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Original research article

A study of morphological classification of anaemia with the help of histogram and its correlation with peripheral smear in a tertiary care hospital

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Abstract

Anemia is one of the most common morbidity associated with altered RBC morphology and content. Presently all hematology laboratories have automated hematological analyser. Histograms obtained from the analyser help us to study the RBC morphological alterations. Hence both histograms and peripheral smear together help in the diagnosis and management of anemias.

Aims and Objectives

1. To study the RBC histogram in Anemia cases.
2. To compare the RBC parameters in anemia patients.
3. To compare the RBC histogram with morphological features noted on a peripheral smear.

Methods: The study is a cross-sectional study in Tertiary Care Hospital in the Department of Pathology, Gandhi Hospital. A total of 500 cases were collected during January 2022 to May 2022. All anemic patients blood samples collected in EDTA vacutainers sent for CBC and Peripheral smears with Hb<12gm% are included in the study.

Results: Total 500 cases are categorized into Normocytic, Microcytic. Macrocytic, Dimorphic anemia morphologically by smear examination. Left shift with broad histograms are seen in Microcytic anemia. Right shift curve are seen in Macrocytic anemia. Bimodal peaks are seen in Dimorphic anemia.

Conclusion: Histogram helps in screening the RBC's and along with peripheral smear examination aids the pathologist in correctly diagnosing the RBC disorder.

Keywords: Histogram, morphological classification of anemia, peripheral smear, microcytic anemia, macrocytic anemia, dimorphic anemia

Introduction

Blood cell analysis progressed from manual procedure to automated instruments which give information on blood cell indices and histograms. This information along with peripheral smear examination helps in interpreting abnormal RBC morphology to classify anemia [1, 3, 2, 4]. Anemias are one of the major public issue in children and females of reproductive age group in developing countries [5]. CBC are routine blood tests done in diagnosing anemias. Clinicians and laboratory personnel utilize RBC indices in categorization of anemias but histograms are less evaluated. Hence in our study we would like to compare peripheral smear findings, RBC indices and histogram of anemic patients and categorize anemias for better treatment. Therefore, making histograms user friendly to narrow down the differentials run before a peripheral smear confirmation [6].

Aims and Objectives

1. To study the RBC histogram for Anemia cases.
2. To compare the RBC parameters in Anemic patients.
3. To compare the RBC histogram with morphological features noted on a peripheral smear.

Materials and Methods

Study design: Cross-sectional study.

Study settings: Department of Pathology, Gandhi hospital, Gandhi medical college, Secunderabad.

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Study period: January 2022 to May 2022
Sample population: Patients sent to hematology for evaluation of anemia.
Study sample: Blood sample of patients sent to the Department of Pathology, collected in EDTA vacutainer for CBC and peripheral smear to evaluate anemia cases.
Sample size: 500 cases.

Inclusion criteria
1. Samples of Patients with Hb<12gm% coming to Department of Pathology, Gandhi hospital.
2. Patients of all age groups are included in the study.

Exclusion criteria
1. Incomplete requisition forms.
2. Patients with Hb>12gm% are excluded in our study.
3. Inadequate blood sample in vacutainer (<3ml).
4. Pregnant women excluded in our study.

Tools and Techniques
A Peripheral smear was prepared for the study samples and stained with Leishman stain according to the standard protocol and analyzed in SYSMEX XN-1000 hematology analyzer following the operational manual are studied.

Results and Analysis
The RBC indices, Histogram and peripheral smear of 500 patients having Hb % <12gm/dl are analyzed.

Table 1: Distribution of Anemia cases based on Gender

Gender	Number	Percentage
Male	195	39%
Female	305	61%
Total	500	100%

Anemia is seen predominantly in female population (61%).

Table 2: Distribution of Anemia cases based on the Age distribution

Age Distribution (years)	Frequency	Percentage
0-20	45	9%
21-40	230	46%
41-60	155	31%
61-80	65	13%
Above 80	5	1%

Table 2, shows the distribution of Anemia based on the Age distribution with increased prevalence in the age group of 21-40yrs (46%).

