



## COMPARATIVE STUDY OF AUTOMATED CELL COUNTER HISTOGRAM AND PERIPHERAL BLOOD SMEAR IN DIAGNOSIS OF ANEMIA

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### ABSTRACT

**Introduction:** Over the past few years, complete blood count, RBC histogram and peripheral blood smear have become the important diagnostic tools to diagnose various haematological conditions. Major public health burden worldwide is anemia with high prevalence in developing countries like India. Red blood cell and histogram are indispensable for diagnosis and management of anemia. The major diagnostic tool for work up of most commercial laboratories has been analysing the blood film routinely. **AIM:** The aim of the study is to compare between automated cell counter histogram and peripheral smear finding in diagnosis of anemia. **Material and method:** A prospective comparative study of RBC histogram and peripheral blood smear in diagnosis of anemia was done on 100 patients of HB<14gm%, over six month time span (June2022-Nov2022) in the central laboratory of Saraswathi Institute of Medical Sciences Hapur, (UP) India. This study included all the age groups. All cases of anemia that have undergone blood transfusion is excluded from this study. **Result:** In our study it was observed that on peripheral blood smear, the most common type of anemia was microcytic hypochromic anemia followed by normocytic normochromic anemia, when we compared with automated cell counter generated histogram most common type of anemia was normocytic normochromic followed by microcytic hypochromic anemia. In our study female population were more than males. The mean age was (32.4yr). **Conclusion:** Automated cell counters generated CBC histograms and peripheral blood smears plays a major role in diagnosis and management of red cell disorder. Our study observed that histogram patterns and their confirmation by peripheral blood smear along with clinical history gives accurate and confirmed diagnosis of various haematological conditions. There is much improvement in accuracy and precision reducing the subjective errors<sup>3</sup>.

**KEYWORDS :** RBC histogram, Peripheral blood smear, Anemia.

### INTRODUCTION

The most commonly ordered blood test in routine practice is CBC/hemogram. Peripheral blood smear is an important part of investigation for various disorders in hematology. Traditional manual methods of measuring hematological parameters have been replaced by automated hematology analyzer. Histogram provided by automated cell counter gives an important clue regarding particle size and volume. Major public health burden world wide is anemia with high prevalence in developing countries like India. Red blood cell and histogram are indispensable for diagnosis and management of anemia. The major diagnostic tool for work up of most commercial laboratories has been analysing the blood films routinely. RBC histogram along with other parameter like RDW, MCV are crucial for diagnosis of RBC disorders.<sup>2</sup> With the use of automated hematology analyzer, there is much improvement in accuracy and precision reducing the subjective errors. Microscopic examination by the pathologist is crucial for primary calibration of the cell counter and to rule out other hematological disorder such as leukemia and leukemoid reaction. Over the past few years complete blood count by the automated hematology analyzer and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample. The present study will compare anemia cases based on peripheral blood smear and cell count generated RBC indices with histogram.<sup>4</sup>

### AIM

To study the comparison of automated cell counter histogram and peripheral blood smear in diagnosis of type of anemia.

### OBJECTIVES

1. Interpretation of histogram in normal persons and patients with different types of anemia.
2. To correlate the findings of peripheral smear examination with histogram.
3. To diagnose the type of anemia by cell counter generated parameters and peripheral blood smear.

### MATERIALS AND METHODS

Source of Data: The present prospective study was undertaken in the Central laboratory, Department of Pathology, Saraswathi Institute of Medical Sciences. A total of 100 patients were studied over a period of six months (June2022-November2022).

Method of collection: 3 ml of blood sample was collected with aseptic precautions by venipuncture in K3 EDTA (Tri-potassium Ethylenediamine tetra-acetic acid) anticoagulant vacutainer. Hematological parameters were obtained by using 5 part automated hematology cell counter ERBA H 560. The peripheral blood smears were made with leishman stain. Anemia typing was first done using automated cell counter generated RBC parameters such as hemoglobin, RBC indices, reticulocyte count and RDW. Then, PBS examined under light microscope.

