ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 01 | January - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

INTERPRETATION OF RBC HISTOGRAMS AND THEIR CORRELATION WITH PERIPHERAL SMEAR FINDINGS IN PATIENTS OF ANEMIA.

Pathology

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ABSTRACT

Introduction: Automated haematological analysers provide blood cell histograms by plotting the sizes of different blood cells on X-axis and their relative number on Y-axis. Histogram interpretation needs careful analysis of Red Blood Cell (RBC), White Blood Cell (WBC) and platelet distribution curves. Histogram analysis is often a neglected and underrated part of the automated haemogram which if interpreted well, has significant potential to provide diagnostically relevant information even before higher level investigations are ordered and may be a time saving tool(1). The study was aimed to correlate between the Peripheral Smear findings in patients of anemia that presented in our tertiary care hospital and the RBC histogram chart plotted by the automated analyser and how far the machine dependent histogram is near to the gold standard of investigation that is microscopy. **Material and method:** Total of 500 blood samples of patients of adult age group admitted in Civil Hospital, Ahmedabad were studied from August 2019 to October 2019. Patients with haemoglobin less than 12.5gm/dl were included. The blood samples were run in Horiba Pentra XLRTM 80, 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 vacutte and stained by Geimsa stain. Further, 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 anemia, followed by normocytic normochromic anemia 19.6%. The histogram suggested 12.6% of patients and 2.2 % of patients suffering from macrocytic anemia and haemolytic anemia 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 anemia, followed by normocytic normochromic anemia at 18.8%. The Peripheral Smear finding suggested 11.6% of patients and 3.6 % of patients suffering from macrocytic anemia and haemolytic anemia 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.

KEYWORDS

Automated haematological analysers, Histogram, Anemia, Peripheral Smear

Introduction:

The complete blood count (CBC) including DLC are backbone of any laboratory evaluation and provide valuable information to diagnose anaemia, acute and chronic illness, white cell disorder, leukemia and platelet disorders. During past 25 years blood cell analysis has progressed from manual procedures to highly automated instruments (2). In fully automated analyzers additional information in form of histogram charts are displayed which provide information about greater number of variables simultaneously.

Principle:

The Horiba Pentra XLR 80TM is based on Coulters principle which was introduced by Wallace coulter in 1956(2) . The automated hematolyzers based on impedance principle and relies on the change in conductance as each cell passes through an aperture. This change in conductance results in development of an electrical pulse which's amplitude is proportional to the cell volume. The results are displayed as histogram and as figures. RBC histogram is a graphic representation of particle size distribution (cell frequencies verses size).

Shift in one direction or another can be of diagnostic importance and in association with other CBC parameters such as RBC distribution width (RDW) and RBC indices (MCV, MCH, MCHC) it has been found abnormal in various hematological conditions.

Normal curve falls within normal range of MCV which is 80-100 fl. The RBC histogram in the hematological analyzer displays the ranges for RBC are between 30 fl and 300fl. The analyzer counts only those RBC's with volume sizes between 36fl to 300fl as red cells and cells which are counted in the range of 30 fl and below are not included in the RBC count and rejected by counter. Normally below 30fl size space is clear and histogram is begin from base line but if histogram begin above the base line indicates the presence of small particles like platelet clumps, malaria parasite, micro-spherocytes, normoblast, elliptocytes, bacteria, etc[3,4].

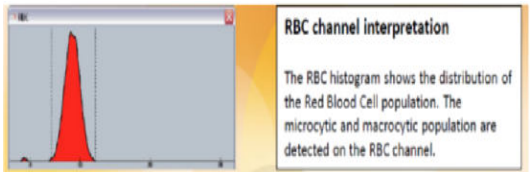


Figure 1: A schematic representation of RBC histogram on modern automated hematological cell counters.

Material and method:

The data for present study was obtained from the Central Hematology Laboratory, Civil Hospital Ahmedabad. Total 500 adult patients' sample were examined with HB < 12.5 gm/dl. Patients suffering from malignancies were excluded. The samples were received in EDTA vacutainer with request form duly filled by the clinician. The samples were then entered into the Lab Information Systems and run in Horiba Pentra XLR 80TM. The samples were used to prepare peripheral blood smear by staining with Geimsa stain. After staining microscopy was

done for peripheral smear examination and the findings were compared with the histogram chart on the Horiba Pentra XLR 80TM. The position (normal, left shift, right shift) and the shape of RBC histogram (normal bell shape or Gaussian, widened base, bimodal peak, skewing to left or right, U shaped curve) (4,5) recorded. The anemia was categorised based on RBC indices as: a) Normocytic normochromic, b) Microcytic hypochromic, c) Macrocytic normochromic when MCV was between 80-100fl, <80fl, >100fl respectively(6) .The RBC indices were seen in association with Histogram pattern. Normally positioned Gaussian bell shaped RBC histogram was considered as normocytic normochromic anemia, left shift indicated Microcytic anemia, right shift indicated macrocytic anemia. A bimodal peak indicated dimorphic anemia and Broad base with a left shift was considered as haemolytic anemia.

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

Observation and result:
The RBC indices, histogram and Peripheral Smear of 500 patients having Hb less than 12.5 gm/dl were analysed. Only adult patients were included for study. Out of 500 patients 54.4% were females and 45.6% 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 anemia while 11 cases (2.2%)of haemolytic 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.

Figure 2: HISTOGRAM SHOWING SHIFT TO RIGHT SUGGESTING MACROCYTOSIS

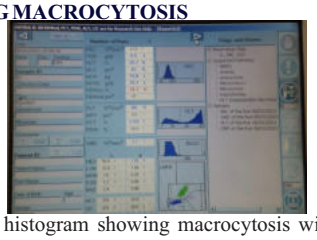


Figure 2 show histogram showing macrocytosis with shift to right which is towards the higher MCV value. Broad base indicates that variability of size among RBC(anisocytosis) along with pokilocytosis implied by high RDW.

FIGURE 3: HISTOGRAM SHOWING SHIFT TO LEFT SUGGESTING MICROCYTOSIS.

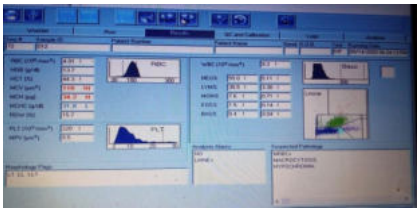


Figure 3 show histogram with microcytosis showing shift to left which is towards the lower MCV value. Narrow base indicates that low variability of size among RBC and implied by normal RDW.

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.

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.

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. [7]	Chavda J et al. [8]	Rao BSS et al. [9]	Shrivastav et al(10)	Present Study
Normal curve	15%	19%	17.7%	18%	16%
Left shift	30%	27%	29.0%	29%	33%
Right shift	6%	07%	5.45%	06%	8%
Broad base	40%	38%	37.72%	40%	34%
Bimodal	4%	3%	7.27%	05%	2%
Short peak	5%	6%	2.7%	02%	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%), Dimorphic (12.2%) and Macrocytic (11.6%). Other studies like sandhya et al.(7) Chavda J et al. (8) & Byna Syam Sundara Rao et al. (9) 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 [10].
The comparative values have been given in table 4.

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

Type of Anemia	Peripheral Smear analysis (n=500)	Histogram and RBC Indices (n=500)
Normocytic Normochromic	94 (18.8%)	98(19.6%)
Microcytic Hypochromic	267 (53.4%)	261(52.2%)
Macrocytic	58 (11.6%)	63(12.6%)
Dimorphic	63 (12.6%)	67(13.4%)
Hemolytic	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[11].

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.

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" (1). 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.

Source of funding: self

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