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RBC Histograms: New Trends in Data Interpretation at a Tertiary Care Hospital

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ABSTRACT

INTRODUCTION: Histograms are graphic representation of cell frequencies verses size. RBC histogram with complete blood cell counts by automated analyzer provides an idea about morphological changes of RBCs in anemia. Peripheral smear examination (PBS) helps in diagnosing anemias by visualizing abnormal shape, size and immature red cells. This study was intended to analyze various types of anemia.
OBJECTIVE: To study automated histogram patterns in various anemias and to know the utility and advantage of red cell histograms.
MATERIALS AND METHODS: The present prospective study was carried out in the Haematology Laboratory of RRMCH, Bangalore from October 2022-March 2023. EDTA blood sample from patients of anemia was run on SYSMEX XN-1000i, six-part analyzer. The hematological investigations and RBC histogram were obtained by analyzer. Peripheral smears were done and stained with Leishman's stain. A detailed history was obtained. Those who had medical treatment for anemia and blood transfusion in last three months were excluded.
RESULTS: The present study included 200cases among which in order of frequency, MHA (64%) was more common followed by NNA (19%), Dimorphic anaemia (10%), Macrocytic anemia (3%), Hemolytic (2%) and pancytopenia (2%) as categorized by peripheral smear examination. Majority of cases were found in the age group, 31-40 yrs. Female cases (57%) were more than Males (43%). In RBC histogram defects, the majority had a broad base (36%) followed by left shift (35%), normal curve (17%), bimodal peak (5%), right shift (5%) and short peak (4%). A correlation between peripheral smear with RBC histogram was statistically significant with a p-value of <0.05 and chi square value 174.21.
CONCLUSION: Findings of the present study shows automated analyzer correlated well with PBS in cases of macrocytic and dimorphic anemia, compared to normocytic normochromic and microcytic hypochromic anaemia. As, RBC histogram with RBC indices and PBS are supplementary to each other, both should be used in conjunction for accurate diagnosis.

Key words: Histograms, Anemias, Automated hematology analyzer, Peripheral smear

INTRODUCTION

Anaemia represents a major public health burden worldwide with high prevalence in developing countries like India. According to the World Health Organization (WHO), almost two billion people have anaemia in the world and half of them are attributable to iron deficiency. The peripheral blood smear has been the main diagnostic aid in establishing the etiology of anemias and interpretation of various hematological disorders[1]. 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. From the earlier instruments that used electrical impedance as the sole counting principle for blood cells, modern day analyzers, in addition, use conductivity differences, cytochemical staining, light scatter, and flow cytometric principles. While enhancing the speed, accuracy and precision of test results, this has also added a new dimension to hematology reporting. The histograms provide major clues in diagnosis and management of significant red cell disorders[2,3].

RBC histogram with complete blood cell counts by automated analyzer provides an idea about morphological changes of RBCs in anemia. The overall pattern of histogram by itself in the red cell distribution curve is meaningless unless it is compared with a reference normal curve and confirmed microscopically. The Normal red cell distribution curve (Histogram) is Gaussian (bell shape) and the peak of the curve should be within the normal MCV of 80.0-100.0 fL.

A histogram can provide useful information for laboratories in[3,4,5,6]:

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 .The red cell distribution curve will get wider as the red cell vary more in size, a narrow distribution curve indicates a homogenous population of red cells; the wider the distribution curve, indicates more heterogenous population of red cells. If the cells are larger than normal, the histogram curve will be more to right, (Shift to right) as in megaloblastic anemia. After appropriate treatment of the underlying cause of an anemia, the curve should move toward the normal range. If the cells are smaller than normal, (Shift to left) the curve will be more to the left, as in untreated iron deficiency anemia. In a dimorphic picture, the histogram may have 2 or more (multiple) red cell populations, whereas in dual populations the histogram has 2 distinct red cell populations (e.g., hypochromic-microcytic and normochromic-normocytic red cells). Multiple peaks (MP): When more than one peak is seen, then it indicates two or more populations of RBCs. Causes for multiple peaks includeIron defiecieny anemia in recovery, Post transfusion, Extreme leucocytosis and Cold agglutinin disease. In leukemia, extended right peak is seen. In reticulocytosis, right shoulder is observed[5,6,7].

AIMS AND OBJECTIVE:

1. To study the histogram patterns in various anemias
2. Comparison of automated histogram patterns with morphological features noticed on peripheral smear examination.

