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Correlation of Peripheral Smear with RBC and WBC Histogram in the Diagnosis of Anemia

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Abstract: ***Introduction: Histograms produced by automated hematology analyzers are the graphical representation of numerical data of different cell populations. The automated hematology analyzer is alternative for traditional manual methods for measuring various hematological parameters as the initial screening method in most of the hospital nowadays. Aims: To correlate RBC and WBC histogram abnormalities with peripheral blood smear findings. Methods: Total of 500 blood samples of patients of the adult age group admitted in Civil Hospital, Ahmedabad were studied from August 2021 to December 2021 were taken. The study is conducted on hematology histograms for RBC generated by 5-part cell counter by Horiba Pentra XLRTM 80 and their degree of concurrence and non-concurrence with (Giemsa stained) peripheral smear findings respectively. Alterations in RBC, WBC histograms were analyzed with their respective peripheral smear findings. Results: In RBC histogram defects, the majority had a left shift followed by right shift and bimodal peak respectively. In WBC Histogram defects, WU defect, WL defect, Leucopenia, Leucocytosis. Conclusion: Histograms should be used as a screening method to pick up pathological samples. These samples should then be followed by a peripheral smear examination for confirmatory diagnosis.***

Keywords: Automated hematological analyses, Histograms, RBC, Peripheral Blood Smear, WBC

1. Introduction

The peripheral blood smear has been the main diagnostic aid in establishing the etiology of anemia. Examining the blood films routinely has facilitated interpretation of various hematological disorders. In this study we have compared the abnormal RBC and WBC histograms with their respective peripheral smear findings and analyzed their utility in diagnosing of anemia.

The automated hematology analyzer has replaced the traditional manual methods for hematological parameters as the initial screening and detection system for hematological abnormalities in modern clinical setups. It produces various histograms, which give ample information even before blood smear is examined.

Principle of Hematology analyzer:
The Hematology analyzer is based on Coulters principle which was introduced by Wallace coulter in 1956(1). 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 amplitude is proportional to the cell volume. The results are displayed as histogram.

Histogram is a graphic representation of different population cell types as haematology analysers count and size thousands of cells to produce a histogram. In a histogram X axis represents the cell size and Y axis represents the number of cells.(2)

The RBC histogram in the hematological analyzer displays the ranges for RBC are between 30 fl and 300fl. Normal curve falls within normal range of MCV which is 80-100 fl. In a normal WBC histogram lymphocytes are distributed between 50-100, mixed cell population (monocytes, basophils and eosinophils) [V] between 100-150, and neutrophils between 150-300.

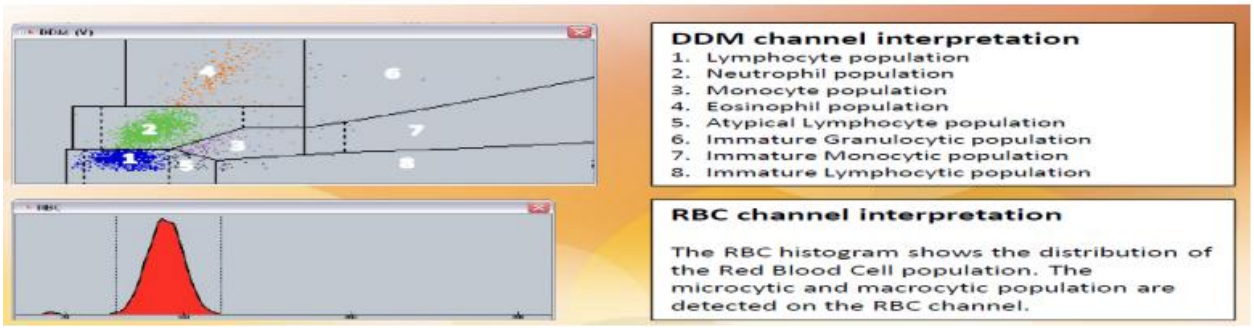


Figure 1: A schematic representation of RBC histogram on modern automated

AIMS: To correlate RBC and WBC histogram abnormalities with peripheral blood smear findings.

2. Material and Methods

Total 500 samples for present study were obtained and analysis was done based on records of the investigations obtained in Central Clinical Laboratory (CCL), B.J Medical College Ahmedabad over a period of four months from January 2022 to April 2022. 3ml of EDTA venous blood sample with request form duly filled by the clinician were received. The data were entered into the Lab Information Systems then check adequate quantity of sample and then run within 2 hours of collection in automated hematology analyzer.

Peripheral smears prepared according to the standard operating procedures and stained by Giemsa stain. The shape, size, chromia and premature precursors of RBCs were noted. The count, morphology and presence of any immature cells were noted for WBCs.

