Golestan University of Medical Sciences
Journal of Clinical and Basic Research
Online ISSN: 2538-3736

Red blood cell histogram in different morphological types of anemia in comparison with peripheral blood smears: A comparative analysis

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Abstract

Background: Anemia is a significant health concern around the world, and it is particularly prevalent in developing nations like India. A red blood cell (RBC) histogram is a graphic representation of particle size distribution (cell frequencies vs size). The goal of this study was to examine various types of anemia in patients and compare RBC histograms and results from peripheral smears.

Methods: The study involved a total of 600 anemic patients. A histogram was produced with the use of a 5-part differential automated analyzer, following the complete mixing of the 3 mL of the EDTA blood sample. Additionally, a peripheral smear was prepared simultaneously and stained using Giemsa stain per standard operating procedures (SOP). The peripheral smear findings were then correlated with the histogram charts from the cell counter, taking into account the relevant clinical history.

Results: Out of 600 cases, 339 were females, with a female-to-male ratio of 1.3:1. The 56.2% showed moderate anemia, 31.3 % showed mild anemia, and 12.5 % showed severe anemia. Among the histogram patterns, 52.7% exhibited a left shift, 35.5% showed the normal curve, 8.6% showed a broad base curve, 1.8% showed a right shift, and 1.3% showed a bimodal curve. Blood samples were evaluated along with the corresponding peripheral smear findings. The type of anemia was diagnosed by peripheral smear. 32.8 % of the cases were normocytic normochromic anemia, and 57.0% were microcytic hypochromic anemia. Macrocytic anemia was observed only in 2.5 % of cases, and dimorphic anemia was observed only in 8.0 % of cases diagnosed with PBS. The majority of times, peripheral smear findings and histogram patterns corresponded in cases of normocytic normochromic, microcytic hypochromic, and macrocytic anemias. In 600 cases, 420 had typing findings that accorded with those of the 2 methods, while 180 had discordant typing.

Conclusion: Through its graphical representation of anemia typing, the automated analyzer helps decrease the total burden; nonetheless, it should always be verified through microscopy. As a result, it is concluded that in the era of molecular analysis and automation, the histogram alone could be used as a screening method. When combined with PBS findings, they act as a useful supplement, and by correlating the results of both methods, we could diagnose the majority of anemias.

Article History

Received: 21 October 2023
Received in revised form: 2 December 2023
Accepted: 16 April 2024
Published online: 23 June 2024
DOI: 10.29252/JCBR.8.2.5

Keywords

Anemia
Haemoglobins
Peripheral Blood Smear

Article Type: Original Article

OPEN ACCESS
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Highlights

What is current knowledge?

The automated analyzer helps to reduce the overall load by providing a graphical representation (histogram) for anemia type; however, microscopy should always be used for confirmation.

What is new here?

It concludes that the histogram by itself could be used as a method of screening in the modern era of molecular analysis and automation. The correlation between histogram patterns and peripheral smear diagnosis in dimorphic anemia highlighted questions regarding the reliability of histograms. When the results of both methods are correlated, we can detect most anemias, especially dimorphic anemia.

Introduction

Anemia is a significant diagnostic and clinical category of hematological disorders prevalent worldwide, affecting 1.62 billion people (24.8%) across the globe. In developing countries like India, anemia is a widespread health issue, particularly among children and reproductive-age women (1). Anemia is defined as a reduction in hemoglobin (Hb) concentration, hematocrit, or the number of red blood cells (RBCs) per liter, falling below the reference range for healthy individuals of similar age, sex, and race under comparable environmental conditions (2).