MATERIALS AND METHODS

Source of Data: The present study was undertaken in the in the Haematology Laboratory of RRMCH, Bengaluru, a tertiary care hospital. A total of 200 patients

with anemia were studied over a period from October 2022-March 2023.

Method of Collection: 3ml of EDTA venous blood sample was collected from the patient and a histogram was obtained after thorough mixing of the sample. The 6 part differential automated analyzer was used for the study. Simultaneously a peripheral smear was prepared according to standard operating procedures and stained by Leishman stain. This peripheral smear was reported by pathologist who was not privy to histogram during the reporting of peripheral smear.

Inclusion Criteria

1. All anemic patients with hemoglobin percentage less than 11.5gm%.
2. Patients of all age groups.

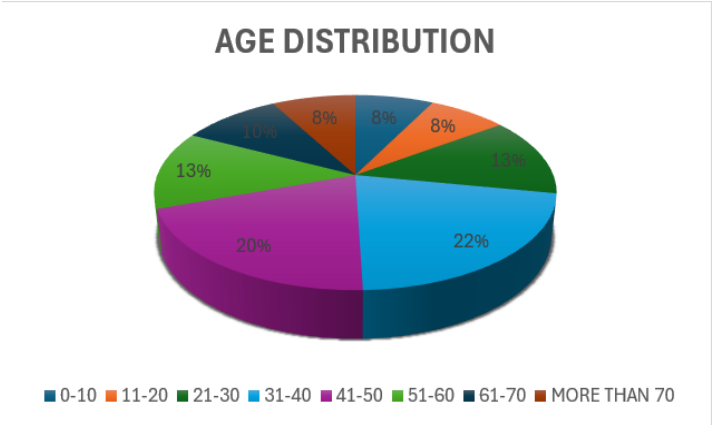
Exclusion criteria

1. All cases of anemia that have undergone blood transfusion.
2. Inadequate quantity of blood sample for automated analyzer (< 3ml)

Statistical Data analysis: A qualitative analysis of the data was done using Pearsons Chi-square test wherever appropriate.

OBSERVATION AND RESULTS:

Our study included patients from the age group 5 years to 80 years. Majority of patients 43 cases (22%) were within 31-40 years of age group followed by 40 cases (20%) were within 41- 50 years. (Graph1).



Gender Distribution: In gender wise distribution, majority were females,113cases(57%) when compared to males 87 cases (43%) as shown in (Table 1).

Table 1: Distribution of study population on the basis of Age and Gender

Age (in yrs)	Sex				Total	
	F		M		Frequency	Percentage
	Frequency	Percentage	Frequency	Percentage		
0-10	12	6%	3	2%	15	8%
11-20	13	7%	2	1%	15	8%
21-30	22	11%	4	2%	26	13%
31-40	39	20%	4	2%	43	22%
41-50	11	6%	29	15%	40	20%
51-60	7	4%	19	10%	26	13%
61-70	5	3%	14	7%	19	10%
>70	4	2%	12	6%	16	8%
Grand Total	113	57%	87	44%	200	100%

In this study, Microcytic Hypochromic anemia constituted the predominant type with 130 cases (65%) followed by Normocytic Normochromic anemia 37 cases (19%), Dimorphic anemia 20 cases (10%), Macrocytic anemia 5 cases (3%). Only 4

cases (2%) each of hemolytic anemia and pancytopenia were seen as in (Graph 2).

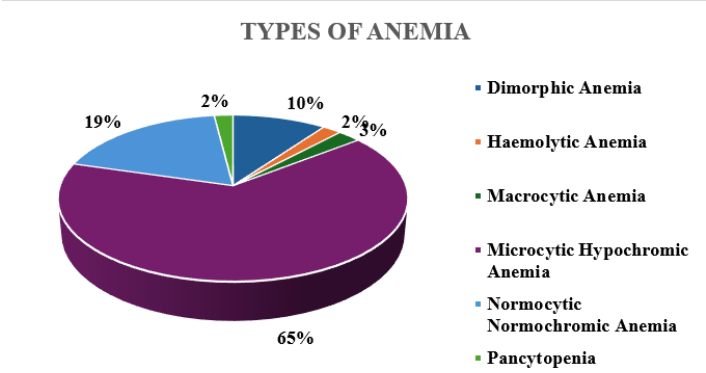


Table 2, shows distribution of RBC histogram curve. Most commonly observed is broad base (36%) followed by left shift (35%). Normal curve observed in 17% of cases. Right shift observed in 5% of cases, bimodal peak in 5 % cases, short peak in 4% cases.

