

Comparative Study of RBC Histogram and Peripheral Smear in Anemias

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Received on 22.06.2019,

Accepted on 10.10.2019

Abstract

Introduction: Red blood cell (RBC) histograms are graphical representations of cell frequencies versus cell sizes. Comparison of size of the patient's cell with the normal population is possible with the help of histogram. RBC histogram along with Red cell indices, Hematocrit and red cell distribution width provides a significant information in various red cell disorders. *Aims and Objectives:* The objectives were to know the advantage and utility of RBC histogram in various anemias and study RBC histogram patterns along with peripheral smear examination. *Material and Methods:* 724 cases of outpatient department with Hemoglobin less than 12 gm% on complete blood count from SMIMER, Surat were included in study. RBC histogram generated in automated hematology analyzer were compared to the peripheral smear findings of the same patient. *Results:* Out of 724 cases, almost equal cases had normal curve and left shift of curve (34.81% and 33.70% respectively). 26.66% cases showed broad based histogram. Histogram of 3.18% cases were shifted to right and 1.65% had bimodal peak. *Conclusions:* Good interpretation of histogram provides significant information in diagnosis and follow-up of many hematological conditions. Red cell histogram can be used as important screening test in diagnosis of anemia before further hematological analysis.

Keywords: RBC histogram; Peripheral smear; Anemia.

How to cite this article:

Toral B Jivani, Rushika P Patel. Comparative Study of RBC Histogram and Peripheral Smear in Anemias. Indian J Pathol Res Pract. 2019;8(6):763-767.

Introduction

Anemia is most frequent and most important hematological disorder all over the world. Almost all modern automated hematology analyzers give RBC histogram along with RBC indices, hematocrit

and RBC distribution width which provides major clues in the diagnosis and management of red cell disorders.^{1,2} Pathology of the blood cells can be identified by shapes of the histograms. Histogram compares the size of a patient's cells with normal populations. Shift of histogram in any direction

has diagnostic significance.³ One histogram graph is worth 1000 numbers. Visual scanning of the histogram gives a good initial sense of the range, size, shape, and other salient features of the red cell morphology.⁴

Aims and Objectives

1. Know the advantage and utility of RBC histogram in various anemias.
2. Study RBC histogram patterns along with peripheral smear examination.

Materials and Method

It is an observational study of 724 patients with hemoglobin less than 12 gm% from the outpatient department of central laboratory of SMIMER, Surat. The study was conducted over the period of 18 months (April, 2017 to September, 2018). The samples were collected in EDTA vacutainer for Complete blood count (CBC). Peripheral smear was made at the time of sample collection and CBC was performed using Beckman coulter Unicel DxH automated hematology analyzer. Sample with hemoglobin <12 gm% were selected and patient's Hb, RBC count, RBC indices and RBC histogram findings were recorded. Peripheral smears were fixed and stained with Giemsa stain for red blood cells morphology. Findings of RBC histogram and red cell morphology on smear were compared for each patient.

Results

In our study, out of total 724 cases of anemia, majority of patients were from age group of 21 years – 30 years. Male accounted for 27.62% of cases and female accounted for 72.38% of cases with male to female ratio 1:2.6. Majority of the males were from age group of 31 years – 40 years and majority of the females were from 21 years – 30 years of age group. 55.5% cases having mild anemia (Hb-10-11.9 gm%), 38.5% with moderate anemia (Hb-7-9.9 gm%) and

6% of cases having severe anemia (Hb-<7 gm%).

Among the RBC indices, 64.09% cases showed decreased MCV value (<80 fl), 32.46% showed normal MCV (80-100 fl) and 3.45% cases showed increased MCV (>100 fl). 59.25% cases have decreased MCH (<26 pg), 7.70% cases have normal MCH (26-34 pg) and 2.75% cases have increased MCH >34 pg). Most of the anemic patients (86.87%) have normal MCHC (<31 gm%), 11.75% have decreased (31-35 gm%) and 1.38% cases have increased MCHC (>35 gm%). 78.73% cases have RDW-CV >14.5% and 21.27% cases have normal RDW-CV (Tables and Figs. 1-4).

Table 1: Age and sex wise distribution of patients with anemia.

