Study of RBC histograms in various anemias: A six months prospective study

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ABSTRACT

Introduction: Complete blood count by automated hematology analyzers and peripheral smear examination complement each other to provide a comprehensive report on patient's blood sample. Data displayed as visual image, can convey information with more impact than numbers. It helps laboratory personnel to diagnose different anemias directly from automated hematology analyzer and correlate with peripheral smear.

Aims & Objectives: To know utility and advantage of red cell histograms and to study automated histogram patterns in various anemias.

Materials and Methods: This is prospective study conducted from January 2017 to June 2017 in Prathima Institute of Medical Sciences on 100 patients of >1year age who were anemic (Hb<12gm%). Complete blood count including hemoglobin, RBC indices, haematocrit ,total WBC count, differential count, platelet countwas obtained from ALFASWELAB,3 part automated hematology analyzer. Peripheral smear was obtained for all cases.

Results: Microcytic hypochromic anemia was the most common (68%). Representing the histogram variation in various anemias: Out of the 7% cases of normocytic normochromic anemia, 5% showed RBC histogram with short peak and 2% showed mild broad base curve histogram. Out of the 68% of microcytic hypochromic anemia, 5% showed normal histogram, 40% showed left shift histogram, 21% showed broad base curve and 2% showed bimodal histogram. Out of 7% cases of Macrocytic anemia, 5% showed right shift with broad base curve, 1% showed bimodal curve and 1% showed short peak histogram. Out of 6% cases of dimorphic anemia 1% showed normal histogram, 1% showed broad base histogram, 1% right and 1% left shift histogram and 2% showed bimodal histogram. Out of the 6% cases of the pancytopenia, 3% showed right shift and 3% showed short peak. Among 4% cases of thalassemia, 2% showed abnormal histogram which was not starting at the baseline with left shift bimodal, broad base curve, 1% showed bimodal curve. 2% cases of sickle cell anemia showed broad based curves with short peak.

Conclusion: Different patterns ofhistograms are obtained in different anemias. Histogram gives information about abnormality of sample and need for follow up on peripheral smear. Histograms can be useful to prioritize cases and help in speedy disposal of samples in laboratorybut will not identify conditions like malarial parasites, membrane abnormalities that cause anemia.

Keywords: Histograms, anemias, automated hematology analyzer.

INTRODUCTION

The well known coulter principle of counting and sizing the cells provides basis of generating a histogram[1,2]. It relies on change in conductance as each cell passes through an aperture.The change in conductance results in an electrical pulse, the amplitude of which is proportional to the cell volume[3].

Histograms are graphic representation of cell frequencies verses size[3]. X axis represents the size of cell and Y axis represents number of cells. They provide major clues in the diagnosis and management of significant red cell disorders.

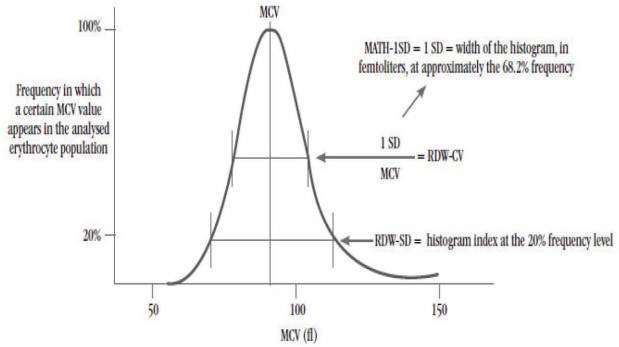


FIGURE 1: NORMAL RBC HISTOGRAM

Normalred cell histograms are symmetric, single-peaked, and Guassian or "bell shaped"curves[3] (Figure 1). The distribution should always start and ends on base line and should be located between the two discriminators[3].

Following are the important points to consider when reviewing/analyzing histograms

1. Position of individual populations compared to normal/ typical positions.
2. Amount of separation between populations compared to normal/typical separation.
3. Relative concentration of each population compared to normal/typical concentrations.
4. Presence of unexpected or non-typical populations.