The peripheral blood smear (PBF) has traditionally served as the primary diagnostic tool for determining the cause of anemia. Routine examination of the peripheral blood film has been instrumental in evaluating various hematological disorders. Laboratory hematology was a time-consuming and labor-intensive process 3 to 4 decades ago. Reagents were prepared in the laboratory from raw materials, and procedures were performed manually. The determination of Hb using the cyanmethemoglobin method, for instance, was a time-intensive procedure. In modern clinical settings, the automated hematology analyzer has replaced traditional manual methods for hematological parameters as the initial screening and detection tool for hematological issues. Modern analyzers incorporate various principles such as electrical impedance (previously the sole principle used by earlier machines to count blood cells), conductivity differences, light scatter, cytochemical staining, and flow cytometric principles. These advancements not only enhance the speed of test results but also improve accuracy and precision, ushering in a new era for hematology reporting. Red blood cell histograms, now available on all automated cell counters, play a crucial role in automated blood analysis. These histograms provide valuable insights into the diagnosis and treatment of significant RBC disorders (3,4).

Automated hematolyzers rely on the impedance principle, where changes in conductance occur as each cell passes through an aperture. This change in conductance results in the generation of an electrical pulse, the amplitude of which is proportional to the cell volume. The analyzer displays histograms and numerical results. Among the standard features provided by automated cell analyzers is the RBC histogram, which is based on the well-established Coulter principle. The generation of a histogram is rooted in the analysis of individual cells (4,5).

Histograms graphically represent the frequency of cells in relation to their size. The Y-axis represents the number of cells, while the X-axis depicts cell size. Prior to analyzing the blood smear, different histogram patterns and shapes offer insights into potential pathology. When coupled with other complete blood cell count (CBC) measures, such as RBC distribution width (RDW) and RBC indices, which have shown abnormalities in various hematological conditions, changes in the histogram can have diagnostic significance in one direction or the other. Histograms provide crucial information for managing and diagnosing significant red cell abnormalities (5,6).

Despite significant technical advancements, the interpretation of test results has continued to rely heavily on numerical data. In most laboratory settings, the primary focus has been on validating automated data. However, this practice has

[DOI: 10.61186/jcbr.8.2.5]

become outdated and lacks practical utility without a comparison to a reference normal curve and microscopic confirmation. The overall pattern of the RBC distribution curve in the histogram becomes meaningful only when contrasted with these elements (3,6).

The precision of automated analyzers has significantly increased with their growing sophistication, leading to a continuous decline in the manual evaluation of blood smears (1,3). Unfortunately, technologists may not fully comprehend how to relate graphical displays to morphological findings. This is possible because graphical results, such as scatter plots and histograms, have often been overlooked in favor of traditional parameters like RDW, Hb distribution width, and reticulocyte Hb content, which provide valuable information (4,7).

Despite the technological advancements of modern instruments, there is still a need to rely on human expertise for primary calibration. This emphasizes the importance of maintaining manual technical skills and ensuring this through appropriate technician training programs, even in the face of the temptation to trust everything to machines (7).

An automated analyzer's RBC histogram with CBC gives insight into RBC morphological changes in anemias. By identifying the abnormal red cell form, size, arrangement, and immature red cells, peripheral smear examination (PBS) aids in the diagnosis of various anemias. Peripheral blood smears make the morphology of peripheral blood cells visible, securing their role in diagnosing various primary and secondary blood disorders and blood-related conditions. The development of molecular and automated hematology has not diminished the diagnostic value of PBF. In recent years, the results from CBC obtained through automated hematology analyzers and microscopic analysis of peripheral smears have been combined to provide a comprehensive analysis of a patient's blood sample.

In the present study, different forms of anemia were classified by comparing the RBC histograms obtained from automated hematology analyzers of anemic patients with the results of peripheral smears and correlated accordingly.

The aim of the present study was to interpret the RBC histogram in anemic patients.

1. Morphological typing of anemia on PBF examination.
2. To compare automated histogram patterns with morphological features seen on peripheral smear examination.

Methods

Source of data:

The present study was conducted in the hematology laboratory of the Department of Pathology at GMERS Medical College and Hospital, Godhra, Panchmahal, Gujarat, India. A total of 600 patients with anemia were studied between January 2023 and June 2023. The study was conducted after obtaining ethical clearance from the institutional ethics committee. The cases included in the study were newly diagnosed cases undergoing treatment and follow-up.