Age (years)	Patient with hemoglobin <12 gm%			Percentage of total (%)
	Male	Female	Total	
0-10	17	16	33	4.55
11-20	18	98	116	16.2
21-30	38	228	266	36.76
31-40	67	124	191	26.38
41-50	32	41	73	10.08
51-60	21	09	30	4.14
>60	07	08	15	2.07
Total	200 (27.62%)	524 (72.38%)	724	100

Table 2: Types of histogram abnormalities observed in present study.

Type of Histogram	No. of cases	Percentage (%)
Normal curve	252	34.81
Left shift	244	33.70
Right Shift	23	3.18
Broad Base	193	26.66
Bimodal peak	12	1.65

Table 3: Distribution of cases as per RBC morphology observed on peripheral smear.

Type of anemia	No. of cases	Percentage (%)
Normocytic	267	36.8
Microcytic	377	52.07
Macrocytic	31	4.28
Dimorphic	49	6.77

Table 4: RBC histogram variation in different anemias.

	Normal curve	Left shift	Right shift	Broad base	Bimodal peak
Normocytic	243(33.56%)	-	03 (0.41%)	21 (2.90%)	-
Microcytic	05 (0.69%)	242 (33.43%)	-	130 (17.95%)	-
Macrocytic	03 (0.41%)	-	19 (2.62%)	09 (1.24%)	-
Dimorphic	01 (0.13%)	02 (0.28%)	01 (0.13%)	33 (4.56%)	12 (1.65%)

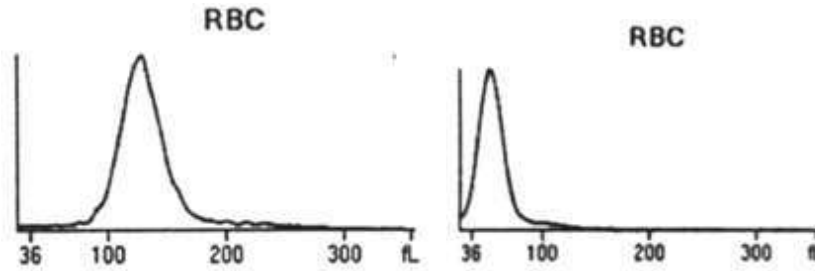


Fig. 1 & 2: RBC Histogram showing right shift with broad base and left shift of the curve. (Beckman Coulter Unicel DxH 800)

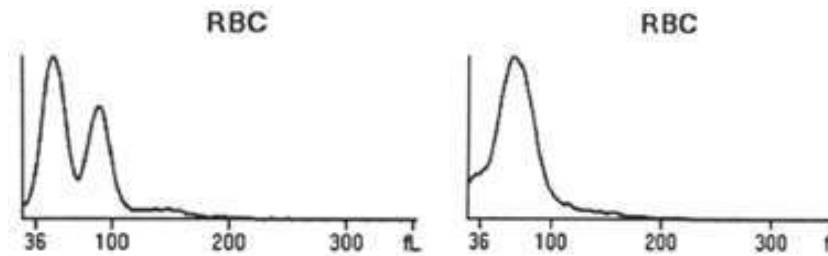


Fig. 3 & 4: RBC Histogram showing bimodal peak and lower discriminator error of the curve. (Beckman Coulter Unicel DxH 800)

RBC histogram variation in different anemias

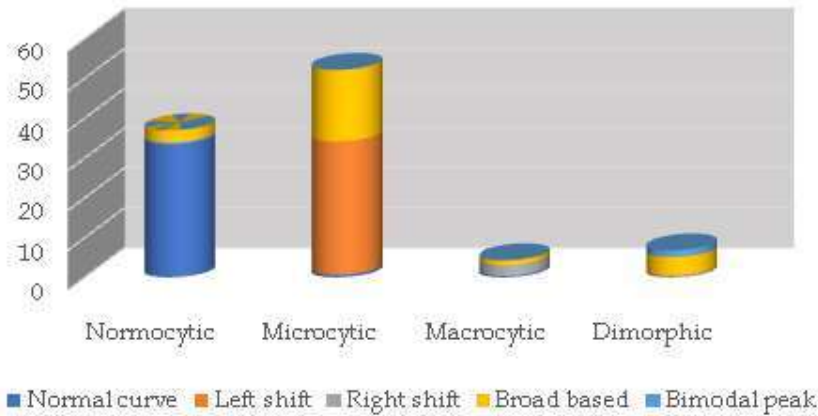


Fig. 5: RBC histogram variation in different anemias

Discussion

The new generation analyzers produce various histograms. These histograms provide significant information even before blood smear is examined.⁵ Histogram is graphical representations of cell frequencies versus sizes. Normally curve is symmetrical bell shaped or Gaussian distribution. The curve is considered symmetric if both sides of the curve coincide when folded in half or are approximately mirror images. When the distribution is not symmetric, it is referred to as skewed.⁶

