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Original Research Article

RBC histogram: Useful diagnostic tool in evaluating anaemia

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ABSTRACT

Background: Anaemia is a widespread public health problem, more common in developing countries. Red blood cell histogram is a vital part for evaluation and diagnosis of anaemia. Histogram can be a useful modality for arriving at the probable diagnosis of anaemia reporting in short span of time.
Aims and Objectives: To observe various RBC Histogram on haematology Analyser in anaemia and analyse the usefulness of RBC Histogram to correlate with peripheral smear.
Materials and Methods: A prospective study was done in Department of Pathology of Secondary Care hospital from January 2024 to March 2024 on 300 anaemia patients. RBC Histogram were observed, analysed and correlated with peripheral smear.
Results: Out of total 300 patients, Most common anaemia is Microcytic Hypochromic (60%), Normocytic normochromic (21.33%), Dimorphic anaemia (12.33%) and macrocytic anaemia (6%). 95.5% microcytic hypochromic anaemia showed left shifted curve, 84.3% Normocytic normochromic anaemia showed normal curve, 88% macrocytic anaemia showed right shift curve.
Conclusion: Considering the speed, reliability and less inter observer variation of the modern haematological analysers RBC histogram are important diagnostic tool for various types of anaemia and provide accurate morphological typing of anaemia in most cases hence presumptive report can be released based on RBC histogram.

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1. Introduction

Anaemia is a widespread public health issue, particularly prevalent in developing countries, with significant repercussions for health, economic stability, and national progress. The World Health Organization (WHO) defines anaemia as a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet the body's physiological needs. Nutritional deficiencies are often cited as the primary cause of anaemia. Histograms can serve as a valuable tool for assessing the reliability of haematology analyzer results, identifying potential causes of discrepancies, and facilitating probable diagnoses.[1]

Red blood cell histogram is a vital part for evaluation and diagnosis of anaemia for management of various red blood cell (RBC) disorders. Parameters reported while performing complete blood count on analyser like red cell distribution width (RDW) and mean corpuscular volume (MCV) are useful along with histogram for interpretation of abnormal red blood cell morphology.[1,2] Normal red blood cell histograms are symmetric, single- peaked, and gaussian or "bell shaped" curves. The distribution should always start and end on base line and should be located between the lower discriminator and the upper discriminators.[3]

Shift in one direction or another can be of diagnostic importance and in association with other parameters such as red cell distribution width (RDW) and RBC indices (MCV, MCH, MCHC) it has been found to

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be very useful in diagnosing abnormal haematological conditions.[1,4] Evaluation of anaemia for diagnosis and management of various red blood cell (RBC) disorders through red blood cell histogram is a vital part. Complete blood count parameters like red cell distribution width (RDW) and mean corpuscular volume (MCV) are useful along with histogram for interpretation of abnormal red blood cell morphology. The narrow red cell distribution curve shows homogenous population of cells and wider red cell distribution curve indicates a heterogenous population of red cells. In macrocytic anaemia where the red blood cells are larger, the histogram curve will shift to right and the curve will move to left if the cells are smaller than normal like in microcytic anaemia. After treatment of underlying cause in anaemia, the curve shifts toward the normal range. In dimorphic anaemia the histogram curve may show multiple peaks due to two distinct red cell populations. Even when MCV is normal, RDW is a good indicator of anisocytosis. Higher RDW represents dual population of cells like small cells, some normal size cells, and immature red cells during degenerative response to anaemia which are larger than normal. Histogram can be useful for monitoring the reliability of results of analyser, potential causes of results and arriving at the probable diagnosis of anaemia.[5]

2. Aims and Objectives

1. To observe the RBC Histogram obtained from automated hematology analyzer in anaemia patients and compare with peripheral smear examination.
2. To study utility of RBC histogram as diagnostic tool for evaluating anaemia and recommend its use.

3. Materials and Methods

This is a prospective study conducted in the Department of Pathology, Acharyashree Bhikshu Government Hospital, Moti Nagar, New Delhi, India from January 2024 to March 2024. A total of 300 samples of anaemic patients sent for Complete Blood Count were used for the present study. All patients who are diagnosed as anaema according to WHO definition were included. For men anaemia is defined as haemoglobin level less than 13.0 gm/dl and in women as haemoglobin of less than 12.0 gm/dl.