When the distribution is not symmetric, it is referred to as skewed[2]. In a normal RBC histogram, the majority of each cell falls between 55 fL and 125 fL. The tail of the distribution consists of coincident doublets and anomalous pulses[2]. The RDW is calculated from the width of the histogram at 1 SD from the mean divided by MCV. The normal RDW-CV is 11.5% to 14.5%. The RDW-SD is the arithmetic width of the distribution curve measured at the 20% frequency curve. The normal RDW-SD is 39 to 47 fL[2]. Increased RDW shows a mild broad base histogram[4,5,6].

Causes of deviation from normal RBC histogram:

1. **Shift to left :**If the cells are smaller than normal, the curve will be more to theleft (Figure2), as in untreated iron deficiency anemia.
2. **Shift to right:** If the cells are larger than normal, the histogram curve will be more to right (Figure 3), as in megaloblastic anemia. After appropriate treatment of the underlying cause of an anemia, the curve should move toward the normal range[2].
3. **RU Flag (Figure 4):** When upper discriminator exceeds preset height by 5% RU flag is seen. Causes of RU flagincludecold agglutination and Rouleax formation.
4. **RL Flag (Figure 5):** When lower discriminator exceeds preset height by 10% RL flag is seen. Causes of RL flag includes Giant platelets, Micro-erythrocytes and Fragmented RBCs.
5. **Multiple peaks (MP):** When more than one peak is seen, then it indicates two or more populations of RBCs. Causes for multiple peaks (Figure 6) includeIron defiecieny anemia in recovery, Post transfusion, Extreme leucocytosis and Cold agglutinin disease.
6. In leukemia, extended right peak is seen. In reticulocytosis, right shoulder is observed (Figure 7).

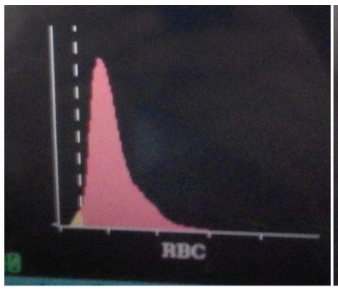


FIGURE 2: MICROCYTIC RBCs SHOWING SHIFT TO LEFT

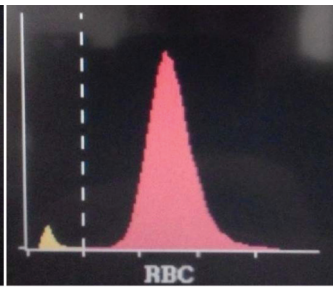


FIGURE 3: MACROCYTIC RBCs SHOWING SHIFT TO RIGHT

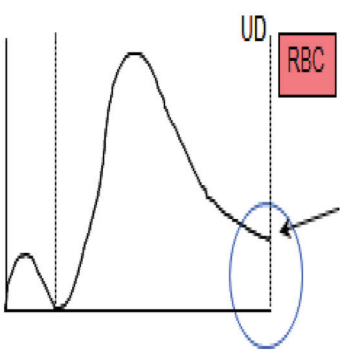


FIGURE 4 : RU FLAG (Upper Discriminator Error)

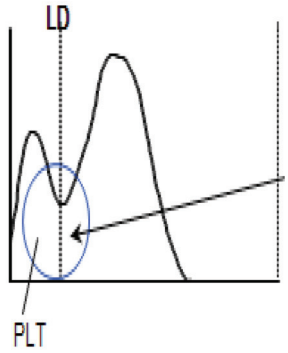


FIGURE 5 : RL FLAG (Lower Discriminator Error)

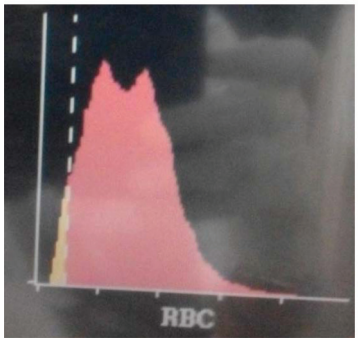


FIGURE 6: RBC HISTOGRAM SHOWING MULTIPLE PEAKS

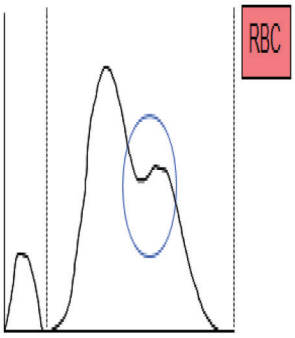


FIGURE 7: RBC HISTOGRAM SHOWING RIGHT SHOULDER

MATERIALS AND METHODS

This is prospective study conducted in Prathima Institute of Medical Sciences on 100 patients of >1year age who were anemic (Hb<12gm%). Red Blood Cell indices, hematocrit value were obtained from ALFA SWELAB three partautomated hematology analyzers.All cases were studied with a correlation of peripheral smear findings.

