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Original Research Article

RBC Histograms and their correlation with Peripheral smear examination in anaemic patients

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Abstract

Introduction: Automated haematology analysers provide the histograms of different blood cells. The interpretation of histograms need careful analysis of all the parameters including red blood cells (RBC), white blood cells (WBC) and platelet distribution curves. This analysis is often neglected and underrated part of the hemogram generated by the analyser. If interpreted properly it has the potential to provide valuable diagnostic information even before a higher level investigation is ordered. This is certainly a time saving tool. This study was conducted to study the correlation between RBC histogram charts plotted by the automated haematology analyser and the peripheral smear findings in patients with anaemia in our tertiary care hospital, and how close are these findings with the gold standard of investigation that is microscopy.

Material and method: 500 sample of patients of adult age group admitted in Chirayu Medical College, Bhopal were studied from March 2023 to August 2023. Patients with haemoglobin less than 12.5gm/ dl were included. The blood samples were run in Mindray BC 6000 plus haematology analyser, which is a 5 part differential counter. The histogram is obtained on the display of the cell counter. Peripheral smears were prepared from same samples of patients received in EDTA vacutainer and stained by Leishman stain. Then microscopy of stained Peripheral smears were done. The peripheral smear findings were then correlated with the histogram charts from cell counter with due knowledge of relevant clinical history.

Result: In our study of 500 patients, the histogram suggested 52.2% of cases as microcytic hypochromic anaemia, followed by normocytic normochromic anaemia 19.6%. The histogram suggested 12.6% of patients and 2.2 % of patients suffering from macrocytic anaemia and haemolytic anaemia respectively. In 13.4 % of cases histogram showed dimorphic picture. On confirming with Peripheral Smear finding we found that 53.4% of cases showed picture of microcytic hypochromic anaemia, followed by normocytic normochromic anaemia at 18.8%. The Peripheral Smear finding suggested 11.6% of patients and 3.6 % of patients suffering from macrocytic anaemia and haemolytic anaemia respectively. In 12.6 % of cases Peripheral smear showed dimorphic picture.

Conclusion: The peripheral blood smear examination along with Histogram analysis can prove a very promising and complementary tool in diagnosis of anemias. Histogram can provide subtle information about the RBC pathology and many a times can act as time saving method.

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Keywords: Automated haematological analysers, Histogram, Anemia, Peripheral Smear

1. Introduction

The complete blood count (CBC) which includes the haemoglobin (Hb), the RBC indices, total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count are the backbone of laboratory evaluation. It provides valuable information in typing anaemia, leukaemia and providing help in diagnosing various disorders. (1)

In the current era fully automated haematology analysers provide additional information in the form of histograms. Histograms are charts which present data on bigger number of variables and hence provide us with extra information in fully automated analysers. MCV and RDW are significant indices for morphology interpretation among others. (2,3)

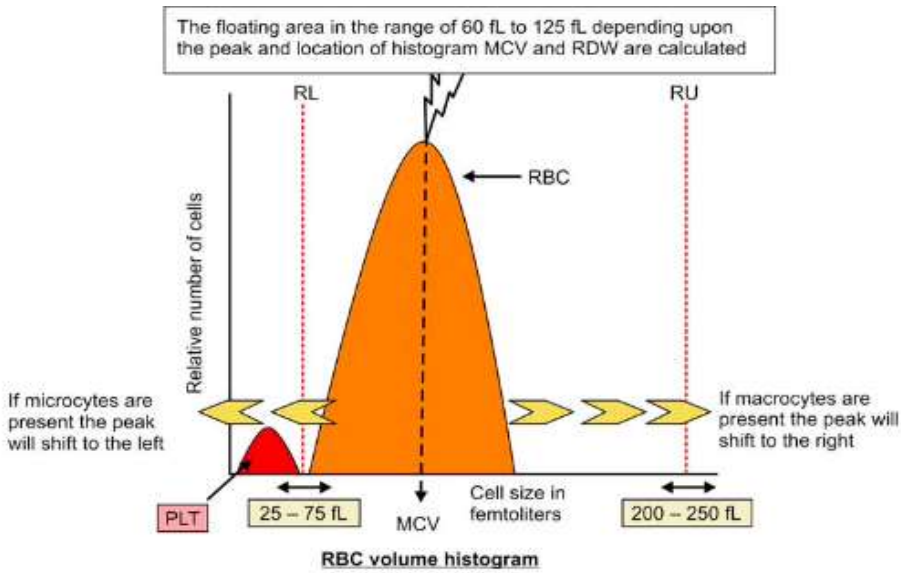
The curve of RDW is bell shaped and peaks within 80 to 100 fl. The RBC histogram in the analyzer displays RBCs in the range between 30 to 300 fl. (4)

Principle:

The Mindray BC6000 plus haematology analyser is based on Coulters principle which was introduced by Wallace Coulter in 1956. These analysers are based on impedance principle and relies on the change in conductance as each cell passes through an aperture. This change in conductance causes an electrical pulse and it's amplitude is proportional to the cell volume. These results are displayed in the form of histograms. RBC histogram provides a graphical representation of cell frequencies versus cell size. (1)

The shift of this graph in one direction or the other is related to CBC parameters such as RDW, MCV, MCH, MCHC and helps in diagnosing various haematological conditions.