Table 2: Histogram pattern observed in the study

Histogram Pattern	Frequency	Percentage
Bimodal	9	5%
Broad Base	72	36%
Left Shift	69	35%
Normal Curve	34	17%
Right Side	9	5%
Short peak	7	4%
Grand Total	200	100%

Table 3: Comparison between Peripheral Smear findings and RBC histogram in different Anemias

	Bimodal Curve	Broad Base	Left Shift	Normal Curve	Short Peak	Right Shift	Grand Total
Dimorphic Anemia	8	1	2	5	4		20
Haemolytic Anemia		2	2				4
Macrocytic Anemia						5	5
Microcytic Hypochromic Anemia	1	50	65	9	5		130
Normocytic Normochromic Anemia		17		20			37
Pancytopenia		2				2	4
Grand Total	9	72	69	34	9	7	200

The study revealed that most of the findings on peripheral smears can be correlated with the histogram patterns as showed in (Table 3). In smears reported as Normocytic Normochromic anemia, 20cases (54.1%) showed normal Gaussian curve and17 cases (45.9%) showed broad base. In cases reported as Microcytic Hypochromic anemia 64 cases (50%) showed left shift, 9 cases (6.9%) normal curve and 50 cases (38.4%) showed broad base curve, 1 case (0.7%) of bimodal curve, 5 cases (3.9%) of short peak. Dimorphic anemia was an exception where we got various patterns of histogram. Bimodal curve was observed only in 8 cases (40%), 5 cases (25%) of normal curve,4 cases (20%) of short peak and 2 cases (10%) showed left shift, while 1 case (5%) of dimorphic anemias showed broad base curve. All 5 cases (100%) of

macrocytic anemia showed right shift Out of 4 cases of pancytopenia 2 cases (50%) showed broad base and 2 cases (50%) showed right shift. In smears reported as hemolytic anemia 2 cases (50%) showed broadbase,2 cases (50%) showed left shift.

Table 4: Diagnostic Accuracy of Different Histograms in Specific Anemias

Histogram in the diagnosis of specific anaemia	Sensitivity (Se)	Specificity (Sp)	PPV	NPV
Normocytic Normochromic anaemia	54.05%	93.25%	60.71%	88.37%
Microcytic Hypochromic anaemia	50.01%	85.71%	86.67%	48.02%
Macrocytic anaemia	100.0%	88.21%	17.86%	100.0%
Dimorphic anaemia	40.02%	88.89%	28.57%	93.02%

(Note:Se: Sensitivity, Sp: Specificity, NPV: Negative Predictive Value, PPV: Positive Predictive Value)

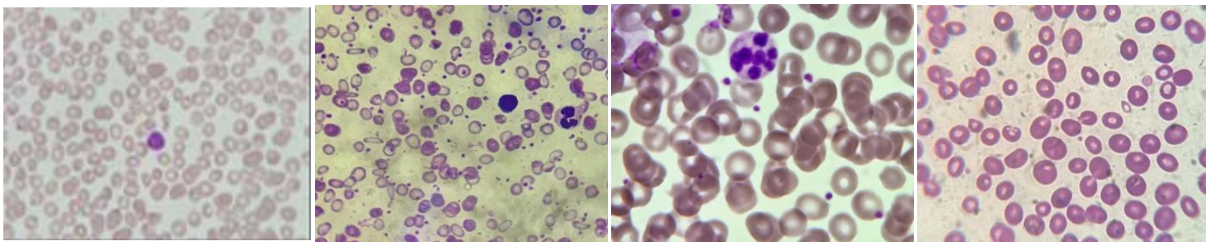


FIG 1 **FIG 2** **FIG 3** **FIG 4**

Figure 1: Peripheral blood smear in Normocytic normochromic anemia; Figure 2: Peripheral blood smear in Microcytic anemia; Figure 3: Peripheral blood smear in Macocytic anemia; Figure 4: Peripheral bloods smear in Dimorphic anemia