RESULTS

Based on Hb%, anemia is classified as mild (9-12gm%), moderate (7-9gm%) and severe (< 7gm%).

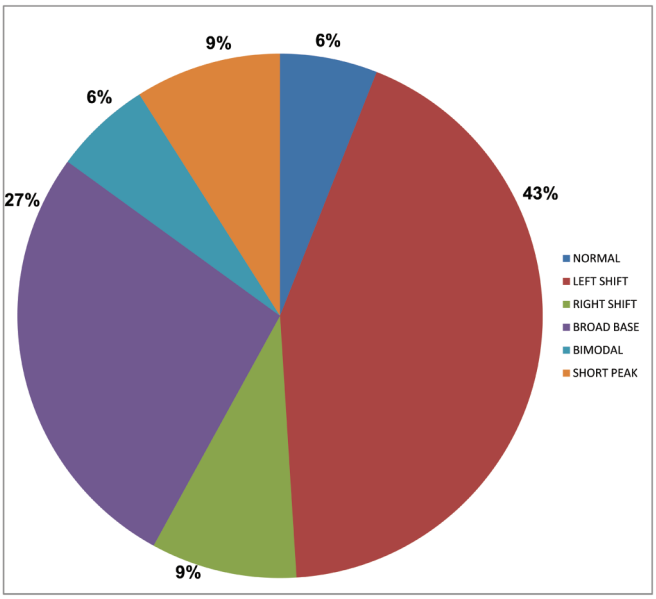
TABLE 1: DISTRIBUTION OF CASES BASED ON HEMOGLOBIN PERCENTAGE.

DEGREE OF ANEMIA	PERCENTAGE OF CASES
MILD	21%
MODERATE	60%
SEVERE	19%

TABLE 2: DISTRIBUTION OF CASES AS FOR PERIPHERAL SMEAR DIAGNOSIS

TYPES OF ANAEMIA	PERCENTAGE OF CASES
NORMOCYTIC	7%
MICROCYTIC HYPOCHROMIC	68%
MACROCYTIC	7%
DIMORPHIC	6%
PANCYTOPENIA	6%
THALASSEMIA	4%
SICKLE CELL ANEMIA	2%

FIGURE 8: PIE CHART REPRESENTATION OF TYPES OF HISTOGRAM IN THE STUDY



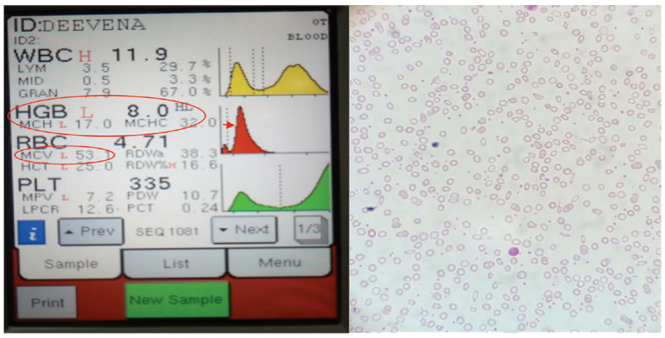


Image 1: Rbc Histogram Showing Left Shiftand Peripheral Smear Of Microcytic Hypochromic Anemia (40x)

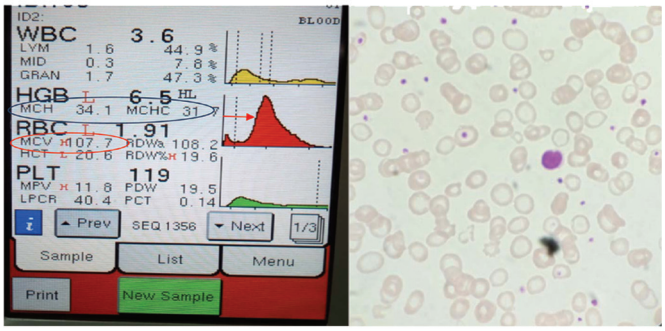


Image 2: Rbc Histogram Showing Right Shift And Peripheral Smear Of Macrocytic Anemia (100x)