3.1. Methodology

The venous blood sample was collected by venepuncture in di potassium EDTA vacutainers and analysed by the haematology analyser, Mindray BC- 6200. Complete blood count which included Haemoglobin estimation, Total leucocyte count, Differential leucocyte count, Platelet count & Red blood cell (RBC) indices along with histograms were obtained. Peripheral blood smears were prepared, dried and stained with Leishman stain and morphological features were observed during microscopic examination. Anaemia typing was done first by parameters obtained by haematology analyser, position and shape of histograms which were then correlated with peripheral blood smear findings.

4. Results

In our study, RBC histograms of various types of anaemia in a total 300 cases were studied. This study includes predominantly females 207 (69%) and males 93 (31%) (Table 1) and maximum number of anaemia cases were noted in 21-30 years of age (37.67%) (Table 2). All cases had anaemia with haemoglobin less than 12 gm/dl in females and less than 13gm/dl in males. The cases were categorised into mild (Hb 8-12gm/dl for females and 8-13 g/dl for males), moderate (Hb 5-7.9 gm/dl) and severe degree of anaemia (Hb <4.9 gm/dl). Maximum number of cases (54.33%) had moderate degree of anaemia, 37.67% of the cases showed mild degree of anaemia, 8% had severe anaemia.(Table 3). The cases consist of Microcytic hypochromic anaemia, Macrocytic anaemia, dimorphic anaemia and Normocytic normochromic anaemia, as diagnosed by peripheral smear. Most common anaemia is Microcytic Hypochromic (60%), Normocytic normochromic (21.33%), Dimorphic anaemia (12.33%) and macrocytic anaemia (6%) (Table 4).

Table 1: Gender distributionof anaemic patients (total 300) in the study

Gender	Total count	%
Male	93	31
Female	207	69

4.1. RBC Histograms observed in the study

In a normal RBC histogram in our haematology analyser, the majority ranges between 50 fL and 130fL.

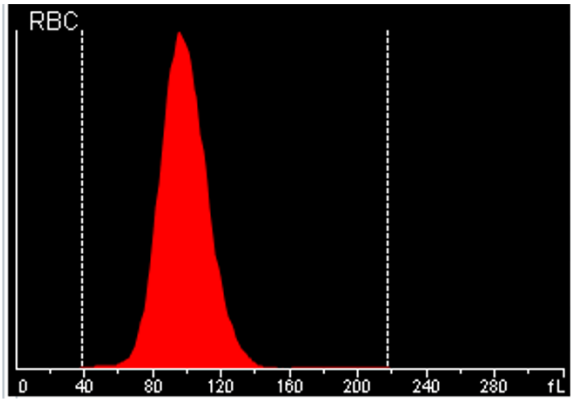

Figure 1: Normal RBC histogram

Table 2: Age distribution of anaemic patients (total 300) in the study

Age range (yrs)	**0-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**>60**
No. of cases	40	51	113	48	24	14	10
Percentage (%)	13.33	17	37.67	16	8	4.67	3.33

Table 3: Severity of anaemia

S. No.	**Severity of anaemia**	**No. of cases**	**Percentage (%)**
1.	Mild (Hb 8-12g/dl for females; 8-13 g/dl for males)	113	37.67
2.	Moderate (Hb 5-7.9 g/dl)	163	54.33
3.	Severe (Hb <4.9 g/dl)	24	8

Table 4: Case distribution as per types of anaemia diagnosed on peripheral smear

S. No.	**Type of Anaemia**	**No. of cases**	**Percentage (%)**
1.	Microcytic Hypochromic	180	60
2.	Macrocytic	18	6
3.	Normocytic	64	21.33
4.	Dimorphic	38	12.67

When the distribution is not symmetric, it is referred to as skewed.

