



Research Article

# A Comparative Study of Red Cell Histogram along With CBC Parameters and Peripheral Blood Smear in Various Anemias

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**Abstract: Background:** Anemia is one of the most common global health problems, particularly in India. It has been associated with significant morbidity and mortality. Laboratory investigations, including a complete blood count (CBC) and differential leukocyte count, are crucial in diagnosing anemia, platelet disorders, white cell disorder, leukemia, and other related conditions. Automated hematological analyzers have become an integral tool in providing accurate and efficient blood cell analysis. These machines not only provide essential information on RBC indices, hematocrit, and RBC distribution width (RDW), but also give a detailed RBC histogram.

**Materials and methods:** The present study was a prospective study and comparative study was conducted in the Department of Pathology, Tertiary care Teaching Hospital over a period of one year. All patients, both male and female with anemia i.e haemoglobin levels below WHO reference values were included. Method of Collection: 3ml of EDTA venous blood sample was collected from the patient and a histogram was obtained after thorough mixing of the sample. The 5 part differential automated analyzer was used for the study. Simultaneously a peripheral smear was prepared according to standard operating procedures and stained by Leishman stain. This peripheral smear was reported by a pathologist who was not privy to histogram during the reporting of peripheral smear.

**Result:** Females were predominantly composed of (57.5%). The age group of patients included in this study ranged from 11 years to 80 years. Majority of patients (30.83%) were within 21-30 years of age and least were 71-80 years (2.5%). Based on hemoglobin values, anemia was divided into following categories: MILD (Hb Out of the 120 subjects majority cases (60%) showed moderate anemia. In this study, Microcytic Hypochromic anemia constituted the predominant type with 79 cases (65.83%) followed by Normocytic Normochromic anemia (17.5%) and 15.83% of Dimorphic anemia. Only 1 case (0.83%) of Macrocytic anemia was encountered in the study with no cases of hemolytic anemia. The histogram pattern of all cases were analysed. Left shift was the most common pattern observed constituting 83 (69.16%) cases.

**Conclusion:** Histogram is an important tool of diagnosis when correct interpretation of the curve is combined with findings of blood count parameters like red cell distribution width and red cell indices. By observing these curves we could give presumptive diagnosis of presence of fragments in blood, microcytic, macrocytic or dimorphic red cells. Histograms are useful tool for technologists as it could guide them that which cases need actual detailed peripheral smear examination by experts.

**Keywords:** Histogram, Anemia, Complete blood count.

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## 1. Introduction

**A**nemia is one of the most common global health problems, particularly in India. It has been associated with significant morbidity and mortality. Laboratory investigations, including a complete blood count (CBC) and differential leukocyte count, are crucial in diagnosing anemia, platelet disorders, white cell disorder, leukemia, and other related conditions. [1] Over the years, blood cell analysis has advanced significantly from manual procedures to automated instruments, providing more accurate and reliable results. [2]

Automated hematological analyzers have become an integral tool in providing accurate and efficient blood cell analysis. These machines not only provide essential information on RBC indices, hematocrit, and RBC distribution width (RDW), but also give a detailed RBC histogram. [3] Such comprehensive analysis plays a crucial role in diagnosing and managing red cell disorders. In fact, for accurate morphological diagnosis of anemia, the histogram provided by these analyzers is particularly important. Therefore, it is evident that the RBC histogram is a critical component in the laboratory evaluation of blood cells. [4]

The peripheral blood smear has been a primary diagnostic tool for identifying anemia and other hematological disorders. The routine examination of blood films has significantly contributed to the interpretation of various hematological conditions. However, with the advancement in technology, automated hematology analyzers have now replaced the traditional manual methods for analyzing various parameters. [5] These instruments have become the go-to tool for initial screening and detection of hematological abnormalities in modern clinical diagnostic laboratories. As a result, the use of automated hematology analyzers has streamlined the process and enhanced the accuracy of hematological analysis. [6]

Over the past few years, automated hematology analyzers have become increasingly popular due to their accuracy and reliability, which has significantly reduced subjective errors in diagnosing anemia. However, it is important to note that the microscopic examination of the peripheral blood smear (PBS) by a pathologist remains a critical step in the primary calibration of cell counters. [7] This examination plays a pivotal role in ruling out other hematological disorders that may go undetected through automated analysis alone. Therefore, despite the remarkable advancements in technology, the role of pathologists in examining the PBS remains invaluable in ensuring accurate and reliable diagnosis. [8]

## 2. Material and methods

The present study was a prospective study and comparative study was conducted in Department of Pathology, Tertiary care Teaching Hospital over a period of one year.

**Inclusion criteria** All patients, both male and female with anemia i.e haemoglobin levels below WHO reference values.

**Exclusion criteria** Patients with normal Hemoglobin levels. (Within the normal range for that particular age.)

**Method of Collection:** 3ml of EDTA venous blood sample was collected from the patient and a histogram was obtained after thorough mixing of the sample. The 5-part differential automated analyzer was used for the study. Simultaneously a peripheral smear was prepared according to standard operating procedures and stained by Leishman stain. This peripheral smear was reported by pathologist who was not privy to a histogram during the reporting of peripheral smear.

RBC histogram from same sample was analysed separately. Evaluation was done by Pathologist.

The results obtained from both methods are then classified into 4 morphological types of anaemia – Normocytic normochromic, microcytic hypochromic, macrocytic and haemolytic anaemias.