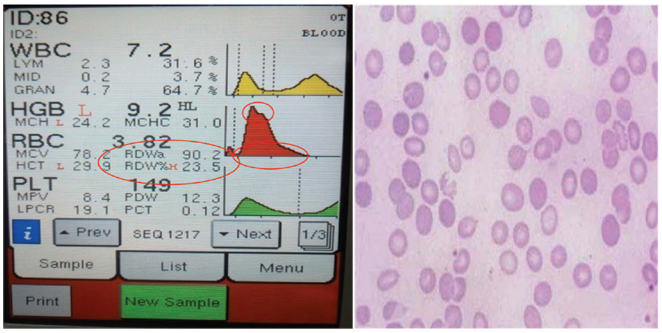


Image 3: Rbc Histogram Showing Double Peaksand Peripheral Smear Of Dimorphic Anemia (100x)

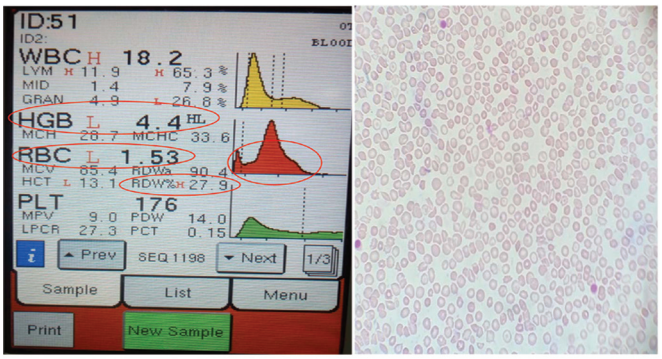


Image 4: Rbc Histogram Not Starting At The Baseline And Peripheral Smear Of Thalassemia (40x)

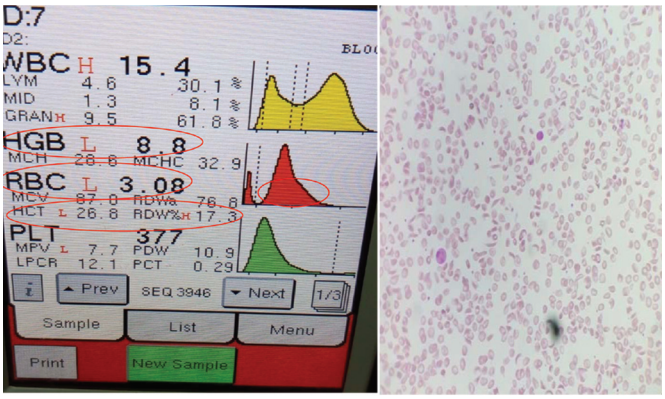


Image 5: Rbc Histogram Showing Short Peak, Broad Base And Peripheral Smear Of Sickle Cell Anemia (40x)

A histogram can provide useful information for laboratories in:

1) Monitoring the reliability of the results generated by the analyzer.

2) Investigating the potential cause(s) of the erroneous automated results.

3) Arriving at the presumptive diagnosis E.g.; certain conditions like the presence of fragmented red cell or red cell agglutination that could not have been identified earlier without blood film examination can now be presumably detected on the red cell histogram[7].

Table 3: Histograms Variations In Various Anaemias

	NORMAL CURVE	LEFT SHIFT	RIGHT SHIFT	BROAD	BIMODAL	SHORT PEAK	TOTAL
NORMOCYTIC	-	-	-	2%	-	5%	7%
MICROCYTIC	5%	40%	-	21%	2%	-	70%
MACROCYTIC	-	-	5%	-	1%	1%	7%
DIMORPHIC	1%	1%	1%	1%	2%	-	6%
PANCYTOPENIA	-	-	3%	-	-	3%	6%
THALASSEMIA	-	2%	-	1%	1%	-	4%
SICKLE CELL ANEMIA	-	-	-	2%	-	-	

DISCUSSION

Histogram can be used for screening but not considered diagnostic for any pathological condition[6]. A CBC report, if abnormal should always be correlated with clinical features and blood smear findings for meaningful conclusion[3].