Various patterns noted were left shift, right shift, Normal, Bimodal peak, broad base, left shoulder and right shoulder. (Figures 2, 3, 4, 5, 6 and 7) Left shift histogram was observed as most common in 57.67% cases of anaemia followed by normal curve (22.67%), right shift and broad based both 7.33%, bimodal peak 4.33% and left and right shoulder both in 0.33 cases. (Table 5)

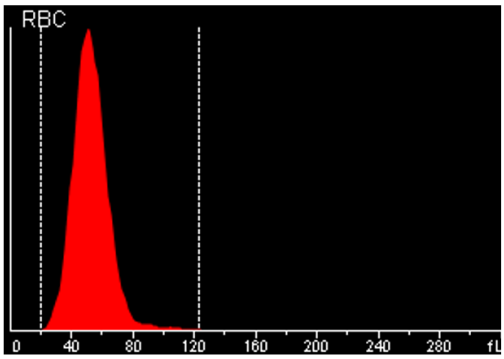

Figure 2: RBC histogram showing left shift

4.2. Correlation of red cells histogram with peripheral smear findings

Maximum number of cases 180 (60%) were of microcytic hypochromic anaemia and showed various types of histograms, 172 (95.55%) were having left shifted curve, 5 histograms were normal, 2 showed broad based curve and 1 of the cases showed right shouldering. Normocytic normochromic anaemia having total 64 cases, 54 (84.37%) showed normal curve, 6 of the cases showed broad based curve, 3 cases showed a slight shift to right and 1 case

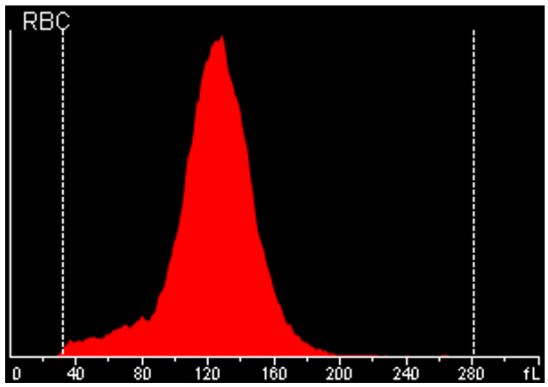

Figure 3: RBC histogram showing right shift

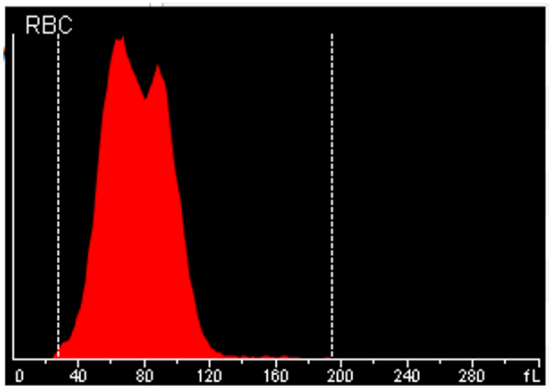

Figure 4: RBC histogram showing double peak

Table 5: Distribution of RBC histogram in present study

S. No.	Type of histogram	No. of cases	Percentage %
1.	Left shift	173	57.67
2.	Normal curve	68	22.67
3.	Right shift	22	7.33
4.	Broad base	22	7.33
5.	Bimodal peak	13	4.33
6.	Left shoulder	1	0.33
7.	Right shoulder	1	0.33

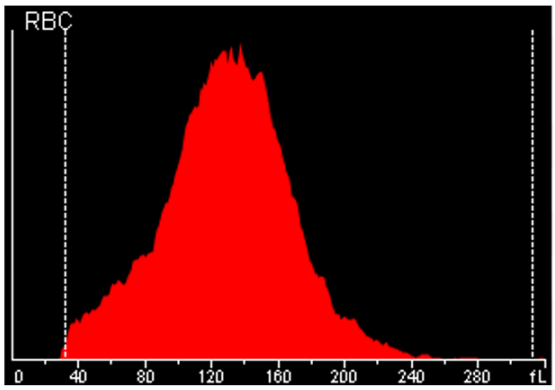

Figure 5: RBC histogram showing broad base

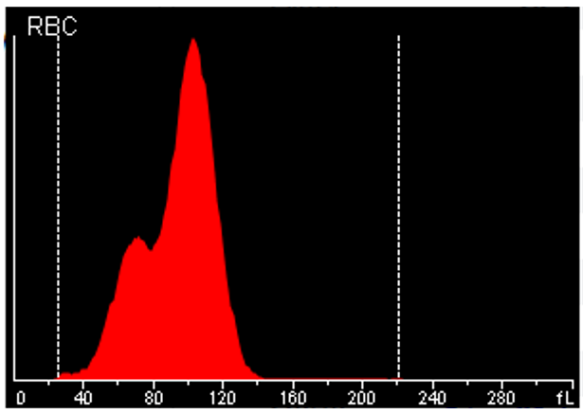

Figure 6: RBC histogram showing left shouldering

presented with left shouldering. 18 (6%) cases of macrocytic anaemia were found on microscopy which showed right shift curve in 16 (88.88%) of the cases and showed broad based curve histogram in rest of 2 cases. 38 cases of Dimorphic anaemia were observed on microscopy, and they showed broad based curve in 12 and bimodal curve in 13 cases, normal curve in 9 cases, left shift curve in 1, right shift curve in 3. (Table 6)

