (n = 300)	Percentage
Normocytic normochromic anemia	60	20%
Microcytic hypochromic anemia	156	52%
Macrocytic anemia	38	12.70%
Dimorphic anemia	40	13.30%
Hemolytic anemia	6	2%

Table 2 shows the distribution of anemia types based on peripheral smear examination among 300 patients. Microcytic hypochromic anemia was the most common, affecting 161 patients (53.7%), followed by dimorphic anemia in 38 patients (12.7%) and macrocytic anemia in 35 patients (11.7%). Normocytic normochromic anemia was observed in 56 patients (18.7%), while hemolytic anemia was the least frequent, seen in 10 patients (3.3%). Overall, microcytic hypochromic anemia predominated in this population.

Table 2: Distribution of Anemia Cases on Peripheral Smear Examination

Diagnosis	No. of cases (n = 300)	Percentage
Normocytic normochromic anemia	56	18.70%
Microcytic hypochromic anemia	161	53.70%
Macrocytic anemia	35	11.70%
Dimorphic anemia	38	12.70%
Hemolytic anemia	10	3.30%

Table 3 presents the distribution of RBC histogram curve types among the study population. The most common patterns were broad base (34%) and left shift (33%), followed by normal curves (16%). Less frequent patterns included right shift (8%), bimodal peak (7%), and short peak (2%). This indicates that the majority of patients exhibited abnormal RBC distribution patterns, with broad base and left shift being the predominant types.

Table 3: Distribution of RBC Histogram Curves

S. No.	Type of histogram	Percentage
1	Normal curve	16%
2	Left shift	33%
3	Right shift	8%
4	Broad base	34%
5	Short peak	2%
6	Bimodal peak	7%

Table 4 compares the classification of anemia by peripheral smear examination versus histogram and RBC indices in 300 patients. Normocytic normochromic anemia was identified in 56 patients (18.7%) by smear and 60 patients (20%) by histogram analysis. Microcytic hypochromic anemia was the most common type, observed in 161 patients (53.7%) on smear and 156 patients (52%) on histogram. Macrocytic anemia was noted in 35 (11.7%) and 38 (12.7%) patients, dimorphic anemia in 38 (12.7%) and 40 (13.3%), and hemolytic anemia in 10 (3.3%) and 6 (2%) patients, respectively. Overall, the two methods showed comparable results, with only minor differences in the classification percentages.

Table 4: Comparison of Peripheral Smear Examination Vs Histogram Analysis (N = 300)

Type of Anemia	Peripheral Smear Analysis (n = 300)	Histogram and RBC Indices (n = 300)
Normocytic Normochromic	56 (18.7%)	60 (20%)
Microcytic Hypochromic	161 (53.7%)	156 (52%)
Macrocytic	35 (11.7%)	38 (12.7%)
Dimorphic	38 (12.7%)	40 (13.3%)
Hemolytic	10 (3.3%)	6 (2%)

Discussion

In this study, the correlation between peripheral smear findings and RBC histograms was analyzed in 300 cases of anemia, with microcytic hypochromic anemia found to be predominant at 52% as estimated by red cell indices and at 53.7% by peripheral smear examination, followed by normocytic normochromic anemia, dimorphic anemia, macrocytic anemia, and hemolytic anemia in descending order. There is agreement with Sandhya and Muhasin (2014) [9] microcytic anemia was the most common, accounting for 54% of cases, followed by normocytic (19%), dimorphic (13%), and macrocytic anemia (12%) using RBC histograms and peripheral smear correlation. Chavda et al. (2015) [10] reported 50% of cases of microcytic anemia, while normocytic and macrocytic types were represented by 18% and 11%, respectively, which matched the prevalence pattern noted in our cohort. Rao et al. (2017) [11] also reported a similar pattern of distribution, with microcytic anemia seen in 51.5% of patients, normocytic in 20%, dimorphic in 14%, and macrocytic in 11%, thus affording support to the consistency of this anemia pattern across diverse demographics. These comparisons suggest that dietary patterns and the prevalence of chronic blood loss maintain iron deficiency anemia as the leading cause of anemia in diverse demographic settings".