In the normal RBC curve, MCV falls between 80-100 fl. The analyser counts all the particles between the size of 36-300fl as RBCs and the graph begins at baseline. If the graph begins above the baseline it indicates the presence of small particles like platelet clumps, malaria parasite, microspherocytes, bacteria, elliptocytes and normoblasts.(5,6)



RBC volume histogram (7)

2. Material and Method

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The study was conducted at Chirayu Medical College and Hospital, Bhopal on 500 patients.
The samples were received in EDTA vacutainers. These were then run in Mindray BC 6000 plus hematology analyzer.
The EDTA samples were used to prepare peripheral smear by staining with Leishman stain. After staining microscopy was done and the findings were compared to the histograms displaed on the Mindray BC 6000 plus hematology analyzer. The shape and the deviation of the RBC curves were recorded. The anemia was categorized using the RBC indices as normocytic normochromic (MCV between 80-100 fl), microcytic hypochromic (MCV <80 fl) and macrocytic normochromic (MCV > 100fl).
These RBC indices were also studied along with the shape of these graphs. Normal gaussian bell shaped curve was considered as normocytic normochromic anemia, left shift as microcytic hypochromic anemia and right shift as macrocytic normochromic anemia. A bimodal peak was categorized as dimorphic anemia and broad base with left shift was considered as hemolytic anemia.

Inclusion Criteria

1. All Blood Sample from patients with age of 15yr and above will be included.

Exclusion Criteria

1. All cases that have undergone blood transfusion will be excluded from the study
2. Inadequate quantity of blood sample for automated analyzer (less than 3ml) will be excluded.

Ethical Considerations: All procedures were in accordance with the ethical standards of the institution.

Observation and Result

The RBC indices, histograms and peripheral smear of 500 patients were analysed. Only pateints above 15 years were included in the study. Out of 500 patients 54.6% were females and 45.4% were males.

Table 1: DISTRIBUTION OF ANEMIA CASES ON RED CELL INDICES AND HISTOGRAMS

DIAGNOSIS	NO OF CASES (N=500)	PERCENTAGE
Normocytic Normochromic anemia	98	19.6 %
Microcytic hypochromic anemia	261	52.2 %
Macrocytic anemia	63	12.6 %
Dimorphic anemia	67	13.4 %
Hemolytic anemia	11	2.2 %

Table 1 shows relative distribution of type of anemias based on RBC indices and Histogram. Out of 500 cases, 98 (19.6%) cases are normocytic normochromic, most common is microcytic hypochromic with 261 cases (52.2%), There are 63 cases (12.6%) of macrocytic

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anemia while 11 cases (2.2%) of hemolytic anemia. Anemia with dimorphic pictures accounted for 67 cases (13.4%).

Table 2: DISTRIBUTION OF ANEMIA CASES ON PERIPHERAL SMEAR EXAMINATION

DIAGNOSIS	NO OF CASES (N=500)	PERCENTAGE
Normocytic Normochromic anemia	94	18.8 %
Microcytic hypochromic anemia	267	53.4 %
Macrocytic anemia	58	11.6 %
Dimorphic anemia	63	12.6 %
Hemolytic anemia	18	3.6 %

Table 2 shows relative distribution of type of anemias based on peripheral blood smear examination. Out of 500 cases, 94 (18.8%) cases are normocytic normochromic, most common are microcytic hypochromic with 267 cases (53.4%), There are 58 cases (11.6%) of macrocytic anemia while 18 cases (3.6%)of hemolytic anemia. Anemia with dimorphic pictures accounted for 63 cases (12.6%).

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 3 shows distribution of RBC histogram curve. Most commonly observed is broad base (34%) followed by left shift (33%). Normal curve observed in 16% of cases. Right shift observed in 8% of cases and bimodal peak in 7 %. Cases.

Microcytic Hypochromic Anemia: It was the most observed common type of anemia**.** Most common histogram pattern seen in microcytic hypochromic was shift to left with broad base. Few cases showed bimodal pattern.

Normocytic Normochromic Anemia: Most common histogram pattern seen in normocytic normochromic anemia was bell shaped curve in majority of cases.

