



**CORRELATION OF PERIPHERAL BLOOD SMEAR WITH RBC HISTOGRAM IN
THE DIAGNOSIS OF ANEMIA-A STUDY IN A TERTIARY CARE CENTER**

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ABSTRACT:

Introduction: Anemia continues to be a major public health problem worldwide, particularly among females of reproductive age in developing countries. RBC histogram is an integral part of automated analysis which in association with RBC indices, hematocrit and RBC distribution width provides major clues in the diagnosis and management of red cell disorders

Methodology: The present study was done as a retrospective comparative study in a total of 100 adult blood samples, in our study all adult patients with hemoglobin level below 11 gm%. were included The histogram were studied in comparison to their respective Peripheral Blood smears The RBC indices were seen in association with Histogram pattern. In this study we have correlated the RBC histogram abnormalities with peripheral blood smear findings.

Results: Out of 100 cases all were anemia cases. Analysis based on RBC indices and Histogram: Of the 100 cases, majority (52%) were microcytic hypochromic anemia. Normocytic macrocytic, dimorphic, macrocytic and hemolytic were 22%, 10%, 14% and 2% respectively. While according to RBC histogram it was mildly different with 54% being microcytic hypochromic anemia. Normocytic macrocytic, dimorphic, macrocytic and hemolytic were 21%, 112%, 12% and 1% respectively.

Conclusion: In the age of molecular analysis and automation using various five, six and seven part analyzers, peripheral smear examination along with clinical history is an important diagnostic tool. RBC histograms can be used as a preliminary screening modality to identify any structural and morphological abnormalities. Histograms along with blood indices and hemoglobin values will guide us towards the RBC morphology.

KEYWORDS: Microcytic Hypochromic anemia, RBC Histogram

INTRODUCTION

Anemia continues to be a major public health problem worldwide, particularly among females of reproductive age in developing countries. Between 1993-2003 World Health Organization's global estimates of anemia prevalence averaged 49 %.¹ Anemias constitutes an important diagnostic and clinical category of hematological disorders prevalent all over the world.

The automated hematology analyzers are based on the impedance principle and rely on the change in conductance as each cell passes through an aperture. This change in conductance results in the development of an electrical pulse the amplitude of which is proportional to the cell volume. The results are displayed as numerical and histograms. RBC histogram is a standard part and is routinely generated by automated cell analyzers. The well-known Coulter principle of counting and sizing the cells provides a basis for generating a histogram. Histograms are the graphical representation of cell frequencies versus size. X-axis represents the size of the cell and Y-axis represents the number of cells. RBC indices (MCV, MCH, and MCHC) have been found abnormal in various hematological conditions. RBC Indices were first introduced by Wintrobe to determine Abnormalities in red cell histogram including left shift of the curve in microcytosis, Right shift of the curve in macrocytosis, and bimodal peak of the curve in double (dimorphic) population of red cells.

RBC histogram is an integral part of automated analysis which in association with RBC indices, hematocrit and RBC distribution width provides major clues in the diagnosis and management of red cell disorders.²it provides valuable information not apparent in the numerical data as in megaloblastic anemia with developing iron deficiency. The small population of microcytic hypochromic cells is identified in the histogram as a double peak³ while MCV being an average value does not reflect the heterogeneity of the RBC population. On the other hand, a double peak histogram curve only indicates the presence of dual cell population which needs to be further correlated with peripheral smear findings. There have been few studies on the utility and comparison of peripheral smear findings with RBC histograms and RBC indices.

Amongst various indices, MCV and RDW were important for interpretation of morphology. 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. The examination of peripheral blood smear has been one of the main stays for the diagnosis of and also to identify the etiology of anemia. The manual methods of determining the hematological parameters have almost been completely replaced by various automated hematology analyzers which also provides the corresponding histograms which can be used to arrive at a preliminary diagnosis even before examining a peripheral smear. In this study we have correlated the RBC histogram abnormalities with peripheral blood smear findings.

MATERIAL AND METHODS

The present study was done as a retrospective comparative study in a total of 100 adult blood samples were collected from patients admitted in Saveetha Medical College and Hospital, Chennai from June 2023 to July 2023. The corresponding histograms were collected from Sysmex XN1000-Six part analyzer. The histogram was studied in comparison to their respective Peripheral Blood smears. The RBC indices were seen in association with Histogram pattern. Normally positioned 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 hemolytic anemia.

Following red cell indices were obtained from the hematology analyzer. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). Peripheral smear was done to subtype the anemia. In our study all adult patients with hemoglobin level below 11 gm% were included whereas All patients with hemoglobin > 11 gm%, normal histograms and normal peripheral blood smears were excluded.

RESULTS

The RBC indices, histogram and peripheral smear of 100 patients having Hb less than 11 gm/dl were analyzed. The age group of patients included in this study ranged from 18 to 80 years. Majority of patients were between 41 and 50 years of age followed by 31- 40 yrs. and 21-30 year age group. Out of 100 patients 59 % were females and 41% were males. Majority of females were in reproductive age group. After 40 years of age males were seen to be affected more than females.

