ORIGINAL RESEARCH

Utility of RBC Histogram Interpretations in Assessing Red Blood Cell Disorders

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ABSTRACT

Aim: To analyse the utility of RBC histogram interpretations in assessing red blood cell disorders. **Material and Methods**: A cross-sectional study to evaluate the utility of automated complete blood count parameters in various haematological disorders was undertaken. Red blood cell indices like MCV, MCH, MCHC, RDW-SD and RBC histogram interpretations were helpful in making the diagnosis on peripheral blood smear examination. The study consisted of 500 cases which were examined using histogram and peripheral smear examination. **Results**: The predominant age group affected was 20–30 years. Peripheral smear finding shows normocytic normochromic anemia was seen in 47.2% cases, microcytic hypochromic anemia seen in 40% cases, macrocytic anemia seen in 10.8% cases and dimorphic anemia seen in 2% cases. 52.8% of cases had normal shift in histogram curves. This peripheral smear finding shows well correlation with findings of histogram pattern and RBC indices. **Conclusion**: Histogram guides a technologist about the cases that need actual detailed peripheral smear examination by experts. It could be used as screening method and when combined with peripheral blood smear findings, they act as useful supplement and by correlating findings of both methods, we could diagnose majority of anemia.

Key Words: Histograms, anaemias, automated haematology analyser.

INTRODUCTION

Three or four decades ago, many procedures performed in the haematology laboratory were manual and time consuming. At that time reagents were prepared in the laboratory, for that qualified worker were required in bulk. Even haemoglobin measurement was done manually by the cyanmethemoglobin method which was labour intensive. In the Modern era the automated haematology analyser has replaced the traditional manual methods and made it simpler for pathologists to diagnose blood cell disorders in haematological reporting. The Automated haematology analyser produces histogram interpretations in the form of graphical images which provide enough information in making diagnosis prior to advance level of investigation[1].

Red blood cell histogram is the essential part of automated haematology analyser which also interprets the other red blood cell indices like MCV, MCH, MCHC and RDW. These RBC indices associated with the histogram interpretations provide significant information which help in diagnosis and management of various types of red blood cell disorders. Red cell distribution curve is a symmetrical bell-shaped curve. The peak area of the curve measures volume of cells ranging from 60 fL to 125 fL, this area reveals mean corpuscular volume and red cell distribution width.

Any small change in the red cell population causes notable variation in the red cell distribution curve. Narrow red cell distribution curve represents the homogenous red blood cells population and the broader curve indicates a heterogenous population of red cells. The variation in the size of red blood cells can shift the curve to the left or right side. In case of microcytic anaemia where the size of RBC is smaller than normal will shift the curve towards the left and in macrocytic anaemia where RBC size is larger than normal will shift the curve to right side.

Histogram also shows bimodal curves in patients with hemolytic anaemia, prior blood transfusion which may be associated with the different size and shapes of red blood cells. Interpretation of RBC histogram associated with major RBC indices like MCV, MCH, MCHC and RDW helps clinicians and pathologists in making accurate diagnosis of different types of red

blood cell disorders and also evade other unnecessary and expensive investigations[2].Evaluation of these values diagnose the different categories of anemia and other red cell disorders.

Histogram correlation with peripheral blood smear could be used as a screening method in haematological reporting. Pathologist can get an idea about the morphology of cells by understanding the graphical images of histogram before focusing the cells under a microscope. Modern analysers can automatically run and report large numbers of blood samples within a fraction of time.

They are enumerated and displayed in the histogram area between the 24 fL and 36 fL range, however, allowing the lower end of the histogram to be monitored. Normally, the space below 36 fL remains clear, but in certain conditions the histogram may begin above the baseline or show a high takeoff on the far left of the curve, which generally indicates the presence of small particles. These particles include red cell fragments, microspherocytes, nucleated RBCs, nonlyzed RBCs, elliptocytosis, macrothrombocytes, platelet clumps, bacteria, parasitic organisms, and other interfering substances such as cryoglobulin, cold agglutinin, and macroglobinemia. The aim and objectives of the study are as follows:

AIM

To Study the Utility of RBC Histogram Interpretations in evaluating the red blood cell disorders.

MATERIALS AND METHODS

The present cross-sectional studywas conducted at the department of Pathology at AIMSR, Bathinda. All the patient's blood sample at random who were attending the Out-Patient Department (OPD) as well as those admitted in hospital and fulfilling the inclusion criteria were taken for the study. Ethical approval from the institutional ethics committee of Adesh University, Bathinda was taken before the start of the study.