Inclusion criteria:

1. All individuals, both sexes, who have anemia-that is, Hb levels below WHO reference levels.
2. Patients of all age groups will be included in the study.

Exclusion criteria:

1. Patients with normal hemoglobin levels (within the normal range for that particular age).
2. All cases of anemia that have undergone blood transfusion.
3. Inadequate quantity of blood samples for automated analyzer.

Tools and techniques.

Venous blood was collected for this study under aseptic conditions in an EDTA vacutainer for hematological examinations. The 5-part automated hematology analyzer ERBA H560 was used for the analysis. Additionally, a peripheral smear was prepared using Giemsa stain following standard operating procedures (SOP). The smear will be evaluated by a pathologist who will not have access to the histogram during the reporting process. Automated histogram patterns will be correlated with morphological features observed on the PBS.

Based on standard reference, the following categories were used to type anemia based on histogram patterns: (i) normal curve, (ii) left shift, (iii) right shift, (iv) broad base, and (v) bimodal.

On the PBS exam, the following categories were used to morphological classification of anemia was: (i) Normocytic normochromic anemia (ii) Microcytic hypochromic anemia (iii). Macrocytic anemia (iv) Dimorphic anemia

In our study, we give the pathologist detailed information to correctly identify and categorize anemia. This includes a thorough clinical history, past medical records, records of blood transfusions, and information about any hemoglobinopathies in the patient's family. Additionally, we give the pathologist pertinent clinical information, such as serum iron levels, vitamin B12 and folic acid levels, liver and kidney function tests, and thyroid hormone levels in suspected cases of anemia.

In our study, RBC histograms generated by cell counters were compared to PBFs in anemia for evaluation. If the results of the 2 methods show the same morphological type of anemia, the results are considered to be concordant; otherwise, they are considered to be discordant.

The comparative analysis of RBC histogram shapes in different studies were done (Table 1). In addition, comparison of concordant and discordant cases of present study with other previous studies were also studied (Table 2).

Table 1. Comparison of red blood cell histogram shapes in different studies

Studies	Normal curve	Left shift	Right shift	Broad base	Bimodal curve
Arvind et al [8]	19.0 %	67.4 %	0.8 %	12.0 %	12.0 %
Chavda J et al [9]	19.0 %	27.0 %	7.0 %	38.0%	38.0 %
Rao BSS et al [10]	17.7 %	29.0 %	5.45 %	37.7 %	7.2 %
Shrivastav et al [11]	18.0 %	59.0 %	6.0 %	19.0 %	5.0 %
Present study	35.5 %	52.7 %	1.8 %	8.6 %	1.3 %

Table 2. Comparison of concordant and discordant cases in different studies

Studies	No. of total cases	Concordant (%)	Discordant (%)
Varghese et al, 2020. [12]	354	266 (75.10%)	88 (24.90%)
Radadiya P et al, 2015. [13]	100	72 (72.0 %)	28 (8.0%)
Farah E. et al, 2013. [14]	350	274 (78.3 %)	76 (21.7%)
Present study, 2023	600	420 (70.0 %)	180 (30.0%)

Statistical data analysis:

The data were entered in Microsoft Excel, and further statistical analysis was done using SPSS version 26. Pearson's chi-square test and Fisher's exact test was used wherever appropriate.

Results

Our study included a total of 600 anemic cases; males were 261 cases (43.5%), while females were 339 (56.5%). The male-to-female ratio was 1:1.3 (Figure 1).