RBC indices in study population: Hemoglobin in study population ranged from 2 to 11 gm % with mean of 7.7 gm%. MCV in study population ranged from 49.5 fl to 139 fl with mean of 81.09 fl. MCHC in study population ranged from 18.7 gm /dl to 42.6 g/dl with mean

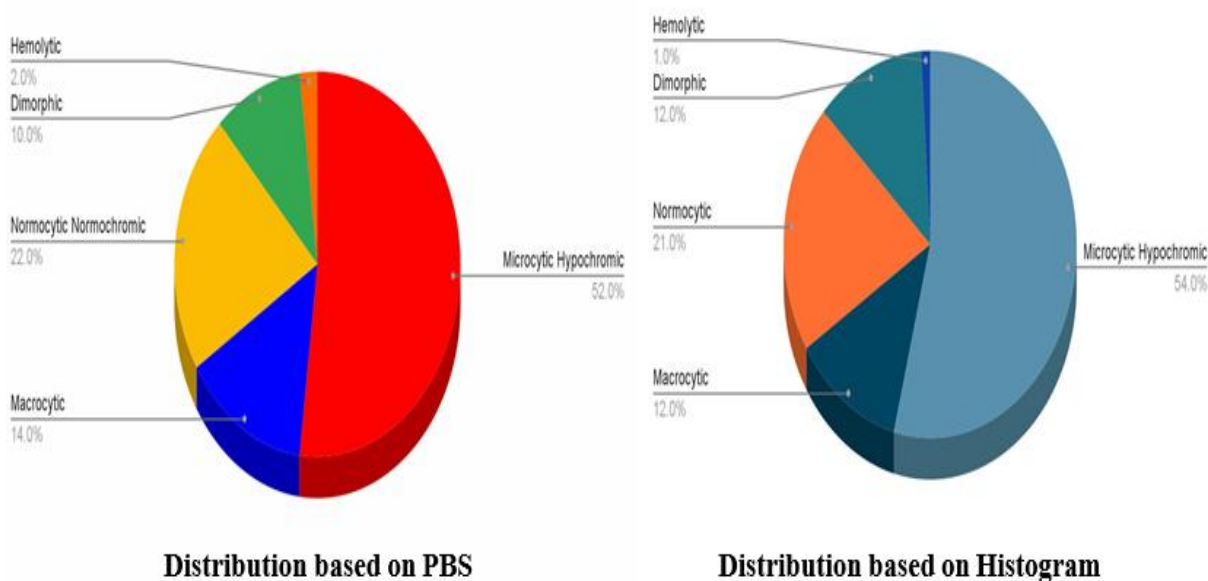
of 31.11 g/dl. MCH in study population ranged from 13.7pg to 44.9 pg with mean of 25.2 pg. RDW in study population ranged from 12.4 % to 39.2 % with mean of 19.3%. PCV ranged from 9.5% to 65% with mean of 27.3 %.

Analysis based on RBC indices and Histogram: Of the 100 cases, majority (52%) were microcytic hypochromic anemia. Normocytic macrocytic, dimorphic, macrocytic and hemolytic were 22%, 10%, 14% and 2% respectively. While according to RBC histogram it was mildly different with 54% being microcytic hypochromic anemia. Normocytic macrocytic, dimorphic, macrocytic and hemolytic were 21%, 112%, 12% and 1% respectively.

Table 1: Distribution based on peripheral blood smear and Histogram

Type Of Anemia	Peripheral Smear analysis	Histogram analysis
Microcytic Hypochromic	52	54
Macrocytic	14	12
Normocytic Normochromic	22	21
Dimorphic	10	12
Hemolytic	2	1
Total	100	100