INCLUSION CRITERIA

1. Every 10^{th} blood sample coming to the laboratory were taken for study.
2. All anemic patients with hemoglobin percentage less than 11.5 gm% were included.
3. Patients of all age groups were included.

EXCLUSION CRITERIA

1. All patients with hemoglobin percentage more than 11.5 gm% were excluded.
2. Inadequate quantity of blood sample for Automated Haematology analyzer.

MATERIALS

1. Dispovan syringe with needle.
2. EDTA vial for CBC.
3. Mindray BC-5380 or Mindray BC-6800 Haematological automated cell Counter 5 part and 6 part respectively.

METHODS

3ml of the EDTA venous blood sample were collected from the patient and the histogram obtained through mixing of samples. The automated haematology analyser Mindray BC- 5380 or Mindray Bc-6800 5 and 6-part differential automated analyser were used for study. The automated blood cell counting process is very fast and can process up to 60-80 blood samples in an hour. There are three detector blocks in an automated haematology analyser RBCs and platelets are counted in the same block whereas WBCs are counted in a separate block. All the red cells in the blood directed towards the WBC counting block are lysed first using the stromatolyser solution. This solution is composed of an organic quaternary ammonium salt (8.5 g/L) and sodium chloride (0.6 g/L). Cells are counted by passing a dilute solution of the blood through an aperture across which an electrical current is flowing. The passage of cells through the current changes the impedance between the terminals *(Coulter principle*). The sizing and counting of blood cells are based on this measurable change in the electrical impedance[1].

METHOD FOR SAMPLE COLLECTION

For taking blood samples from the patient, SOP (Standard Operational Procedure) was used as below:

1. Venous blood samples were collected from all the subjects in both groups under proper aseptic precautions for complete blood count (RBC indices), Hb levels and Haematocrit estimation.
2. Approximately 3 ml of blood were collected in a vial containing EDTA for CBC (RBC indices).
3. All the samples are maintained at room temperature and tested within 1 hour of collection to minimize variations due to sample ageing.
4. RBC indices were analysed by Mindray BC -5380 or Mindray-BC 6800 Haematological Automated cell counter 5 part and 6 part respectively.

All the data were recorded, documented, and subjected for statistical analysis.

RESULTS

A total of 500 cases were recruited for the present study. There were 252 (50.4%) males and 248 (49.6%) females in sample studied. Majority of the patients belonged to the age group of 21years to 30years (104) followed by >60years (58) with a mean age of 33.34years as shown in table 1.

Table 1: Age & Sex distribution of the sample

Age(years)	Sex		Total	Percent
	Male	Female		
<1	15	2	17	3.4
1-10	39	31	70	14
11-20	27	34	61	12.2
21-30	33	71	104	20.8
31-40	40	35	75	15
41-50	31	32	63	12.6
51-60	27	25	52	10.4
>60	40	18	58	11.6
Total	**252**	**248**	**500**	**100**

Table 2 shows distribution of RBC histogram curve. Most observed is Normal curve observed in 52.8% of cases. Right shift observed in 9% of cases, lest shift observed in 37.4% cases and bimodal peak in 0.8 % cases.

Table 2: Distribution of RBC histogram curves

Type of histogram curves	Percentage
Bimodal curve	.8
Left shift	37.4
Normal	52.8
Right shift	9.0
Total	**100**

Table 3 shows relative distribution of type of anemias based on RBC indices &Histogram and peripheral smear examination. Out of 500 cases, 236 (47.2%) cases are normocytic normochromic, most common is microcytic hypochromic with 200 cases (40%), There are 54 cases (10.8%). Anemia with dimorphic pictures accounted for 10 cases (2%) when seen on RBC indices. When seen by peripheral smear examination it was found that 12 cases (2.4%) were dimorphic, 52 cases (10.4%) had macrocytic anemia, 198 cases (39.6%) had microcytic hypochromic anemia and 238 cases (47.6%) had normocytic normochromic anaemia.