Histograms were studied based on their shape, size, and center of spread along with the starting and end points of curve. Presence of any histogram defects in RBC (left shift, right shift and bimodal peak)(3, 4), WBC (WU, WL, F1, F2, F3, T1, T2) were noted. 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(5).

Interpretation of histograms was done in all the cases, after which a concordance with their peripheral smear was done to find out the percentage of concordance, various causes of flags and possible causes of non-concordance.

Inclusion Criteria

All adult patients with haemoglobin level below 11 gm /dl were included

Exclusion criteria

1) All patients with normal histogram and normal PBS, leukemoid reaction, leukemia along with pediatric patients were excluded from the study.
2) Inadequate quantity of blood sample for automated analyzer (< 3ml)

Study Design

It is a comparative study of 500 cases being performed on automated hematology analyzer (5-part cell differentiation counter).

3. Results

All cases had hemoglobinlevel less than 11 gm/dl.

Table 1: Distribution of study population on basis of age and gender

Age (years)	Male	Female	Total	Percentage (%)
18-30	49	83	127	26.40%
31-40	53	97	148	30%
41-50	37	35	72	14.40%
51-60	50	33	83	16.60%
61-70	25	22	47	9.40%
71-80	7	16	23	4.60%
Total	221	286	500	100

The age group of patients included in this study ranged from aged 18 year to 80 years. Majority of patients were within 18-40 years of age (56.4%) (Table 1)

On peripheral smear examination Normocytic normochromic anemia, Microcytic hypochromic anemia, dimorphic anemia and pancytopeniawere diagnosed.

Microcytic Hypochromic Anemia : Most common histogram pattern was shift to left with broad base. Few cases showed bimodal pattern. In RBC Indices MCV, MCH were decreased with increased RDW seen.

Normocytic Normochromic Anemia: Most common histogram pattern seen in normocytic normochromic anemia was bell shaped curve in majority of cases. In RBC Indices MCV, MCH were Normal with normal RDW seen.

Macrocytic anemia: Histogram pattern showed shift to right in majority of cases. Also seen was bimodal pattern. Very few cases showed broad base curve. In RBC Indices increased MCV, MCH, RDW with normal MCHC was seen.

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). MCV, MCH, MCHC were within normal limits and RDW was increased due to high degree of ansiopoikilocytosis in Dimorphic anemia.

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.

Pancytopenia only change noticed in red cell indices are increased RDW with normal MCV, MCH, and MCHC.

Table 2: Distribution of anemia cases based on peripheral smear

Type of anemia	Cases	Frequency (Percentage)
Normocytic normochromic	119	23.80%
Microcytic hypochromic	252	50.40%
Macrocytic	64	12.80%
Dimorphic	38	7.60%
Hemolytic	19	3.80%
Pancytopenia	8	1.60%
Total	500	100%

In our study 119 (23.8%) cases were normocytic normochromic anemia, 252(50.4%) cases were of microcytic hypochromic anemia, 62 (12.4 %) cases were of macrocytic anemia and 38(7.6%) cases were of dimorphic anemia, 19 (3.8%) cases were hemolytic anemia. Pancytopenia was seen in 8(1.6%) cases (Table 2) based on Peripheral smear examination.

Table 3: Distribution of anemia cases on red cell indices and histogram

Type of anemia	cases	Frequency (Percentage)
Normocytic normochromic	112	22.4%
Microcytic hypochromic	262	52.4%
Macrocytic	64	12.8%
Dimorphic	43	8.6%
Hemolytic	13	2.6%
Pancytopenia	6	0.6%
Total	500	100%

In our study of 500 cases, 112 cases were normocytic normochromic anemia, 262 cases were of microcytic hypochromic anemia, 64 cases were of macrocytic anemia and 43 cases were of dimorphic anemia, 13 cases were hemolytic anemia. Pancytopenia was seen in 6 cases (Table 3) based on red cell indices and histogram.

Table 4: Histogram pattern observed in the study

Histogram Pattern	Cases	Frequency (Percentage)
Normal curve	97	19.40%
Left shift	153	30.60%
Right shift	54	10.80%
Broad base curve	178	35.60%
Bimodal	18	3.60%
Short peak	5	1%
Total	500	100%

In our study of 500 cases, 19.4% cases showed normal curve, left shift were seen in 30.6% cases, right shift in 10.8% cases, broad base in 35.6% cases, short peak in 1% cases and bimodal curve in 3.6 % cases (Table 4).