On correlating the red blood cell histogram with the peripheral smear findings, we observed that 276 out of 300 cases i.e. 92% cases showed positive correlation, on comparing the histogram along with the RDW values and

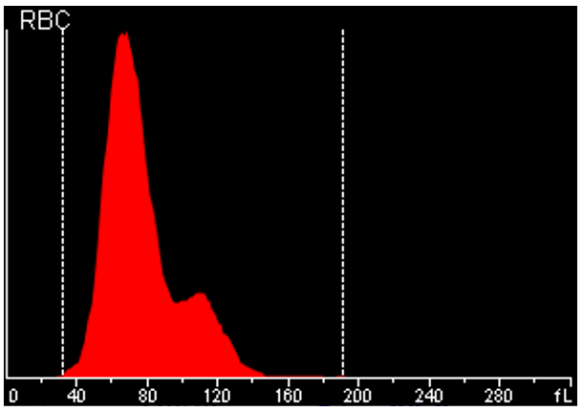

Figure 7: RBC histogram showing right shouldering

the peripheral smear findings, we observed about 291 (97%) cases showing positive correlation. (Table 7). Of the 24 (8%) cases that did not show positive correlation, when compared with the red cell distribution width (RDW) values, 15(62.5%) of the cases showed direct correlation.(Table 8)

5. Discussion

Anaemia is a major public health problem world over. It causes increased morbidity and mortality, especially in children and pregnant females. Timely and accurate diagnosis is very important. Peripheral blood smears (PBS) are being used as a diagnostic tool for the classification of anaemia. The introduction of hematology analyzer has improved the accuracy of reporting complete blood count parameters and significantly reduced the workload in the laboratory. The present study was conducted to evaluate whether the anaemia parameters and histograms obtained by the haematology analyser correlated well with the observations made after examining peripheral blood smear manually and in case a good correlation between the two can be established, whether haematology analyser can replace the manual confirmation of the result.

Histogram is a graphical representation obtained from automated haematology analyser. The RBC histogram is a graphic representation of particle size distribution in automated cell analyser. It helps in diagnosis of various disorders and gives valuable information regarding the RBC

Table 6: Presentation of RBC histograms in different types of anaemia

Type of anemia on morphology (PS findings)	Normal curve	Left shift	Right shift	Broad base	Bimodal peak	Right shoulder	Left shoulder	Total
Microcytic hypochromic	5	172	-	2	-	1	-	180
Normocytic	54	-	3	6	-	-	1	64
Macrocytic	-	-	16	2	-	-	-	18
Dimorphic	9	1	3	12	13	-	-	38

Table 7: RBC histogram correlation with peripheral smear findings

Histogram correlation with Peripheral Smear microscopy	**No. of Cases**	**Percentage**
Yes	276	92%
No	24	8%
Histogram and RDW correlation with Peripheral Smear microscopy	No. of Cases	Percentage
Yes	291	97%
No	9	3%

Table 8: Red cell distribution width (RDW) correlation with peripheral smear findings

RDW correlation with Peripheral Smear	**Yes**	**No**
No. of Cases	15	9
Percentage	62.5%	37.5%

parameters like RDW, MCH and MCV. Normal curve is symmetrical bell shaped or Gaussian distribution and falls within normal range of MCV which is 80-100 fl. The RBC histogram in the hematology analyzer displays the ranges for RBC are between 24fl and 360fl. The analyzer counts only those RBC's with volume sizes between 36fl to 360fl as red cells and cells which are counted in the range 24fl to 36 fl are not included in the RBC count and rejected by counter. Normally below 36fl size space is clear and histogram begins from base line but if histogram begin above the base line indicates the presence of small particles like platelet clumps, malaria parasite, microspherocytes, etc.