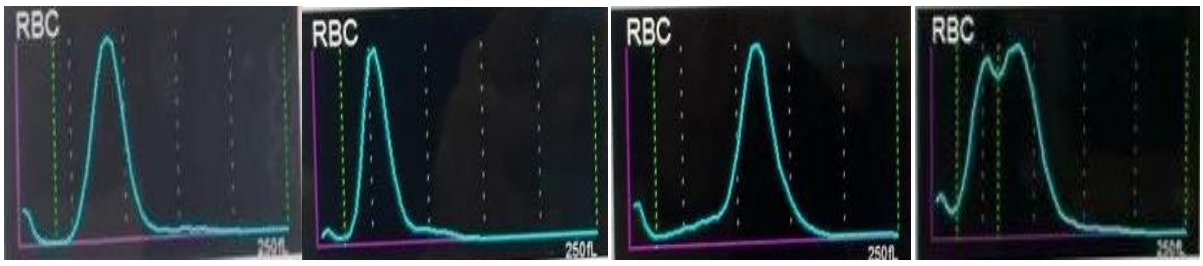


FIG 5 **FIG 6** **FIG 7** **FIG 8**

Figure 5: Normal curve seen in Normocytic normochromic Anemia; Figure 6: Histogram showing right shift in microcytic anemia; Figure 7: Left shift of Histogram in Macrocytic anemia; Figure 8: Bimodal Histogram Pattern in Dimorphic Anemia

DISCUSSION

Age: The study showed a predominance of age group between 31-40years.This can be comparable to the age group in other studies, (Graph1)[1,2].

Gender Distribution: The female preponderance in the study is comparable to the studies done earlier (Table1). In our study it was seen that out of 200 cases majority of cases fall in the adult age group of 31 to 40 years and among 113 female patients majority fall in reproductive age group. These results were in concordance with the studies conducted by Aravinda et al., Rahul Sinha et al and Rose et al. This can be explained as the period of adolescence and adult group has high iron demand. The body needs more iron when it grows rapidly and when frequent blood loss occurs (menstruation). Thus women in reproductive age group are at high risk of developing iron deficiency anemia[1,2].

Analysis of cases of Anemia based on Peripheral Blood Smear examination: The most common morphological type of anemia in the study (Graph 2) was Microcytic hypochromic anemia (64%) followed by Normocytic normochromic

anemia (19%). This is in concordance with various other studies, Aravinda et al and Thomas et al. Iron deficiency anemia is the most common cause of microcytic hypochromic blood picture. WHO has estimated that prevalence of anemia is in all the groups and is higher in India as compared to other developing countries. In our study, Dimorphicanemia, 20 cases (10%), Macrocytic anemia 5 cases (3%) and 4 cases (2%) of hemolytic anemia were diagnosed, which was comparable to the studies[1,2,4].

Comparison between Anemias Diagnosed on Peripheral Smears and Automated Cell Counter Histograms: In the present study 200 patients of anemia were analyzed and their peripheral smear report was compared with the red blood cell histogram pattern obtained from a 6 part differential automated analyzer. We noticed that in smears reported as microcytic hypochromic anemia 50% histograms showed left shift, 54.05% of normocytic normochromic smears showed normal curve and all the smears having macrocytic blood picture showed right shift pattern of histogram (Table 3). Thus we can see that histograms are useful diagnostic aid when it comes to normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic anemia. However the dimorphic anemia showed different histogram patterns from simple curve to complex curves[1,2,3].

In the smears reported as dimorphic anemia, we noticed that 40% of histograms showed bimodal curve and 5% showed broad base. Broad base curve can be explained by the presence of multiple populations of cells of varying sizes (i.e. normocytic, microcytic and macrocytic). Our study was in concordance with the study conducted by Aravinda et al and Thomas et al. In Dimorphic anemia histogram pattern, the centeredness and the width showed the variations in the RBCs. The dimorphic blood picture will look like a dual population of microcytic and normocytic or normocytic and macrocytic red cells or admixture of small, normal and large cells of different sizes and forms with or without normal red blood cell indices which can mislead the diagnosis if we rely on automated values alone. Practically since dimorphic anemia is usually associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results[1,2,3].

Diagnostic accuracy was evaluated in this study and it was observed that histogram showed high sensitivity and high specificity for macrocytic anaemia with right shift curve followed by Normocytic Normochromic with normal curves and left shift for microcytic hypochromic anaemia, (Table 4)[2,3,4].

CONCLUSION

Red cell histogram from automated hematology analyzer provides valuable information regarding the hematological conditions. Our study showed a significant correlation between RBC histograms and peripheral smear diagnosis in Microcytic Hypochromic, Normocytic Normochromic and Macrocytic Anemia. However the correlation between histogram patterns and peripheral smear diagnosis in dimorphic anemia posed queries regarding the authenticity of histogram. Hence it is concluded that in the age of molecular analysis and automation, peripheral smear examination along with clinical history is an important diagnostic tool while treating patients. The use of automated hematologyanalyzers should be encouraged and appropriate training should be made available for its efficient utilization.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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