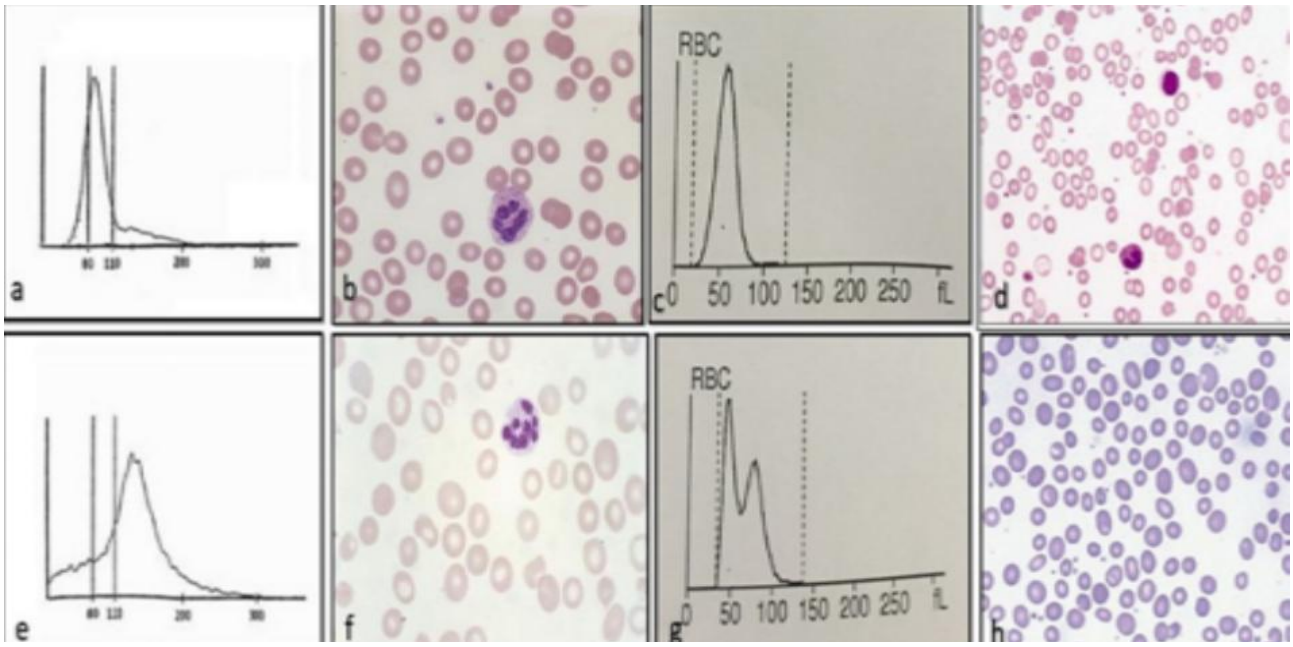
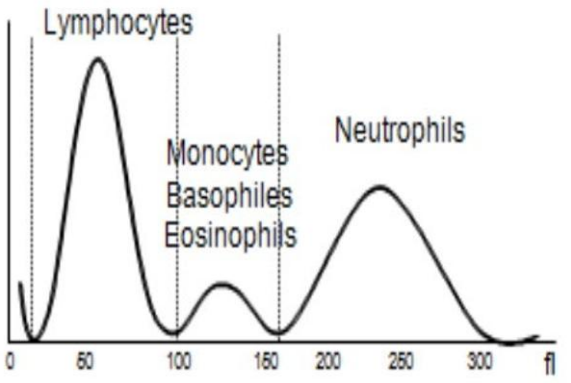


Figure 2: Correlation of RBC histograms with PBS.

a. RBC histogram showing a normal bell shaped curve in normocytic normochromic anemia
b. PBS showing Normocytic normochromic anemia
c. RBC histogram showing Shift to left in microcytic hypochromic anemia
d. PBS showing Microcytic hypochromic anemia
e. RBC histogram showing Shift to right in macrocytic anemia
f. PBS showing Macrocytic anemia
g. RBC histogram showing a bimodal peak in Dimorphic anemia
h. PBS showing Dimorphic anemia

Correlation between Peripheral Smear Findings and WBC Histogram pattern:

The WBC histogram defects give a clue to the presence of any immature cell or presence of any particular cell population in excess or low amounts. WBC morphology can be studied using a peripheral blood smear.