In present study of 300 cases, maximum numbers of cases have microcytic anaemia (60%) followed by normocytic (21.33%), Dimorphic (12.67%) and Macrocytic (6%). Other studies like sandhya et al.[6] Chavda J et al.[7] Atul Shrivastava et al.[4] & Byna Syam Sundara Rao et al.[5] also found having similar findings regarding distribution of anaemia cases. Our study of RBC histogram showed normal curve (22.66%), left shift (57.66%), right shift (7.33%) Broad base (7.33%) and bimodal (4.33%) and these findings regarding to RBC histogram were also in concordance with other studies like Sandhya et al.[6] Chavda J et al.[7] Atul Shrivastava et al.[4] & Rao BSS et al.[5] Gupta V et al. studied 900 cases of anaemia, most common was microcytic hypochromic anaemia that showed most common pattern as left shift and all cases of macrocytic anaemia showed Right shift on RBC histogram. Their study showed that the haematology analyser provided accurate morphological categorization in most cases of microcytic hypochromic, normocytic normochromic and macrocytic anaemia.[1]

Shifa et al observed that histogram can also be used in differentiating various types of anaemia. Histogram changes correlated well with peripheral smear findings in majority of the cases. Histogram analysis is often a neglected part of the automated haemogram which if interpreted well, has significant potential to provide diagnostically relevant information even before higher level investigations are ordered.[2] Shrivastava A et al concluded RBC Histogram is an important tool of diagnosis. By observing RBC histogram 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 gives information about RBC morphology. Histograms are useful tool for technologists as it could guide them that which cases need actual detailed peripheral smear examination by experts. Findings of automated analyser 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 anaemia.[4]

Rao BSS et al. found that Peripherial smear interpretation was significantly correlated with histogram curve in 220 anaemia cases (p value <0.001, chi square value 74.28) and concluded Histogram Changes correlated well with peripheral smear findings in majority of the cases.[5] Singhal S et al recommended that Red blood cell (RBC) parameters and histograms are indispensable for the diagnosis of anaemia; however, peripheral blood smear

(PBS) examination is also very important as it provides crucial information about patients' clinical conditions. Hence, a combined approach to diagnosis of anaemia is considered the gold standard and essential for definitive diagnosis.[8]

Shashidhar et al concluded that the histogram may be used as a screening tool that can minimize the use of peripheral smear examinations, and by comparing the results of both approaches, we can diagnose the majority of anaemia cases.[9] Korgaonker KA et al. studied 500 patients of anaemia and found the histogram patterns correlated with the peripheral smear findings in majority cases of normocytic normochromic anaemia, microcytic hypochromic anaemia and macrocytic hypochromic anaemia.[10] Erythrocyte size and haemoglobin distribution cytograms provide very useful qualitative information, which is an adjunct to the numerical data.[11] Neelima M et al found Histogram 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 anaemias that are diagnosed on peripheral smear. Hence, peripheral smear still stands as gold standard method in categorizing anaemia based on morphology and histogram study helps as an additional tool to describe RBC morphology.[12] The peripheral blood smear examination along with Histogram analysis can prove a very promising and complementary tool in diagnosis of anaemias. Histogram can provide subtle information about the RBC pathology and many a times can act as time saving method.[13] Our study correlates well with the above mentioned studies and we observed about 291 (97%) cases showing positive correlation when compared with RBC histogram.

6. Conclusion

In our study RBC histogram changes correlated well with peripheral smear findings in majority of the cases. Hence, we conclude RBC histogram is an important tool of diagnosis and it can also be used in differentiating various types of anaemia. Histogram analysis is generally a neglected part of the automated hemogram which if interpreted well, has significant potential to provide diagnostically relevant information even before higher level investigations are ordered. The speed and the reliability of the modern haematological analysers allow technologists, time to evaluate abnormal blood films, consider diagnostic clues and correlate clinical findings to histograms and other hematologic parameters with greater confidence and efficiency, all of this helps in providing better patient health care. Though, peripheral smear examination even today cannot be totally replaced by automated haematology analysers as they provide so much additional information, the present generations of automated haematology analysers are well at par and provide accurate morphological typing of anaemia in most cases thus reduce the workload of Pathologist, laboratory staff and thereby increasing the efficiency of a laboratory.

7. Source of Funding

None.

8. Conflict of Interest

None.

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