### Inclusion Criteria

1. All patients with hemoglobin percentage less than 14gm%.
2. Patients of all age groups.

### Exclusion criteria

1. All cases of anemia that have undergone blood transfusion.
2. Inadequate quantity of blood sample for automated analyzer and PBS preparation (<3ml)

**Statistical Analysis:** Data was entered in Microsoft excel and statistical analysis between various parameters was done, using statistical IBM software statistical package of social science (SPSS) for windows version 25.0. Different statistical tools were used for analysis like mean, and different statistical tests like chi-square test, Fisher's exact test and correlation coefficient were used.

### OBSERVATION AND RESULTS

**Age:** This study included all the age groups. Majority of patients (28%) were in 20-30 years of age (Table1). Mean age was (32.3yr).

**Gender Distribution:** This study showed majority of females (51%) (Fig1)

**Age and Gender wise Distribution of study subjects:** It was observed that females who were in reproductive age group affected more and males were affected more after 20 years of age.

**Classification of anemia based on Hb value:** Based on haemoglobin value anemia was divided into mild (Hb<12gm%) moderate (Hb7-10gm%) and severe (Hb<7gm%). Out of 100 cases majority showed moderate anemia (38%) (table2).

Anemia based on histogram pattern: The histogram pattern for all cases were analysed. The most common pattern observed was normal bell shaped curve followed by left shift, bimodal pattern and right shift. Cases of anemia based on peripheral blood smear examination: In peripheral blood smears microcytic hypochromic anemia was the predominant type with 37 cases followed by normocytic normochromic anemia with 36 cases and 20 cases of dimorphic anemia. Only 7 cases of macrocytic anemia observed in the study and there was no case of haemolytic anemia. In our study P value(0.926) showed no significant difference. (table4)

**Comparative study between peripheral blood smear and histogram:**

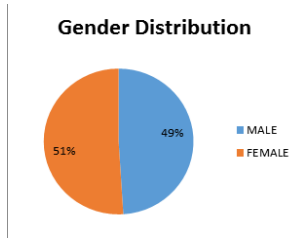
In our study when we compared peripheral blood smear with histogram patterns, we observed that, in microcytic hypochromic anemia most common pattern of histogram was left shift and few cases showed normal curve, while in case of normocytic normochromic anemia most common histogram pattern was normal bell shaped curve. In macrocytic anemia most common pattern was shift to right in majority of cases. In case of dimorphic anemia most common pattern observed was bimodal pattern with few case of left shift and right shift.

**TABLE 1 Age Distribution Total Cases (n= 100)**

AGE	COUNT	PERCENTAGE %
0 - 10	8	8
10 -20	13	13
20 - 30	28	28
30 - 40	19	19
40 - 50	13	13
50 - 60	12	12
60 - 70	5	5
70 - 80	1	1
80 - 90	1	1

**TABLE 2 ANEMIA BASED ON HB LEVEL (n=100)**

ANEMIA BASED ON HB LEVEL	GENDER		Total(
	FEMALE	MALE	
Normal(>12 m%)	15	14	29(%)
Mild(<12gm%)	1	3	4(%)
Moderate(710gm%)	20	18	38(%)
Severe(<7gm%)	15	14	29(%)

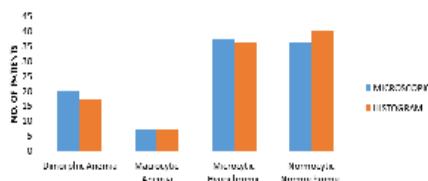


**TABLE 4 COMPARISON BETWEEN HISTOGRAM AND MICROSCOPIC ANALYSIS**

	Microcytic Hypochromic	Normocytic Normochromic	Dimorphic Anemia	Macrocytic Anemia	TOTAL
MICROSCOPIC	37	36	20	7	100
HISTOGRAM	36	40	17	7	100

Chi -square test  $X^2_{cal} = 0.4675$ , P value = 0.926 NOT SIGNIFICANT

**Graph 1 COMPARISON BETWEEN HISTOGRAM AND MICROSCOPIC ANALYSIS**



**TABLE 5 Histogram pattern in different type of anemia**

HISTOGRAM	Bimodal Curve	Left shift	Normal curve	Right shift	Total
Normocytic Normochromic	1	2	36	1	40
Microcytic Hypochromic	0	32	4	0	36
Dimorphic Anemia	15	1	1	0	17
Macrocytic Anemia	0	1	0	6	7
TOTAL	16	36	41	7	100