Figure 1: Comparison based on PBS and Histogram

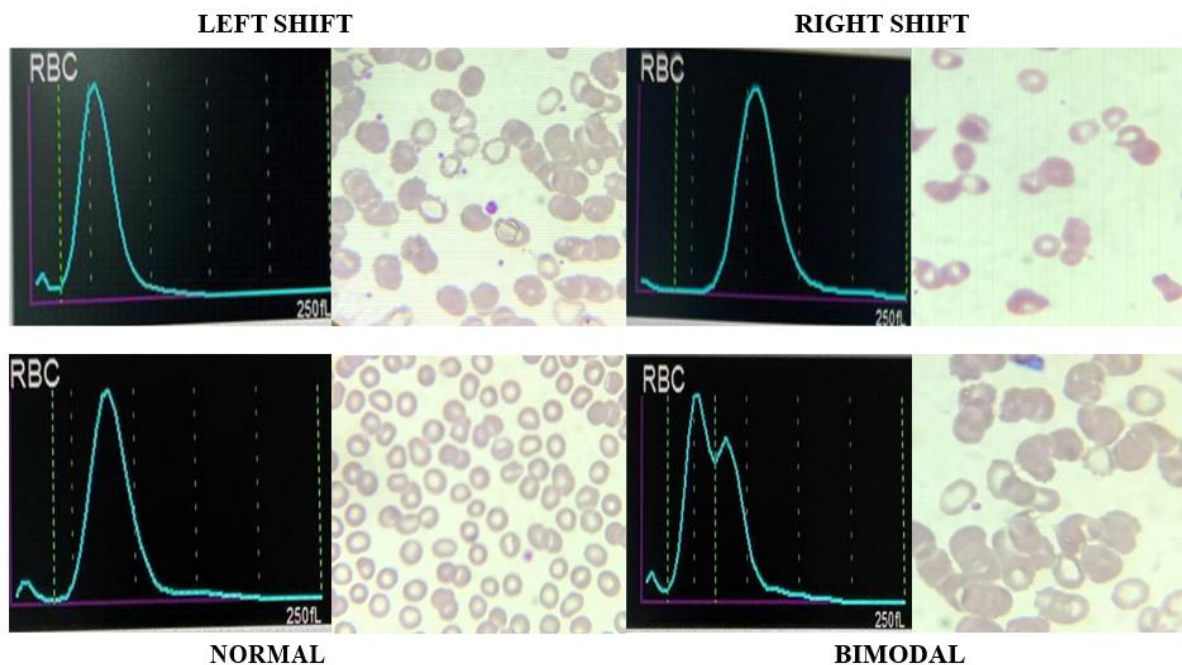


In microcytic hypochromic anemia, most common histogram pattern seen in microcytic hypochromic was shift to left with broad base. Few cases showed bimodal pattern. In macrocytic anemia, Histogram pattern showed shift to right in majority of cases. Also seen was bimodal pattern. Very few cases showed broad base curve.

In dimorphic anemia, a bimodal peak with a broad base was seen in all the cases. In addition to the bimodal pattern, 50 cases showed right shift with skewing of the histogram to the left indicative of combined nutritional deficiency (macrocytes with few microcytes). The histogram patterns seen in hemolytic anemia was shift to left with broad base (Fig3) with a few cases showed bimodal pattern and shift to right.

The impression made by Peripheral smear vs RBC histogram and indices were statistically analyzed. The correlation between the diagnosis made by Peripheral smear vs RBC histogram and indices was analyzed using Cramer's V which was statistically significant with p value of 0.0001 Kappa statistics for agreement between the two methods=0.518 had moderate agreements with p value of 0.0001.

Figure 2: RBC Histograms



DISCUSSION

A major public health problem worldwide, particularly among females of reproductive age in developing countries is anemia. Constantanio² and others have compared

Red cell histogram and RBC indices while others have correlated RBC indices and peripheral smears.⁴ But a correlation between all three parameters have been infrequently analyzed. The present study was thus undertaken to correlate these in providing a better approach to the diagnosis of anemia.

In our study predominant age group was between 41 to 50 years and there was female preponderance. As in other studies by Japeth E and others⁵ on peripheral smear examination 52 of 100 cases were normocytic normochromic anemia. On histogram analysis 54 cases showed position within normal limits and MCV ranged from 82-92fl. So diagnosis based on RBC indices and histograms were comparable to diagnosis with peripheral smear examination. It is comparable to study done by Kumar and others.⁴

Macrocytic anemia was seen in 14 of 100 cases on peripheral smear examination. But histogram and RBC indices showed 12 cases as macrocytic anemia. This difference in the categorization was as a result of the interpretation of cases of hemolytic anemia as macrocytic anemia due to the presence of polychromatophils. Though MCV by automated blood cell counter is rarely inaccurate, hyperglycemia, marked leukocytosis and cold agglutinins may result in false elevation of the MCV.

Few cases diagnosed as macrocytic on histogram and RBC indices analysis were diagnosed as dimorphic on peripheral smear examination. 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 and others.⁽⁸⁾ Only few cases were diagnosed as hemolytic anemia on Histogram and Indices analysis as compared to peripheral smear because fragmented RBCs were counted as microcytes and polychromatophils seen in hemolytic anemia were counted as macrocytes by cell counters. These findings points to the limitation of RBC histograms and RBC indices in the diagnosis of Hemolytic Anemia's.

A pure bimodal histogram is associated with therapeutic transfusion while a bimodal curve with a right shift and skewing towards left indicates a combined nutritional anemia. These findings seen in our study are in agreement with those described by previous studies⁷ However since dimorphic anemia is associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better

interpretation of results.

CONCLUSION

The study revealed that most of the findings on peripheral smears can be correlated with the histogram patterns. In the age of molecular analysis and automation using various five, six and seven part analyzers, peripheral smear examination along with clinical history is an important diagnostic tool. RBC histograms can be used as a preliminary screening modality to identify any structural and morphological abnormalities. By observing these curves we could give presumptive diagnoses based on the presence of RBC fragments, microcytic, macrocytic or dimorphic red cells. Histograms along with blood indices and hemoglobin values will guide us towards the RBC morphology. This in conjunction with Peripheral blood Smear examination will help in improving the diagnostic accuracy of the pathologist.

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