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Haematology

# COMPARATIVE STUDY OF AUTOMATED CELL COUNTER HISTOGRAM AND PERIPHERAL BLOOD SMEAR IN DIAGNOSIS OF ANAEMIA AT TERTIARY CARE HOSPITAL

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**ABSTRACT** **Introduction:** In recent years, complete blood count, red blood cell histogram and peripheral blood smear have become important diagnostic tools for diagnosing various blood diseases. Anemia is a major public health problem worldwide, with high prevalence in developing countries like India1. Red blood cell count and histograms are essential for the diagnosis and management of anemia2. The most important diagnostic tool for testing in most commercial laboratories is the periodic analysis of blood films. AIM: the aim of this study was to compare the role of automated cell count histograms and peripheral blood smear results in the diagnosis of anemia.. Material and method:A prospective comparative study of RBC histogram and peripheral blood smear in diagnosis of anemia was done on 900 patients of HB<14gm%, over two month time span(September 2022-October 2022) in the central laboratory lab, Department of pathology, Gajra Raja Medical College, Gwalior, Madhya Pradesh. All cases of anemia who received blood transfusions were excluded from this study. Result:It was observed in our study that on peripheral blood smears, the most common type of anemia was microcytic hypochromic anemia, followed by normochromic normocytic anemias. When we compared with the histogram generated by the automated cell counter, the most common type of anemia was hypochromic microcytic anemia, followed by normochromic normocytic anemia. In our study, the female population was more numerous than the males. The average age is (23 years old). Conclusion:CBC histograms and peripheral blood smears generated by automated cell counters play an important role in the diagnosis and management of red blood cell disorders. Our study observed that the histogram pattern and its confirmation by the peripheral blood smear along with the clinical medical history allow an accurate and confirmed diagnosis of various blood disorders. Accuracy and precision have been greatly improved resulting in reducing the subjective error3 .

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

## INTRODUCTION

Anemia is a common condition characterized by a decrease in the number of red blood cells (RBCs) or the amount of hemoglobin in the blood, leading to a decrease in the oxygen-carrying capacity of the blood. Anemia can have various causes, including nutritional deficiencies, chronic diseases, genetic disorders, and medications.

The diagnosis of anemia involves a comprehensive evaluation of the patient's clinical history, physical examination, and laboratory tests. One of the essential laboratory tests for the diagnosis and management of anemia is a complete blood count (CBC) performed on an automated hematology analyzer.

Automated hematology analyzers are sophisticated instruments that use advanced technology to provide accurate and rapid analysis of blood cells' morphology and count. These instruments can analyze various blood components, including RBCs, white blood cells, platelets, and hemoglobin. The automated hematology analyzer provides a detailed report of the patient's blood counts, including the number of RBCs, hemoglobin levels, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), among others.

In addition to automated hematology analyzers, peripheral smear examination is also an essential diagnostic tool for anemia patients. Peripheral smear is a microscopic examination of a blood smear slide that allows the physician to visualize the blood cells' morphology and identify any abnormal cells or cell inclusions. The peripheral smear can provide additional information about the type and severity of anemia and help to guide further diagnostic tests and treatment decisions.

Overall, the use of automated hematology analyzers and peripheral smear examination is crucial in the diagnosis and management of anemia patients, providing valuable information about the patient's blood counts and morphology, and guiding further diagnostic and therapeutic interventions. This study will compare cases of anaemia based on peripheral blood smear and cell count generated RBC indices with histogram.

## AIM:

To study the comparison of automatic cell count histogram and peripheral blood smears in the diagnosis of anemia types.

## OBJECTIVES

1. Interpretation of histograms of normal subjects and patients with different types of anaemia.
2.Correlation of peripheral smear results with histogram.
3.The type of anaemia was diagnosed 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 lab, Department of Pathology, Gajra Raja Medical College, Gwalior. A total of 900 patients were studied over a period of Two months(September2022-October2022).

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. Haematological parameters were obtained by using 3 part automated hematology cell counter hemax 330x. The peripheral blood smears were made with leishman stain. Anaemia typing is first performed using automated cell counter generated RBC parameters such as haemoglobin, RBC indices and RDW. Then, PBS examined it under a light microscope.