Table 3: Distribution of Anemia cases on the basis of red cell indices and histograms and peripheral smear examination

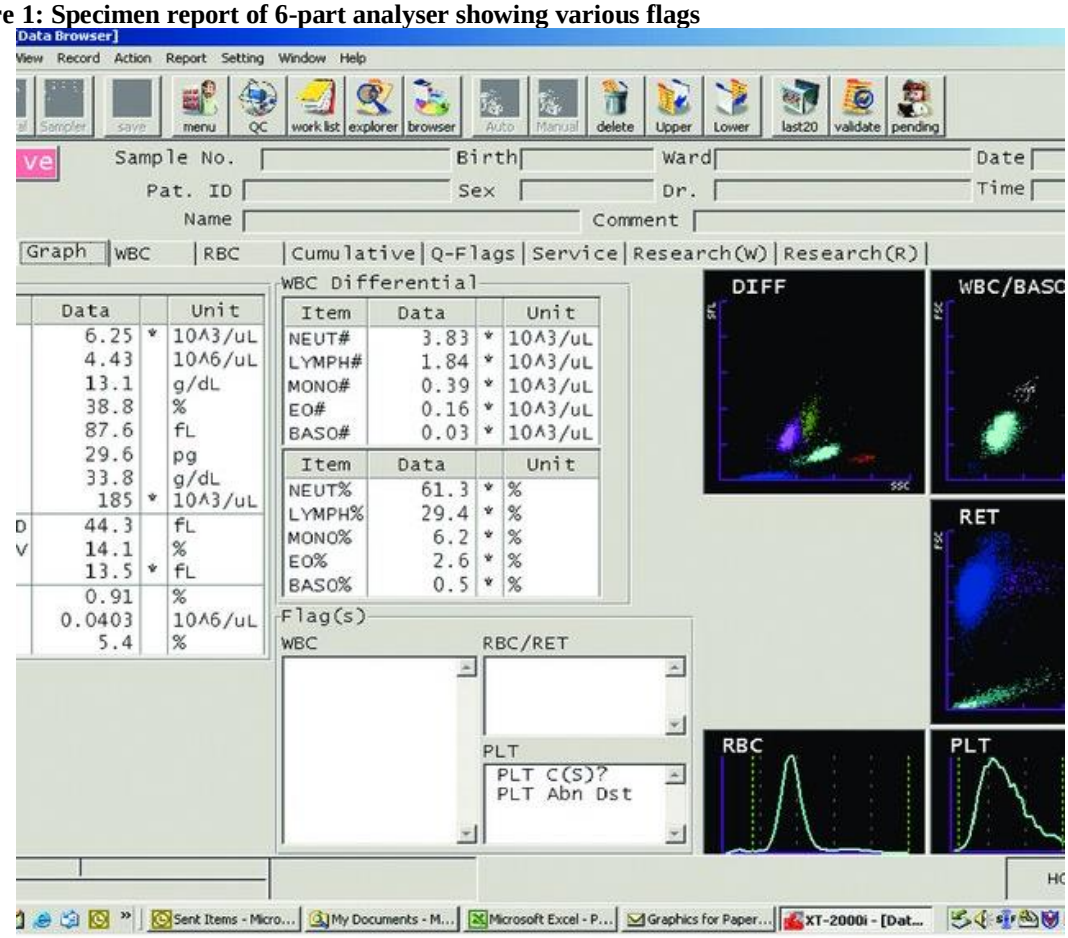
Type of anemia	RBC indices & histograms	Peripheral smear examination
Dimorphic	10 (2)	12 (2.4)
Macrocyctic	54 (10.8)	52 (10.4)
Microcytic hypochromic	200 (40)	198 (39.6)
Normocytic Normochromic	236 (47.2)	238 (47.6)
Total	**500 (100)**	**500 (100)**

RBC histogram variation in different anemias and RBC histogram curves was found in table 4.

Table 4: Shows RBC histogram variation in different anemias

Type of anemia	Right shift (%)	Left shift (%)	Normal (%)	Bimodal (%)
Dimorphic	1	-	0.2	0.8
Macrocyctic	2	3.2	5	-
Microcytic hypochromic	5	21.9	32.3	-
Normocytic Normochromic	1	12.3	18.6	-

Figure 1: Specimen report of 6-part analyser showing various flags



2. Example of positive XT-2000*i* screen print. See Figure 1 legend for abbreviation defi

DISCUSSION

It is already stated in various literatures that RBC Histogram is a graphical representation obtained from automated hematology analyzer. RBC histogram is standard part of complete blood picture which provides clues in diagnosis of various RBC disorders and give valuable information regarding to RBC parameters like RDW, MCH and MCV. Normally curve is symmetrical bell shaped or Gaussian distribution. Normal curve falls within normal range of MCV which is 80-100 fL. The RBC histogram in the hematology analyzer displays the ranges for RBC are between 24fL and 360fL. The analyzer counts only those RBC's with volume sizes between 36fl to 360fl as red cells and cells which are counted in the range 24fl to 36 fl are not included in the RBC count and rejected by counter.

WBC's does not affect the RBC count until their number is increased by more than 50,000.