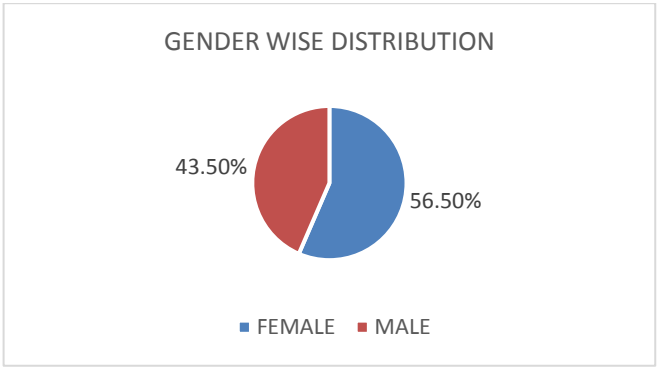

Figure 1. Gender distribution of study samples

In our study, it was found that, out of 600 cases, the majority of cases fall in the adult age range of 21 to 49 years and that the majority (69.3%) of 339 female patients fall in the reproductive age group. Patients aged 21 to 30 constitute the majority of the population (21.8%), closely followed by patients in the 31 to 40 age range (16.0%). This can be explained by the high requirement for iron during adolescence and adulthood, which are stages of rapid growth and development. The body requires extra iron during periods of rapid growth and frequent blood loss (such as during menstruation). Consequently, the risk of getting iron deficiency anemia is higher in women who are of childbearing age. Men were more frequently impacted than women beyond the age of 40. These findings highlight the need to use the right screening tools and treatment plans throughout one's life, as anemia is a condition that affects people of all ages (Table 3).

Table 3. Distribution of study population based on age and gender

Age (Years)	Male; No. (%)	Female; No. (%)	Total
1-10	41 (51.9%)	38 (48.1 %)	79 (100%)
11-20	26 (38.2%)	42 (61.8%)	68 (100%)
21-30	40 (30.5 %)	91 (69.5 %)	131 (100%)
31-40	34 (35.4 %)	62 (64.6%)	96 (100%)
41-50	49 (56.3%)	38 (43.7%)	87 (100%)
51-60	37 (59.7 %)	25 (40.3%)	62 (100%)
61-70	22 (44.9%)	27 (55.1%)	49 (100%)
> 70	12 (42.9%)	16 (57.1%)	28 (100%)
Total	**261 (43.5 %)**	**339 (56.5 %)**	**600 (100%)**

Hemoglobin readings were used to categorize anemia into 3 different levels: mild (< 11 gm% Hb), moderate (Hb 7-10 gm%), and severe (< Hb 7 gm%). The majority of cases (56.2%) of moderate anemia, 188 (31.3%) of mild anemia, and 75 (12.5%) of severe anemia were found among the 600 patients (Table 4a).

A significant proportion of the study population, or 342 out of 600 patients,

[DOI: 10.61186/jcbr.8.2.5]

had been detected by PBS testing as having microcytic hypochromic anemia. By PBS examination, normocytic normochromic, dimorphic, and macrocytic anemias were found in 32.8%, 8.0%, and 2.5% of the cases in our study, respectively (Table 4b).

Table 4. Categorization of anemia based on hemoglobin values and peripheral blood smear findings

4a. Categorization of anemia based on Hb values.			
Hemoglobin (gm%)	Females; Count (%)	Males; Count (%)	Total; Count (%)
Mild	103 (30.4%)	85 (32.6%)	188 (31.3%)
Moderate	185 (54.6 %)	152 (58.2 %)	337 (56.2%)
Severe	51 (15.0%)	24 (9.2 %)	75 (12.5%)
Total	339 (100%)	261 (100%)	600 (100%)
4b. Categorization of anemia based on PBS findings.			
PBS findings		Frequency (n)	Percentage (%)
Normocytic normochromic		197	32.8 %
Microcytic hypochromic		342	57.0%
Macrocytic		13	2.5%
Dimorphic		48	8.0%
Total		600	100%

Our study's findings show that a normal histogram (bell shape) was only seen in 35.5% of cases, the majority (52.7%) of which showed a left shift and only 1.8% of which showed a right shift. The broad base was identified in 8.6% of cases, while bimodal histograms were seen in 1.3% of cases (Table 5). These findings demonstrate the wide range of the RBC size and shape in our study population and may have significant therapeutic ramifications for the identification and treatment of underlying diseases that impact RBC morphology.