Table 3: Frequency of Anemia based on the severity

Severity of anemia	Frequency	Percentage
Mild	130	26%
Moderate	280	56%
Severe	90	18%
Total	500	100%

Table 3, shows the frequency of Anemia based on the severity showing moderate Anemia being 56%.

Table 4: Distribution of cases as per Morphological types of Anemia

Type of anemia	Frequency	Percentage
Dimorphic anemia	5	1%
Macrocytic anemia	30	6%
Microcytic Hypochromic anemia	210	42%
Normocytic Normochromic anemia	255	51%
Total	500	100%

The distribution of Anemia based on Morphology with Normocytic Normochromic Anemia more predominant followed by Microcytic Hypochromic Anemia.

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Table 5: RBC Indices in relation with morphological classification of Anemias

	MCV	MCH	MCHC	RDW
Normocytic Normochromic	Normal	Normal	Normal	Normal/Increased
Microcytic anemia	Decreased	Decreased	Normal	Increased
Macrocytic anemia	Increased	Increased	Normal	Increased
Dimorphic anemia	Normal	Normal	Normal	Increased

In our study Normocytic, Normochromic anemia has MCV, MCH, MCHC within normal limits and normal or increased RDW. Microcytic anemia have decreased MCV, MCH than normal range, Normal range MCHC and increased RDW. Macrocytic anemia have increased range of MCV, MCH normal range MCHC and increased RDW. Dimorphic anemia has normal range MCV, MCH, MCHC and increased RDW. Increased RDW seen due to marked anisopoikilocytosis.

Table 6: RBC Histograms in the study

Type of histogram	Frequency	Percentage
Left shift	15	3%
Normal	80	16%
Normal (Short Peak)	100	20%
Left shift (Broad Base)	130	26%
Left shift (Short Peak)	35	7%
Normal (Broad Base)	105	21%
Right shift (Short Peak)	30	6%
Double Peak	5	1%
Total	500	100%

Table 7: Shows RBC histograms with left shift broad base more predominant

Type of histogram	Percentage %
Normal Curve	16%
Left Shift	24%
Right Shift	6%
Broad Base	26%
Short Peak	27%
Bimodal Peak	1%

Table 8: Shows Types of histograms

	Normal curve	Left shift	Right shift	Broad base	Short peak	Bimodal
Normocytic	16%	--	--	21%	20%	--
Microcytic	--	3%	--	26%	7%	--
Macrocytic	--	--	6%	--	--	--
Dimorphic	--	--	--	--	--	1%

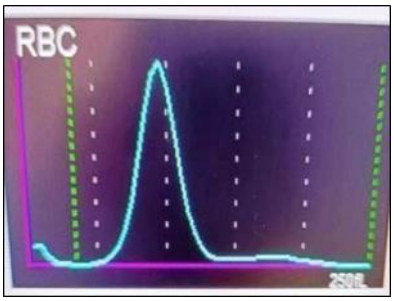

Fig-1 : Normal RBC Histogram.

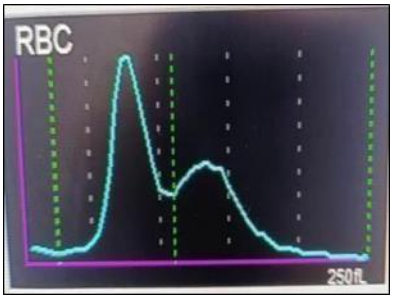

Fig -2 : Histogram showing double peak

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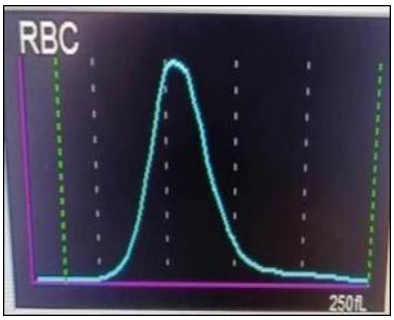

Fig-3 : Histogram showing broad base with right shift.