In the current study, analysis of RBC histograms demonstrated left-shifted curves in 33% of cases, indicating a predominance of microcytic red cells, and right shifts indicative of macrocytic cells in 8% of cases, 16% had normal curves, 34% showed broad-based histograms indicative of anisocytosis, 7% showed bimodal peaks, and 2% short peaks. These findings closely parallel those of Sandhya and Rashmi (2017) [12] who reported left-shifted histograms in 31% of cases, right shifts in 9%, broad-base histograms in 33%, normal curves in 17%, and less frequent bimodal patterns at 3%. Sandhya and Muhasin (2014) [9] similarly reported 35% left shifted and 32% broad-based curves. Minor variations in the percentage of cases between studies may be due to population characteristics and the relative frequency of dimorphic or mixed anemias. Additionally, Kakkar and Makkar (2009) [8] have pointed out that broad-base histograms reflect, as a rule, anisocytosis and a mixed cell population that can be accurately confirmed only by the examination of peripheral smears. The present study supports this view as the frequency of

dimorphic anemia was noted in 13.3% of cases by indices and 12.7% by smear, similar to earlier reports of 12.2–13% by Chavda et al. (2015) and Rao et al. (2017) [10,11].

Comparisons in the present study between peripheral smear examination and automated indices revealed minor differences. Microcytic hypochromic anemia was slightly more frequent on smears whereas macrocytic and normocytic anemias were slightly more prominent in histogram analysis. Various studies have reported this, including the work of Poonam and Nandita 2011 [13] who identified that automated analyzers at times misclassify giant platelets, fragmented RBCs, or hemolytic artifacts as microcytic cells when, in fact, peripheral smear review differentiates these morphological features accurately. Kumar et al., in 2013 [14], also identified similar observations and noted that smear examination remains an important modality for correct classification of anemia, especially in cases with complex or mixed morphology. These findings emphasize the complementary nature of histogram analysis and smear review, with the former offering rapid quantitative assessment and the latter ensuring morphological verification, especially for dimorphic or hemolytic anemias.

Macrocytic anemia in our present study constituted 12.7% by red cell indices and 11.7% by smear, which is comparable to previous reports by McKenzie (1996) [15] and Begemann & Rastetter (2016) [16], who reported macrocytic anemia in 11-13% of adult populations. Right-skewed histograms, representing larger cell volumes, were found in 8% of our cases, very close to the 7-9% found by Sandhya & Muhasin, 2014 [9]. Dimorphic anemia was found in 13% of our cases; one common feature in both our study and those of Constantino (2011) [1] is the broad-based histogram, as RDW increases in the presence of mixed populations of RBCs. Hemolytic anemia was infrequent, found in only 2-3.3% of cases, which compares favorably to Bessman (1988) [7], who stated that fragmented red cells or hemolytic artifacts are unusual but may distort automated RBC indices if unsupported by smear examination.

The overall correlation obtained in this study between peripheral smear findings and RBC histograms is in concurrence with several previous studies, which state that the analysis of histogram results is a valuable method for reflecting underlying red cell morphology in the majority of cases but should be interpreted in conjunction with microscopic examination for final diagnosis. Therefore, the current study also endorses the application of combining the diagnostic approach, including red cell indices, histogram, and peripheral smear morphology to arrive at more accurate anemia classification, identification of complex morphological patterns, and appropriate clinical management, similar to the literature reported by Sandhya and Muhasin (2014), Chavda et al. (2015), and Kumar et al. (2013) [9,10,14].

Conclusion

The study revealed a strong correlation between the findings from peripheral smear examination and RBC histogram analysis in the differential diagnosis of various anemias. Examination of peripheral smears and RBC indices, along with histogram pattern interpretations, had very consistent trends in identifying normocytic normochromic, microcytic hypochromic, macrocytic, dimorphic, and hemolytic anemias. Microcytic hypochromic anemia was the most common type, followed by dimorphic and macrocytic anemias; hemolytic anemia appeared to be quite rare. Evaluation of RBC histograms typically showed characteristic curve patterns representative for different types of anemia, including left-shifted and broad-based distributions that corresponded well to the morphological features documented on the peripheral smears. It seems that RBC histograms offer useful supplemental information to the traditional smear examination, elaborating on the diagnosis and allowing a quick, automated estimation that can help in clinical decision-making. In summary, the integration of peripheral smear examination with RBC histogram analysis could be a trustworthy method for comprehensive anemia typing and may be useful in further investigations and management.

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