### Inclusion Criteria

1. All patients with a haemoglobin percentage below 14gm%.
2. Patients of all ages.

### Exclusion criteria

1. All cases of anaemia who received blood transfusion.
2. Insufficient 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 coefcient were used.

## OBSERVATION AND RESULTS

Age: This study included all the age groups. Majority of patients (45%) were in 20-30 years of age (Table1).Mean age was (23yr).

Gender Distribution: This study showed majority of females (55%) (Fig1)

Distribution of study subjects by Age and Gender wise: It has been observed that women of childbearing age are more affected and men more affected after 20 years.

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 900 cases majority showed moderate anemia(42%) (table2).

Anemia based on histogram pattern: The histogram pattern for all cases were analysed. The most common pattern observed was left shift curve followed by normal bell shaped, right shift and bimodal pattern.
Cases of anemia based on peripheral blood smear examination: In peripheral blood smears microcytic hypochromic anemia was the predominant type with 371 cases followed by normocytic normochromic anemia with 285 cases and 68 cases of dimorphic anemia . 93 cases of macrocytic anemia observed in the study and there was no case of haemolytic anemia.

Comparative study between peripheral blood smear and histogram:In our study when we compared peripheral blood smears with histogram patterns ,we noticed that ,In microcytic hypochromic anemia most common pattern of histogram was shift to left and few cases showed normal curve,while in case of normocytic normochromic anemia most common histogram pattern is a 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 a bimodal pattern with few cases of left shift and right shift.

Table 1 Age distribution Total Cases (n=900)

| Age | Count | Percentage |
|---|---|---|
| 0-10 | 135 | 15 |
| 11-20 | 180 | 20 |
| 21-30 | 315 | 35 |
| 31-40 | 198 | 22 |
| 41-50 | 54 | 6 |
| 51-60 | 9 | 1 |
| 61-70 | 9 | 1 |

Table 2 Anemia Based on HB Levels (n=900)

| Anaemia Bases on HB Level | GENDER | | TOTAL |
|---|---|---|---|
| | FEMALE | MALE | |
| Normal (>12mg%) | 105 | 147 | 28% |
| Mild (<12mg%) | 60 | 48 | 12% |
| Moderate (7-10mg%) | 205 | 173 | 42% |
| Severe(<7gm%) | 85 | 77 | 18% |

Table 3 Gender Distribution

| | |
|---|---|
| Male | 45% |
| Female | 55% |

Table 4 Comparision between Histogram and Microscopic Analysis

| | Microcytic hypochromic Anaemia | Normocytic Normochromic Anaemia | Dimorphic Anaemia | Macrocytic Anaemia | Total |
|---|---|---|---|---|---|
| Microscopic | 371 | 285 | 152 | 92 | 900 |
| Histogram | 404 | 335 | 68 | 93 | 900 |

Table 5 Histogram Pattern in Different type of anemia

| Histogram | Bimodal Curve | Left Shift | Normal Curve | Right Shift | Total |
|---|---|---|---|---|---|
| Normocytic Normochromic | 3 | 26 | 315 | 8 | |
| Microcytic Hypochromic | - | 370 | 15 | - | |
| Dimorphic Anemia | 65 | 6 | 3 | - | |
| Macrocytic Anemia | - | 2 | 2 | 85 | |
| Total | 68 | 404 | 335 | 93 | |

Table 6 Comparison of Histogram in Various Studies

| Histogram | Present Study 2022 | Ranul et al 2020[9] | Shrivastav et al 2019[10] | Rao Bss et al 2017[4] | Chawda et al 2014-15[11] | Sandhya et al 2014[6] |
|---|---|---|---|---|---|---|
| Normal Curve | 37.2% | 16% | 18% | 17.7% | 19% | 15% |
| Left Shift | 44.89% | 33% | 29% | 29.0% | 27% | 30% |
| Right Shift | 10.33% | 8% | 8% | 5.45% | 7% | 6% |
| Bimodal | 7.55% | 2% | 5% | 7.27% | 3% | 4% |