Table 5. Histogram pattern observed in the present study

Histogram pattern	Frequency (n)	Percentage (%)
Normal curve	213	35.5 %
Left shift	316	52.7 %
Right shift	11	1.8 %
Bimodal curve	08	1.3 %
Broad Base	52	8.6 %
Total	600	100 %

If we compare the anemia detected by histograms and by manual examination of peripheral smear, we get a very high significant difference ($P < 0.001$) when using Fisher's exact test on the 2 variables (Table 6 & Figures 2-6).

In the present study, 420 patients (70.0%) had concordant typing, while 180 cases (30.0%) had discordant typing. In concordant cases, 146 were normocytic, 257 were microcytic, 9 were macrocytic, and 8 were dimorphic. Discordance may be caused by the presence of fragmented, agglutinated, or abnormal blood cells

Table 6. Comparison between peripheral blood smear findings and histogram patterns

PBS findings	Normal curve	Left shift	Right shift	Broad base	Bimodal curve	Total
Normocytic normochromic	146 (74.1%)	46 (23.4%)	02 (1.0%)	03 (1.5%)	00 (0%)	197 (100%)
Microcytic hypochromic	58 (16.9%)	257 (75.1%)	00 (0%)	27 (7.9%)	00 (0%)	342 (100%)
Macrocytic	04 (30.8%)	00 (0%)	09 (69.2%)	00 (0%)	00 (0%)	13 (100%)
Dimorphic	05 (10.4%)	13 (27.0%)	00 (0%)	22 (45.8%)	08 (16.7%)	48 (100%)
Total	**213 (35.5%)**	**316 (52.7%)**	**11 (1.3%)**	**52 (8.6%)**	**08 (1.3%)**	**600 (100%)**

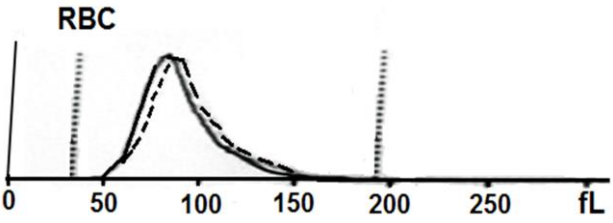

Figure 2. Normal curve in normocytic normochromic anemia

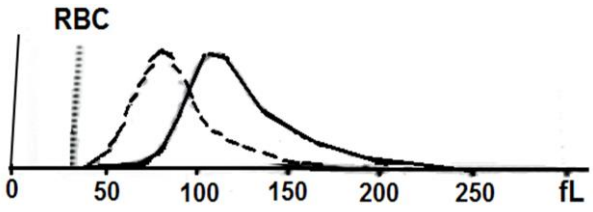

Figure 3. Left shift of histogram in microcytosis

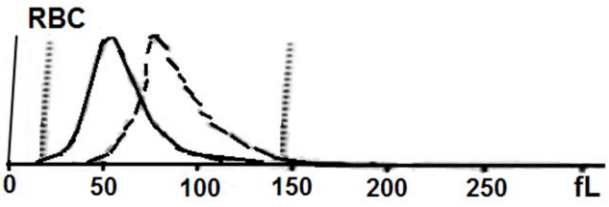

Figure 4. Histogram showing a right shift in macrocytic anemia

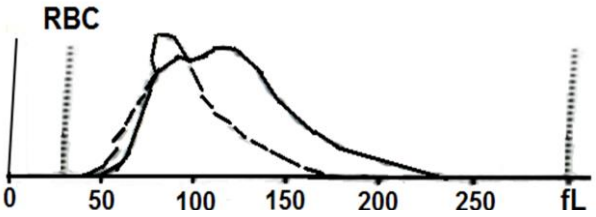

Figure 5. Bimodal histogram pattern in dimorphic anemia

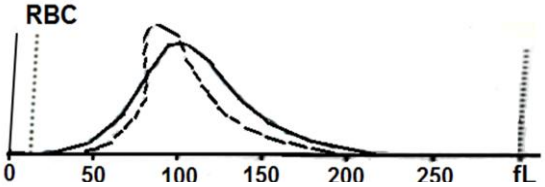

Figure 6. The broad base curve in dimorphic anemia

Table 7. Correlation of automated and manual morphological typing of anemia

Types of anemia	Concordant	Discordant
Normocytic normochromic	146	51
Microcytic hypochromic	257	85
Macrocytic	09	04
Dimorphic	08	40
Total	420 (70.0%)	180 (30.0%)