**TABLE 6 Comparison of histogram in various studies**

HISTOGRAM	Present study 2022	Rahul et al <sup>8</sup> 2020	Shrivastava et al <sup>11</sup> 2019	Rao Bss et al <sup>13</sup> 2017	Chavda J et al <sup>10</sup> 2014-15	Sandhya et al <sup>9</sup> 2014
NORMAL CURVE	41%	16%	18%	17.7%	19%	15%
LEFT SHIFT	36%	33%	29%	29.0%	27%	30%
RIGHT SHIFT	7%	8%	6%	5.45%	7%	6%
BIMODAL	16%	2%	5%	7.27%	3%	4%

**Discussion:**

In the world, Anemia is the most common health problem among people, so adequate diagnosis is required for treatment of anemia. Now a days automated cell counter generated histograms are popularly used world wide but the importance of peripheral blood smear cannot be neglected so automated cell counter and peripheral blood smear plays a important role in diagnosis of various type of haematological conditions. Our study which was conducted on 100 patients (Hb<14gm%) in a time span of six months(June2022- November2022) showed concordance with the studies conducted by Chanchal et al<sup>5</sup> and Arvind P et al<sup>6</sup> regarding distribution of sex where female preponderance was present. In our study 51% were females and 49% were males population, In our study it was observed that on the basis of haemoglobin value majority of patient showed moderate anemia. In previous study of Bhargava et al<sup>7</sup>, done on 400 patients observed that most common type of anemia in females is microcytic hypochromic. In our study it was observed that, on histogram normocytic normochromic anemia was the most common type followed by microcytic hypochromic anemia, dimorphic anemia and macrocytic anemia. There is no case of haemolytic anemia, when we compared it with peripheral blood smear, the most common type of anemia was microcytic hypochromic followed by normocytic normochromic. It shows that automated cell counter generated histograms finding and their correlation with PBS is very necessary for correct diagnosis, It shows that importance of PBS cannot be ignored. Out of 100 cases of anemia 40% cases showed normocytic normochromic anemia in which 36 cases showed normal curve and two cases showed left shift. The second most common type of anemia was microcytic hypochromic anemia with 36% cases out of which 32 cases showed left shift. Dimorphic anemia in which 15 cases showed bimodal pattern on histogram. Out of 7 cases of macrocytic anemia 6 showed right shift on histogram. Dimorphic anemia shows various patterns on histograms as dual population of RBC either microcytic and normochromic or normochromic and macrocytic red blood cells so it is very important to examine the peripheral blood smear for all various type of red blood cells, It was indicated in 17 cases on histogram and one each showed left shift and normal curve pattern. Previous studies showed in the (table 6) that most common type of anemia was microcytic hypochromic anemia followed by normocytic normochromic anemia on histograms. On the other hand when we evaluate these histogram findings with peripheral blood smear we observed that most common type of anemia on microscopic examination was microcytic hypochromic with 37% cases followed by normocytic normochromic with 36% cases (Table 4), which gives us very important clue that Peripheral blood smear evaluation under microscope is always gold standard for correct diagnosis and management for haematological conditions. There are wide reasons for dimorphic blood picture including nutritional anemia, recent blood transfusion or therapy response to nutritional anemia and sideroblastic anemia.<sup>12</sup> In previous studies and our study also women were mainly affected with microcytic hypochromic anemia in their reproductive age. Female are most affected in pregnant state due to more demand of iron in pregnant and in non pregnant women due to blood loss during menstruation.

**CONCLUSION:**

Automated cell counter generated histogram provide a valuable information about various condition in hematology like anemia. Histogram is a early indicator of almost all types of anemia like microcytic hypochromic, normocytic normochromic and macrocytic anemia. Our study observed a significant relation between peripheral blood smear and RBC histogram in the diagnosis of normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic anemia. The variation seen in dimorphic anemia where various patterns of histogram were present, so the peripheral blood smear has become the main diagnostic tool in case of dimorphic anemia. The RBC histogram is essential part of automated hematology analyzer and now routinely available on all automated cell counters. It provides a very important clue in hematology conditions. Although peripheral blood smear is the gold standard for diagnosis of anemia but histogram provided by automated analyzer gives an important and early clue to diagnosis so that early diagnosis and early management can be done.

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