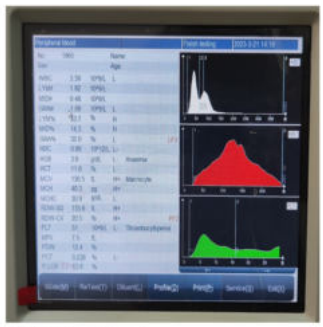

Figure 1: Macrocytosis; Shift to right

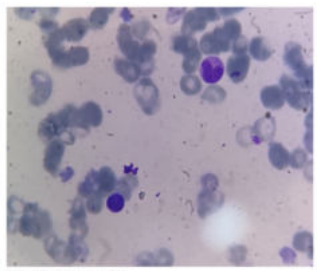

Figure 2: Macrocytic anemia PBS

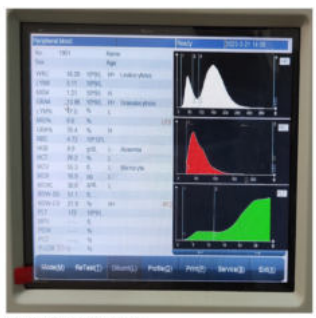

Figure 3: Microcytosis; Shift to left

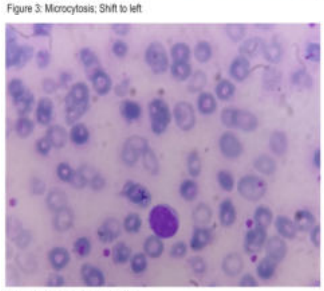

Figure 4: Microcytic anaemia PBS

## Discussion:

In the world, Anemia is the most common health problem among people so adequate diagnosis is required for treatment of anemia .Nowadays, histograms generated by automatic cell counters are widely used in the world, but the importance of peripheral blood smears cannot be ignored, so automatic cell counters and peripheral blood smears play an important role in the diagnosis of various blood

diseases.Our study which was conducted in 900 patients (Hb<14gm%) in a time span of two months(September 2022-October 2022) showed concordance with the studies conducted by chanchal et al5 and Arvind P et al6 regarding distribution of sex where female preponderance was present. In our study 55% were females and 45% 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 al7 ,done in 400 patients observed that most common type of anemia in females is microcytic hypochromic.
In our study it was observed that, on histogram microcytic hypochromic anemia was the most common type followed by normocytic normochromic anemia , macrocytic and dimorphic 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 can not be ignored. Out of 900 cases of anemia 41.22% cases showed microcytic hypochromic anemia in which 371 cases showed left shift.

The second most common type of anemia was normocytic normocrmic anemia with 31.6%% cases out of which 315 cases majority showed normal curve.Out of 93 cases of macrocytic anemia 85 showed right shift on histogram. Dimorphic anemia in which 65 cases showed bimodal pattern 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 68 cases on histogram . 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 41.22% cases followed by normocityc normochromic with 31.67% 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 response to treatment for nutritional anemia and sideroblastic anemia12. In previous studies and our study also women of childbearing age were also predominantly affected by hypochromic microcytic anemia. Women are most affected during pregnancy due to the increased iron needs of pregnant and non-pregnant women due to blood loss during menstruation.

**Conclusion:**

Histograms generated by automated cell counters provide valuable information about various conditions in hematology, such as anemia. Histogram is an early indicator of almost all types of anemia such as microcytic hypochromic, normocytic normochromic and macrocytic anemia. Our study observed a significant association between peripheral blood smear and red blood cell histogram in the diagnosis of normochromic normocytic anemia, microcytic hypochromic anemia and macrocytic anemia. The most important diagnostic tool for dimorphic anemia. Red blood cell histograms are an essential component of automated hematology analyzers and are now routinely available on all automated cell counters.

This provides very important clues to hematological status. Although the peripheral blood smear is the gold standard for diagnosing anemia, the histogram provided by the automated analyzer provides important clues for early diagnosis, enabling early diagnosis and early management.

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