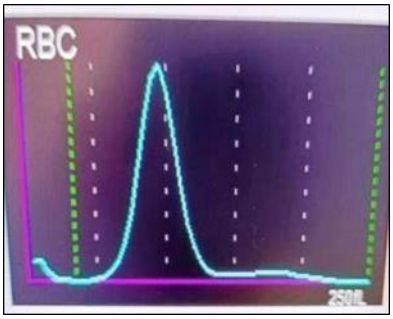

Fig-4 : Histogram with left shift

Discussion

Histograms are graphical representation of particle size distribution. Normally the RBC curve is symmetrical bell shaped or Gaussian distribution.7 the area of the peak is used to calculate the MCV and RDW. Size (volume) is on X-axis and number of cells passed per channel on Y-axis. So, if the RBC'S are smaller i.e.; MCV<80fl microcytic, the curve will shift towards left [8, 9, 10]. If the RBC'S are larger i.e.; MCV>80fl macrocytic, the curve will shift towards right [8, 9]. macrocytosis is seen in number of conditions of all age groups predominantly seen in old age group [11, 12, 13]. The cells with volume size between 36-360fl are counted as RBC'S. The space below 36fl is clear normally but if the baseline is above it could be due to small particles like microspherocytes, norm oblasts, malarial parasites, platelet clumps, bacteria, elliptocytes, agglutinates etc. [9, 14, 15, 16] Normally WBC'S also pass through RBC channel and are counted as RBC and it does not affect the counts as RBC counts are in millions. But we need to consider if the WBC counts are high mainly when above 50,000 it may alter the histogram [9]. Normally RBC give a narrow distribution curve but the curve is broad if there is more anisopoikilocytosis along with high RDW as seen in iron deficiency anemia [17, 18, 19]. The curve is bimodal if there is dimorphic anemia with both left and right shifting of curve. Causes of Dimorphic anemia could be nutritional anemia, recent blood transfusion, sideroblastic anemia or if the patient is in treatment and is responding to it [20, 21].

Table 9: Results of the present study are compared with other studies and are as follows:

Histogram	Sandhya *et al.*	Chavda J *et al.*	Rao BSS *et al.*	Shrivastav *et al.*	Rahul Sinha *et al.*	Present study
Normal curve	15%	19%	17.7%	18%	16%	16%
Left shift	30%	27%	29.0%	29%	33%	35%
Right shift	6%	07%	5.45%	06%	8%	6%
Bimodal	4%	3%	7.27%	05%	02%	1%
Broad base	40%	38%	37.72%	40%	34%	31%
Short peak	5%	6%	2.7%	02%	7%	11%

In the present study of 500 cases maximum number of cases had left shift (35%) followed by broad base (31%), Normocytic (16%), right shift (6%) Dimorphic (1%). Other studies like Sandhya *et al.* [22, 23], Chavda *et al.* [24], Rao *et al.* [11], Shrinivas *et al.* [25], Rahul Sinha *et al.* [26] are all correlating to our present study with maximum microcytic anemia (left shift) cases.

Table 10: Comparison and correlation of histogram and peripheral smear

Type of anemia	Peripheral smear analysis (n=500)	Histogram and RBC indices (n=500)
Normocytic normochromic	177(35.4)	210(41%)
Microcytic anemia	268(53.6)	255(51%)
Macrocytic anemia	42(8.4)	30(6%)
Dimorphic anemia	13(2.6)	5(1%)

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Present table shows the importance of histogram with indices in diagnosing anemia's, compare and correlate with diagnosis made by peripheral smear Though Peripheral smear examination remains the definitive diagnostic test for evaluation of anemia's.

Conclusion

Histogram study acts as supplementary tool to diagnose various RBC disorders along with blood indices, HB value and peripheral smear examination. There can be overlapping in correlation of histograms with different anemias that are diagnosed on peripheral smear. Hence peripheral smear still stands as gold standard method in categorizing anemia based on morphology and histogram study helps as an additional tool to describe RBC morphology.

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