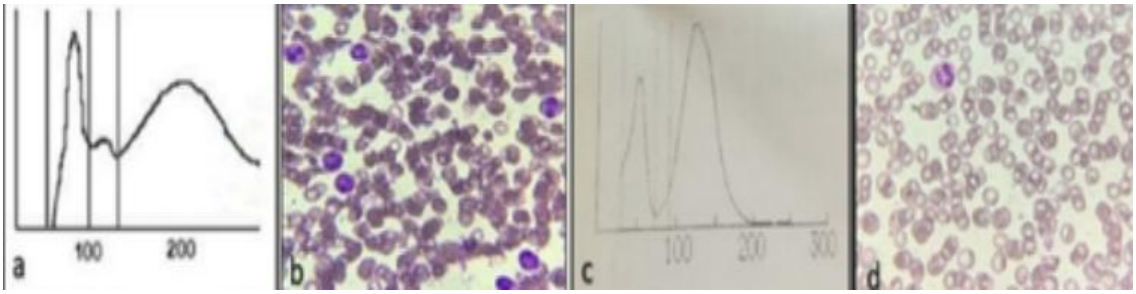


Figure 4: Correlation of WBC histograms with PBS
a. WBC histogram showing WU defect (Abnormal Curve in Front of the Upper Discriminator)
b. PBS showing Leukocytosis (neutrophilia)
c. WBC histogram showing WLdefect (Abnormal Curve in Front of the Lower Discriminator)
d. PBS showing leucopenia

Table 5: Correlation between WBC histogram defects and WBC abnormality on PBS

WBC histogram	PBS abnormalities (count)			
defects	Increased	Decreased	Normal	Total
WL	5	4	0	9
WU	15	0	0	15
Normal	63	22	415	476
Total	83	26	391	500

We found 24 WBC histogram defects 9 cases with WL type and 15 cases with WU type whereas PBS examination revealed 83 cases with leukocytosis and 26 cases with leukopenia. (table 5)

WU defect occurs when the height of the upper discriminator (UD) is [11] greater than the preset 10% on the Y-axis. The cause for this defect was extreme leukocytosis which was evident on the peripheral smears. There were 15 cases of WL defects in our study.

WL defect appears when there is an abnormal curve in front of the lower discriminator. The common causes being unlysed RBC (osmotic resistance), platelet clumps, EDTA-incompatibility, erythroblasts (NRBC), cold agglutinins and coagulated blood. There were 9 cases of WL defects in our study.

4. Discussion

Our study comprised of adult patients ranging from 18 to 75 years. Majority of anemic patients were between 21-40 years of age.

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

Table 6: Comparison of Histogram Shape in Various Studies

Histogram	Sandhya et al. (6)	Chavda J et al. (7)	Rao BSS et al. (8)	Shrivastav et al (9)	Present Study
Normal curve	15%	19%	17.70%	18%	19.40%
Left shift	30%	27%	29%	29%	30.60%
Right shift	6%	7%	5.45%	6%	10.80%
Broad base	40%	38%	37.72%	40%	35.60%
Bimodal	4%	3%	7.27%	5%	3.60%
Short peak	5%	6%	2.70%	2%	1%

Our study of RBC histogram showed normal curve (19.4%), left shift (30.6%), right shift (10.8%) Broad base (35.6%), short peak (1%) and bimodal (3.6%) and these findings regarding to RBC histogram were also correlated with other studies like sandhya et al. (6) Chavda J et al. (7) Rao BSS et al.(8) and Shrivastav et al (9).

Table 7: Correlation between Peripheral Smear Findings and RBC Histogram pattern:

Type of Anemia	Peripheral Smear analysis (n=500)	Histogram analysis (n=500)
Normocytic Normochromic	119 (23.8%)	**112**(22.4%)
Microcytic Hypochromic	252(50.4%)	**262** (52.4%)
Macrocytic	64(12.8%)	**64**(12.8%)
Dimorphic	38 (7.6%)	**43** (8.6%)
Hemolytic	19(3.8%)	13 (2.6%)
Pancytopenia	8 (1.6%)	6 (1.2%)
Total	500	500

The study revealed that most of the findings on peripheral smears can be correlated with the histogram patterns. (Table 5). 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 auto analyser considers it as microcyte. So peripheral smear rules out these errors.

5. Conclusion

RBC and WBC histogram is an important tool of diagnosis when correct interpretation of histogram is combined with findings of peripheral smear. 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.

Haematopathology has made a lot of progress due to introduction of five part and seven part counters in conjunction with different principles of working and various flagging pattern, which are usually overlooked by pathologists. Based on our study, we conclude that these flags are an important part of this study and hence should never be ignored. In the age of molecular analysis and automation, peripheral smear examination along with clinical history is an important diagnostic tool while treating patients.

Histogram if well interpreted can have a good potential in providing diagnostically relevant information about various disease process. We should use histograms as a screening method to pick up pathological samples quickly and follow them up with peripheral blood smear examination for definitive diagnosis.

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Declaration of Conflicts of Interest: The authors declare that they have no conflicts of interest.

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