THE ERYTHROCYTE HISTOGRAM: The analyzer counts those cells as red cells, volume of which ranges between 36 fL and 360 fL[3].Causes of lower discriminator flag include red cell fragments, microspherocytes, nucleated RBCs, nonlyzed RBCs, elliptocytosis, giant platelets, platelet clumpsand causes of upper discriminator flag are interfering substances such as cryoglobulin, cold agglutinin, and roulaex formation[8]. MCV is perpendicular line from peak of curve to base[9].

ANALYSIS AND INTERPRETATIONS OF RBC HISTOGRAM:

Homogenous population of RBC give narrow distribution curve while if more anisopoikilocytosis, then curve will be broad[10]. The curve is said to be symmetric if both sides of the curve coincide when folded in half or are mirror images to one another[2].

A histogram distribution that is bimodal can be seen in various situations, are usually associated with therapeutic transfusion and/or hematinic agent response to microcytic and macrocytic anemia, but they may also indicate other hematological disorders like cold agglutinin disease, in the presence of erythrocyte fragments, in IDA (microcytic) with recent blood transfusion, in sideroblastic anemia especially in acquired forms, and megaloblastic anemia (macrocytic anemia) with recent blood transfusion[2,11].

NORMOCYTIC ANEMIA: Out of the 7% of normocytic normochromic anemia 5% showed normal histogram with short peak and 2% showed mild broad base curve histogram correlating well with the increased RDW.

The population of the cells would be variable in size. For example, some microcytic or small cells and some normal size cells would result in a higher deviation and hence a higher RDW.

MICROCYTIC ANEMIA(Image 1): Out of the 68% of microcytic hypochromic anemia, 5% were normal histogram due to mild anemia, 40% were left shift histogram, 21% were broad base curve histogram due to increase RDW and 2% with bimodal histogram, as here the patient was on treatment.

MACROCYTIC ANEMIA(Image 2): Samples with macrocytic RBC generate histograms exhibiting a longer slope on the right [12].

Among 7% cases of Macrocytic anemia 5% showed the right shift broad base curve histogram with 1% short peak histogram due to reduced RBC count and 1% bimodal curve as the patient was receiving treatment.

DIMORPHIC ANEMIA(Image 3): In a dimorphic picture, the histogram may have two or more (multiple) red cell populations[12].

In our study Dimorphic anemia showed high discrepencies between histogram curves and peripheral smear examination. Out of 6% cases of dimorphic anemia 1% showed normal histogram, 1% showed broad base histogram, 1% right, 1% left shift histogram and 2% showed bimodal curve [13-16].

PANCYTOPENIA : Out of the 6% cases of the pancytopenia, 3% were right shift curve due to severe megaloblastic anemia and remaining 3% were short peak histogram correlating well with decreased RBC count [17].

THALASSEMIA(Image 4): Among 4% cases of thalassemia, 2% showed abnormal histogram which was not starting at the

baseline with left shift, broad base curve, correlating well with low MCV, MCH, HB and red cell count and increased RDW, 1% showed bimodal curve and peripheral smear also showed two populations of RBCs indicating that the patient had received recent blood transfusion [18,19]. These results correlated well with study conducted by Sandhya et al[1].

SICKLE CELL ANEMIA (Image 5): 2% cases of sickle cell anemia showed broad based curve with short peak correlating well with increased RDW and decreased RBC count[20].

CONCLUSION

Histogram can also be used in differentiating various types of anemia. Histogram changes correlated well with peripheral smear findings in majority of the cases.

Histogram analysis is often a neglected part of the automated haemogram which if interpreted well, has significant potential to provide diagnostically relevant information even before higher level investigations are ordered.The speed and the reliability of the modern analyzers allow technologists, time to evaluate abnormal blood films, consider diagnostic clues and correlate clinical findings to histograms and other hematologic parameters with greater confidence and efficiency, all of which produce high returns in terms of patient health care.

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