[DOI: 10.61186/jcbr.8.2.5]

that were not detected by an automated analyzer (Table 7).

We included every case with Hb less than or equal to 11 gm% in our study; 8.93 gm% was the mean Hb level. Hemoglobin levels between 7 and 10 g/dL were present in the majority of cases (56.2%). As shown in the figures below, this was compared to previous research studies (Figure 7).

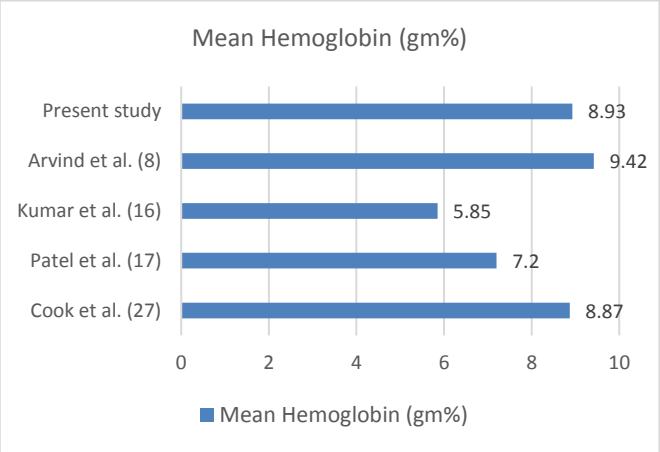

Figure 7. Comparison of mean hemoglobin with other studies

In the present study, PBF evaluation revealed that microcytic hypochromic anemia (57.0%) was the most prevalent morphological type of anemia, followed by normocytic normochromic anemia (32.8%), and dimorphic and macrocytic anemia (8% and 2.5%, respectively). This is consistent with several other studies, including those by Mukaya et al (15), Kumar et al (16), Patel et al (17), and Arvind et al (8) (Table 8).

Table 8. Comparison of types of anemia based on PBS in different studies

Studies	Normocytic Normochromic	Microcytic Hypochromic	Macrocytic	Dimorphic
Mukaya et al. [15]	31.0%	54.5%	10.0%	8.6%
Kumar et al. [16]	31.7%	33.3%	21.7%	13.3%
Patel et al. [17]	24.0%	72.0%	4.0%	-
Arvind et al. [8]	14.0%	72.2%	0.4%	13.4%
Yoginder et al. [18]	-	66.8%	22.2%	11.0%
Present study	32.8%	57.0%	2.5%	8.0%

Discussion

Anemia is a significant health issue, and it is especially prevalent in India (1). The use of automated hematology analyzers and their generated RBC parameters play an essential role in typing the anemia, even though PBS examination and CBC reports can be utilized as preliminary diagnostic tools to diagnose anemia. Through the use of these characteristics, physicians can more accurately diagnose patients with various forms of anemia and decide the most effective course of treatment (19,20).

Clinicians are more impacted by visual representations than merely numbers, such as RBC histograms. Even before examining a PBF, new-generation hematology analyzers produce a variety of histograms that offer important and crucial information about a patient's blood profile (21).

When measuring the size and quantity of RBCs in a blood sample, an automated hematology analyzer that uses modern technology generates an RBC histogram (6). By examining whether the 2 sides of the curve are mirror images of one another, a histogram curve's symmetry can be identified. If both sides of the curve have comparable frequency distributions, the curve is said to be symmetrical and follows a Gaussian distribution (22). The range of the mean corpuscular volume (MCV) between 80 and 100 fl is shown by this normal curve (10). However, a curve is deemed skewed if one side of it is more obvious than the other. It is adjusted in specific circumstances and displays RBC flags (23).