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Macrocytic Anemia: Histogram pattern showed shift to right in majority of cases. Also seen was bimodal pattern. Very few cases showed broad base curve.
Dimorphic Anemia: A bimodal peak with a broad base was seen in all the cases. In addition to the bimodal pattern, few cases showed right shift with skewing of the histogram to the left indicative of combined nutritional deficiency (macrocytes with few microcytes). Few cases showed a pure bimodal pattern indicative of anemia treated with hematinics transfusion.
Hemolytic Anemia: The histogram patterns seen in hemolytic anemia was shift to left with broad base with a few cases showed bimodal pattern and shift to right.

3. Discussion

Results of present study are compared with other studies. They are as follows:

Table 4: Comparison of Histogram Shape in Various Studies

HISTOGRAM	Sandhya et al (8)	Chavda et al (9)	Rao BSS et al (10)	Shrivastav et al (11)	PRESENT STUDY
Normal curve	15%	19%	17.7%	18%	16%
Left shift	30%	27%	29%	33%	33%
Right shift	6%	7%	5.45%	8%	8%
Broad base	40%	38%	37.72%	34%	34%
Short peak	4%	3%	7.27%	2%	2%
Bimodal peak	5%	6%	2.7%	7%	7%

It has been already stated in various literatures and research articles that RBC Histogram is a graphical representation obtained from automated hematology analyser. In present study of 500 cases, maximum numbers of cases are having Microcytic anemia (94%) followed by normocytic (18%),(7) Dimorphic (12.2%) and Macrocytic (11.6%). Other studies like sandhya *et al*. (8) Chavda J *et al*. (9) & Byna Syam Sundara Rao *et al*.(10) were also found similar findings regarding distribution of anemia cases. Our study of RBC histogram showed normal curve (16%), left shift (33%), right shift (8%) Broad base (34%), short peak (2%) and bimodal (7%) and these findings regarding to RBC histogram were also correlated with other studies like sandhya *et al*. Chavda J *et al.* Rao BSS *et al*. and Shrivastav *et al* (11)
As stated previously that RBC histograms are the graphical representation obtained from the automated haematology analysers.
In our study of the 500 cases, majority of the cases have Microcytic hypochromic anemia (94%), followed by normocytic normochromic anemia (18%), dimorphic anemia (12.2%) and macrocytic anemia (11.6%)

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TABLE 5 - COMPARISION OF PERIPHERAL SMEAR EXAMINATION VS HISTOGRAM ANALYSIS (TOTAL 500 CASES)

TYPE OF ANEMIA	PERIPHERAL SMEAR ANALYSIS (n=500)	HISTOGRAM & RBC INDICES (n=500)
Normocytic Normochromic anemia	94 (18.8%)	98 (19.6%)
Microcytic hypochromic anemia	267 (53.4%)	261 (52.2%)
Macrocytic anemia	58 (11.6%)	63 (12.6%)
Dimorphic anemia	63 (12.6%)	67 (13.4%)
Hemolytic anemia	18 (3.6%)	11 (2.2%)

Table 5 shows importance of histogram and red cell indices in diagnosis of anemia and how close they are to the actual diagnosis made after peripheral smear examination. It is comparable to study done by Kumar et al (12).

The mild difference in the analysis of microcytic anemias by peripheral smear examination and by RBC indices/histogram can be explained by the presence of giant platelets and platelet clumps, fragmented RBCs in hemolytic diseases, when the autoanalyser considers it as microcyte. So peripheral smear rules out these errors. This study was in concordance with study done by Poonam et al (12). Homogenous RBC population gives narrow distribution curve and a broad base curve usually denotes presence of more anisocytosis, which can be cross checked by microscopic examination. Shift of RBC histogram graph depends upon on the size/volume of RBC, if cell size is more than normal (macrocytic RBC) shift is toward right and when size of cell is less than normal (microcytic RBC) shift is toward left. Broad base curve is obtained because of high RDW which suggest presence of anisocytosis.

4. Conclusion

To paraphrase an adage, ‘’one histogram graph is worth 1000 numbers. A large collection of data, displayed as a visual image, can convey information with far more impact than the numbers alone. In hematology, these data take on several forms, one of which is the RBC histogram’’. RBC 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 Hb value will guide us about RBC morphology. Histograms are useful tool for technologists it could guide them that which cases need actual detailed peripheral smear examination by experts. In our study findings of automated analyzer was very well correlated with the microscopic examination. Histogram alone could be used as screening method and when combined with PBS findings, they act as useful supplement and by correlating findings of both methods we could diagnose majority of anemia. Alternatively the peripheral smear examination can also be used as one of the quality control parameter for automatic analysers.

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