**Statistical Analysis:** A qualitative analysis of the data was done using Pearsons Chi square test and Fisher exact test wherever appropriate

### 3. Results

**Table 1.** Distribution of Gender

Gender	Frequency	Percentage
Male	51	42.5
Female	69	57.5
Total	120	100

In table 1, females were predominantly comprised of (57.5

**Table 2.** Distribution of Age group

Age (Years)	Frequency	Percentage
11-20	19	15.83
21-30	37	30.83
31-40	21	17.5
41-50	19	15.83
51-60	12	10
61-70	9	7.5
71-80	3	2.5
Total	120	100

The age group of patients included in this study ranged from 11 year to 80 years. Majority of patients (30.83%) were within 21-30 years of age and least were 71-80 years (2.5%) (Table 2)

**Table 3.** Categorization of anemia based on Hemoglobin values

Hemoglobin (gm%)	Frequency	Percentage
Mild	37	30.83
Moderate	72	60
Severe	11	9.16
Total	120	100

Based on hemoglobin values, anemia was divided into following categories: MILD (Hb Out of the 120 subject's majority cases (60%) showed moderate anemia in table 3.

**Table 4.** Peripheral Smear findings of study population

Type of anemia	Frequency	Percentage
Normocytic normochromic	21	17.5
Microcytic hypochromic	79	65.83
Macrocytic	1	0.83
Dimorphic	19	15.83
Total	120	100

In this study, Microcytic Hypochromic anemia constituted the predominant type with 79 cases (65.83%) followed by Normocytic Normochromic anemia (17.5%) and 15.83% of Dimorphic anemia. Only 1 case (0.83%) of Macrocytic anemia was encountered in the study with no cases of hemolytic anemia (Table 4).

**Table 5.** Histogram pattern observed in the study

Histogram Pattern	Frequency	Percentage
Normal curve	24	20
Left shift	83	69.16
Right shift	1	0.83
Bimodal curve	2	1.66
Broad base curve	10	8.33
Total	120	100

The histogram pattern of all cases were analysed. Left shift was the most common pattern observed constituting 83 (69.16%) cases (Table 5).

#### 4. Discussion

In our study female preponderance comparable to the studies done earlier. In our study it was seen that out of 120 cases the majority of cases fall in the adult age group of 21 to 49 years. This results were in concordance with the studies conducted by Singh T et al.[9], Kumar et al. [10] and Cook et al. [11] This can be explained as the period of adolescence and adult group is a period of intense growth and development and iron is in high demand as it is present in all body cells and is fundamental for basic physiological processes such as hemoglobin formation. The body needs more iron when it grows rapidly and when frequent blood loss occurs (menstruation). Thus, women in the reproductive age group are at high risk of developing iron deficiency anemia. After the age of 40 years males were seen to be more affected than females.

In this study the mean hemoglobin in the study was 10.02 gm%. Majority (60%) of cases had hemoglobin 7-10gm/dl. This was comparable to studies done by Patel et al. [12] In our study the most common morphological type of anemia in the study was Microcytic hypochromic anemia (65.83%) followed by Normocytic normochromic anemia (17.5%). This is in concordance with various other studies. Iron deficiency anemia is the most common cause of microcytic hypochromic blood picture. WHO has estimated that prevalence of anemia in pregnant women is 14 per cent in developed countries and 51 per cent in developing countries and 65-75 percent in India. About one third of the global population (over 2 billion) are anaemic. [13] Prevalence of anemia in all the groups is higher in India as compared to other developing countries. [14]

In the present study 120 patients of anemia were analysed and their peripheral smear report was compared with the Red blood cell histogram pattern obtained from a 5 part differential automated analyser. In view of maximizing the usefulness of the histogram a dotted line depicting a reference normal curve was drawn super imposed on every red cell histogram so that any discernible deviation from that curve can be clearly delineated for contrast. We noticed that in smears reported as microcytic hypochromic anemia 69.16% histograms showed left shift, 17.5% of nomocytic normochromic smears showed normal curve and all the smears having macrocytic blood picture showed right shift pattern of histogram. Thus, we can see that histogram are useful diagnostic aid when it comes to normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic anemia However the dimorphic anemia showed different histogram patterns from simple curve to complex curves.

In the smears reported as dimorphic anemia we noticed that only 1.6% of histograms showed bimodal curve, whereas majority 8.3% showed broad base histogram pattern and 69.16% showed left shift histogram curve. The broad base curve can be explained by the presence of multiple populations of cells of varying sizes (i.e. normocytic, microcytic and macrocytic). Our study was in concordance with the study conducted by Constantino et al. [15] Using Fisher Exact test and comparing the two variables i.e peripheral blood smear reports with histogram patterns the p values showed very high significant difference between the two variables. This difference was largely due to dimorphic anemia cases which was in concordance with Yogender

P et al. [16] In Dimorphic anemia the histogram pattern, the centeredness and the width shows the variations in the RBCs.

The dimorphic blood picture will look like a dual population of microcytic and normocytic or normocytic and macrocytic red cells or a admixture of small, normal and large cells of different sizes and forms with or without normal red blood cell indices which can mislead the diagnosis if we rely on automated values alone. Thus it is important to examine the peripheral blood smear to examine all the populations of the cells. Practically since dimorphic anemia is usually associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results

## 5. Conclusion

Histogram is an important tool of diagnosis when correct interpretation of curve is combined with findings of blood count parameters like red cell distribution width and red cell indices. By observing these curves we could give presumptive diagnosis of presence of fragments in blood, microcytic, macrocytic or dimorphic red cells. Histograms along with Blood indices and hemoglobin value will guide us about RBC morphology. Histograms are useful tool for technologists as it could guide them that which cases need actual detailed peripheral smear examination by experts. Microscopic examination of peripheral blood smear still remains gold standard for diagnosis of various hematological conditions.

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**Conflicts of Interest:** Write conflict of interests or write "The authors declare that they do not have any conflict of interests."

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