A helpful tool for assessing a patient's cell size in relation to a normal population is the RBC histogram. Large changes in the histogram curve can be seen if the number of cells is plotted on the Y-axis and the size or volume of the cells is plotted on the X-axis (24). The curve is shifted to the left by smaller cell sizes and to the right by larger cell sizes. These changes in the histogram's direction can offer insightful diagnostic information. Shifts in either direction are crucial for diagnosis and have been linked to abnormalities in a variety of hematological illnesses, along with other blood count metrics like RDW and RBC indices (25,26).

We found that females had a higher frequency of anemia among the 600 patients in our study, with 69.3% of the cases being female and a female-to-male ratio of 1:0.8. The majority (339) of the female patients are in the reproductive age group (69.3%). These findings coincided with studies done by Mukaya et al (15), Kumar et al (16), and cook et al (27). Because iron is present in all body cells and is essential for fundamental physiological processes like the synthesis of Hb, the era of adolescence and the adult group is one of tremendous growth and development. Because the body requires more iron during periods of rapid growth and frequent blood loss (such as menstruation), women in the reproductive age group are particularly vulnerable to iron deficiency anemia. We found that between age groups of 41 and 60, the male frequency was higher (53.2%). Men were shown to be affected more than women after the age of 40 years (28,29).

In our study, iron deficiency anemia was the most frequent cause of microcytic hypochromic anemia, which was the most prevalent form (57.0%). The most prevalent type of anemia worldwide is iron deficiency anemia, which can occur for a number of reasons. Inadequate nutritional intake, poor intestinal absorption, increased demand, particularly during pregnancy and lactation, chronic blood loss, etc, are some causes (25,16). According to estimates from the WHO, anemia is prevalent among pregnant women at a rate of 14% in affluent nations, 51% in poor nations, and 65%-75% in India. According to data from the WHO, approximately 2 billion people on earth are anemic. In comparison to other emerging nations, anemia is more common in all demographics in India (28,29).

It has already been stated in various research articles that an RBC histogram is a graphical representation obtained from an automated hematology analyzer and is available routinely on all automated cell counters. The histogram, in association with other CBC parameters (such as RDW, MCV, etc), has been found to be abnormal in various hematological conditions (30,31).

In the present study, histogram showed 35.5% normal curve, 52.7% left shift, 8.6% broad base, 1.8% right shift and 1.3% bimodal curve (Table 6) Which was compared with other studies like Arvind et al (8), Chavda J et al (9), Rao BSS et al (10), and Shrivastav et al (11), (Table 1). In PBS, microcytic hypochromic anemia predominated (57.0%), which in our study was associated with a larger formation of the left shift (75.1%) pattern in the RBC histogram. The presence of giant platelets and platelet clumps, as well as fragmented RBCs in hemolytic diseases, when the autoanalyzer considers it to be a microcyte, can be used to explain the little variation between the analysis of microcytic anemias by PBS and RBC indices/histogram. Thus, peripheral smear eliminates these types of errors.

In the present study, a PBF analysis of 197 cases (32.8%) of normocytic normochromic anemia revealed 146 cases (74.1%) with normal curves and 46 (23.4%) with left shifts, whereas right shift and broad base were only observed in 1% and 1.5% of cases, respectively. In our study of 13 cases of macrocytic anemia, 9 (69.2%) cases displayed a right shift, and 4 (31.8%) displayed a normal curve, which is consistent with studies by Patel et al (17) and Shrivastava et al (11). As a result, we can observe that histograms are a helpful diagnostic tool when it comes to macrocytic, microcytic hypochromic, and normocytic anemia.