The presence of right sided curve usually corresponds to reticulocytosis and a flagging of erythrocyte population on the far right of the histogram correlates to red cell agglutination. This study is done to define the utility of histogram with RBC indices in anemia typing and to establish it as a routine diagnostic tool in laboratories.

The total number of cases of anemia in our study was 500. Male predominance was observed in the present study with 252 of the cases which was dissimilar to study done by Singhal et al., (64.9%)[5] and Garg et al., (62.9%)[6].

In the present study, the most affected age group was 21–30 years followed by 31–40 years which was comparable to the results of similar studies done by Korgaonker and Shashidhar[7] Kumar et al.,[8] Cook et al.[9] (Table 5)

Table 5 : Comparison of age group for anemia in previous studies

Age group	Present study (%)	Korgaonker and Shashidhar[7] 2019 (%)
<30	50.4	46.3
31-40	15	14.9
41-50	12.6	14.1
51-60	10.4	9.98

>60	11.6	14.37

Our study of RBC histogram showed normal curve (52.8%), left shift (37.4%), right shift (9%), and bimodal (0.8%) and these findings regarding to RBC histogram were also correlated with other studies such as Sandhya and Muhasin,[10] Rao et al.,[11] and Shrivastav et al.[12]

Table 6 : Comparison of histogram pattern in previous studies

Histogram pattern	Present study (%)	Sandhya and Muhasin 2014 (%)	Chavda et al.,2015 (%)
Normal	52.8	15	19
Left shift	37.4	30	27
Right shift	9	6	7
Bimodal	0.8	4	3
Broad	-	40	38
Short peak	-	5	6

The most common type of anemia observed in our study was normocytic normochromic anemia (combined nutritional deficiency) followed by microcytic hypochromic anemia indicative that the most common anemia observed in our population as comparable to studies done bya Rao et al[11] & Chavda et al[12], which shows normocytic normochromic anemia as the most common distribution pattern.

Table 7 : Types of anemia on peruipheral smear compared to other studies

Anemia	Present study (%)	Rao et al (%)	Chavda et al (%)
Normocytic	47.2	19.54	17.4
Microcytic	40	63.63	65
Macrocytic	10.8	2.2	3.6
Dimorphic	2	12.72	14
Pancytopenia	-	1.8	-

In our study out of 47.2% cases of normocytic normochromic anemia, 18,6% showed normal curve and 12.2% cases showed left shift curve. These finding were correlated with study carried by Chavda J. and Bynasyam Sundara Rao et al. MCV and MCH were decreased in microcytic hypochromic anemia but MCHC was normal. RBC with low MCV had shown shift to left. [11,12]

In hypochromic microcytic anemia as there was micocytosis and RDW was increased. On peripheral smear also, hypochromic microcytic anemia showed anisopoikilocytosis. Out of 40% cases of microcytic hypochromic anemia, 32.3 %cases were normal, 5% were right shift and rest were left shifted curve. These finding were correlated with study carried out by Sandhya [10] and Chavda J. [12] The short peak were well correlated with low haemoglobin and red cell count.

In Dimorphic anemia the histogram may have 2 or more red cell populations, whereas in dual populations, the histogram had 2 distinct red cell populations. In dimorphic blood picture there may be dual population of microcytic & normocytic or normocytic & macrocytic red cells or admixture of small, normal, and large cells of different sizes or admixture of patient and donor red cells. In our study in dimorphic anemia, a MCV, MCH and MCHC were normal and increased RDW due to marked ansiopoikilocytosis was noted. The dimorphic RBC showed bimodal curve (0.8%). These finding were correlated with study carried out by Sandhya[10] and Chavda J[12].

In macrocytic anemia right shift with broad based curve means low Hb and macrocytic blood picture. Macrocytosis could be seen in many conditions so proper approach was required for diagnosis. [11,12] Macrocytosis might occur at any age, but it was more prevalent in old age. [3,13] Increased MCV, RDW, MCH with normal MCHC was seen in macrocytic anemia. 10.8% cases of macrocytic anemia showed right shift curve (2%), left shift curve (3.2%) and normal curve (5%). This finding was correlated with Sandhya et al[10]. Increased MCV and MCH were correlated with right shift curve.

The morphological findings should be correlated with the graphical and numerical data for better interpretation of results. Recent blood transfusion may be a limitation for typing of anemia

CONCLUSION

RBC histogram is an important and extremely time saving tool of diagnosis when correct interpretation of curve is combined with findings of blood count parameters such as red cell distribution width and red cell indices. Histograms guides a technologist about the cases that need actual detailed peripheral smear examination by experts. Present study shows a well correlation in findings of automated hematology analyzer 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.

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