With the help of histogram size of patient's cells can be compared with normal populations. Shift of curve in one direction is of diagnostic importance. Size (volume) is put on X axis and no. of cells per channel on Y axis. There is left shift in curve when cell size decreases and right shift when cell size increases.^{6,3,7} The RBC histogram in the cell counter displays the cell ranges for RBC histograms are between 24 fl and 360 fl, the instrument counts only those cells with volume sizes between 36 fl and 360 fl as red cells. The area of the peak is used to calculate the MCV and RDW. This area represents 60 fl to 125 fl.^{8,9}

Table 5: Histogram findings in anemia compared to other studies.

Histogram	Shruti singla <i>et al.</i> ¹⁰	Rao <i>et al.</i> ⁹	Chavda <i>et al.</i> ¹¹	Present study
Normal curve (%)	19	17.7	19	34.81
Left shift (%)	68.2	29	27	33.70
Right shift (%)	0.4	5.45	07	3.18
Broad base (%)	11.6	37.72	38	26.66
Bimodal (%)	0.8	7.27	06	1.65
Short peak (%)	-	2.7	03	-

Table 6: Types of anemia on peripheral smear compared to other studies.

Anemia	Shruti singla <i>et al.</i> ¹⁰	Rao <i>et al.</i> ⁹	Chavda <i>et al.</i> ¹¹	Present study
Normocytic	13.9	19.54	17.4	36.88
Microcytic	72.2	63.63	65	52.07
Macrocytic	0.4	2.2	3.6	4.28
Dimorphic	13.4	12.72	14	6.77
Pancytopenia	-	1.8	-	-

In Iron deficiency anemia and beta thalassemia trait, the red cell distribution curves are shifted to the left. A right shift histogram with broad base curve indicates the macrocytic anemia. Histograms with bimodal peak are usually associated with therapeutic transfusion or hematinic agent response to microcytic and macrocytic anemia.³

In present study of histogram of 724 cases, 34.81% showed normal curve, Left shift in 33.70% cases, Broad based in 26.66%, Right shift in 3.18%, Bimodal peak in 1.56% which were in accordance with other studies like Rao *et al.*⁹, Chavda *et al.* The higher incidence of normal curve in present study is due to inclusion of only outpatient anemic patient and higher incidence¹¹ of mild anemia (Table 5).

In present study majority of patient (52.7%) were of microcytic anemia on peripheral smear examination which was comparable to other studies Shruti Singla *et al.*¹⁰, Rao *et al.*⁹, Chavda *et al.*¹¹ Second most common anemia noted is Normocytic anemia which is also in concordance with above mention studies (Table 6).

Out of total 724 cases, 33.56% of normocytic anemia showed normal curve, 0.41% cases showed normocytic RBCs with right shift and 2.90% of cases showed normocytic cells with broad based curve. Out of total anemic patients, 33.43% cases showed left shifted curve on histogram and microcytic picture on peripheral smear. 17.95% cases had microcytic RBCs with broad based histogram and 0.69% case showed normal curve in microcytic cases. 2.62% of cases have macrocytic red cells with right shifted histogram. 1.24% and 0.41% case with macrocytic cases showed broad based curve and normal curve on histogram respectively.

Peripheral smear with dimorphic red cells showed all types of abnormal histogram with broad based curve in 4.56% (maximum) of cases. 1.65% cases with bimodal peak, 0.28% cases with right shift, 0.13% cases with left shift and 0.13% of cases with normal curve.

Conclusion

RBC histogram analysis is an often a neglected part of automated hemograms. Shapes of histograms can identify pathology to some extent and give hint for that which cases need actual detailed peripheral smear examination. Besides this, histogram also provides some idea about RBC count, MCV and RDW by its various shape and shift in any direction. A subsequent histogram after iron therapy shows the progressive appearance of a new erythrocyte population much before numerical parameters. In present study, histogram changes correlated with peripheral smear findings in majority of the cases. So, along with other RBC parameters, Red cell histogram can be used as important screening test in diagnosis of anemia before further hematological analysis.

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