The existence of greater anisocytosis is typically indicated by a broad base curve in a histogram, which may be cross-checked by microscopic examination. A narrow distribution curve indicates a homogenous RBC population. However, in dimorphic anemia, the width, centeredness, and histogram pattern all reveal differences in the RBCs. Different histogram patterns, ranging from simple curves to complicated curves, were present in the dimorphic anemia. In the smears reported as dimorphic anemia, we noticed that only 8 (16.7%) cases of histograms showed a bimodal curve, whereas 22 (45.8%) cases showed broad base histogram patterns, and 13 (27.1%) cases showed a left shift histogram curve. The broad base curve can be explained by the presence of multiple populations of cells of varying sizes (ie, normocytic, microcytic, and macrocytic). Our study was in concordance with the study conducted by Arvind et al (8), Shrivastav et al (11), and Constantino et al (32).

The histogram patterns of the cell counter and the PBF reports were compared using Fisher's exact test, and p values indicated a highly significant difference between the 2 variables. This difference was largely due to dimorphic anemia cases, which is in concordance with Constantino et al (32).

In the present study, the results of histogram and peripheral smear were varied. Concordant typing was present among 420 (70.0%) cases, and discordance was present among 13 (30.0%) cases. Our study was in agreement with the study conducted by Varghese et al. (12), Radadiya P et al. (13), and Farah E. et al. (14). Discordance can be due to the presence of agglutinated RBCs, fragmented RBCs, or abnormal blood cells, not detected by an automated analyzer and later corrected by smear examination.

The dimorphic blood picture will either show a dual population of normocytic and macrocytic or microcytic and normocytic red cells or a mixture of small, normal, and large cells that vary in size and shape with or without normal RBC indices. Because abnormal red cell populations are frequently associated with dimorphic anemia, morphological observations should be compared with graphical and numerical data to help interpret the findings. Such a diagnosis should not be made solely on the results of an automated cell counter, as this could produce false results. To examine all cell populations, it is crucial to examine the PBF (11).

It is possible to distinguish between distinct morphological types of anemia, hemoglobinopathies, and other blood disorders using PBFs. Every laboratory should have an automated hematology analyzer employing a peripheral smear validation method to guarantee the best possible patient care.

Conclusion

An automated hematology analyzer's RBC histogram provides significant information about hematological conditions. Histograms are useful tools for

[DOI: 10.61186/jcbr.8.2.5]

technologists since they can help them determine which cases require true, in-depth peripheral smear evaluation by experts. According to our study, the diagnosis of microcytic hypochromic, normocytic normochromic, and macrocytic anemia was significantly correlated with RBC histograms and peripheral smear results. However, concerns about the reliability of histograms were raised by the relationship between histogram patterns and peripheral smear diagnosis in dimorphic anemia. Anemia may be misclassified as a result of the difference in RBC morphology analysis between automated and manual scans of peripheral blood.

Consequently, it may be stated that we are now in an era of automation and molecular analysis. By connecting the results of the two approaches, we could diagnose the majority of cases of anemia. The histogram alone could be used as a screening method, and when paired with PBS findings, they act as a beneficial addition.

Acknowledgement

The authors are grateful to the present and past staff of the pathology department of GMERS Medical College and Hospital, Godhra, Panchmahal, Gujarat, India.

Funding sources

For their research, writing, and/or publication of this work, the authors got no financial funding.

Ethical statement

The Ethics Committee of the GMERS Medical College and Hospital, Godhra, India, gave its approval to the study protocol.

Conflicts of interest

Regarding the publication of this work, the authors affirm that there is no conflict of interest.

Author contributions

DP and JP collected data, designed the experimental design, and conceptualized the study. PD and DP contributed to drafting and critically revising the article. The work was revised and expanded upon by all other authors after being first written by AP and analyzing the data. The final version of the text was reviewed and approved by all authors.

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How to Cite:

Patel DD, Desai PN, Prajapati JA, Patel AB, Patel AD. Red blood cell histogram in different morphological types of anemia in comparison with peripheral blood smears: A comparative analysis. *JCBR*. 2024;8(2): 5-9.

[DOI: 10.61186/jcbr.8.2.5]