

Original Article

Correlation of Red Blood Cell Indices and RBC Histogram with Peripheral Blood Smear Findings in Anemia

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The advent of automation has made diagnosis of Anemia and to some extent its underlying cause easy. The objective of our study is to correlate RBC indices and RBC Histogram findings with peripheral smear to provide a better approach in accurate diagnosis of Anemia and to analyze their limitations. All cases of Anemia as per WHO reference range of Hemoglobin levels were included in our study. Patients who have received medical treatment for Anemia in past three months, patients having recent history of blood transfusion, patients having Leukemia or Leukemoid reactions were excluded from the study. Venous blood samples collected from these patients were run in SYSMEX automated hematology analyzer and complete blood count, Red Blood Cell (RBC) indices ie, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW) and RBC Histogram were obtained and peripheral smears were examined. Anemia typing was done based on RBC indices and position, shape and skewing of RBC histograms, followed by peripheral smear examination for morphological typing of Anemia. The diagnoses made by peripheral smear versus RBC histogram and indices were compared and analysed.

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Key words : Automated hematology analyzers, Microcytic hypochromic anemia, Macrocytic anemia, Dimorphic population.

Anemia is not a disease but manifestation of disease and is major global health condition, especially in India and other developing countries, despite the fact that this problem is largely preventable & easily treatable. About 30%, ie, 1/3rd of World's population is suffering from Anemia owing to various causes¹. According to the 5th National Family Health Survey (NFHS-5) data, released by the Ministry of Health and Family Welfare, India has the highest total prevalence of Anemia at 39.86 per cent in the World. NFHS-5 data also suggested that more than half of the children and women are anemic in 13 out of the 22 States/ Union territories in the country².

Hemoglobin binds with oxygen in the lungs and carries it to various tissues and organs throughout the body. In simple words anemia is defined as decrease in the capacity of the blood to carry oxygen due to decrease in hemoglobin concentration of the red blood

Editor's Comment :

- RBC indices along with the Histogram patterns give an idea about the type and to some extent the likely cause of anemia. However, peripheral smear examination is always must to confirm the diagnosis.

cells, decrease in the packed cell volume below the lower limit of the reference interval for the individual's age, gender, geographical location and physiological status^{3,4} as per WHO reference range.

Technology and quality control play an important role towards meeting the targets of diagnosis and treatment of Anemia. The conventionally used manual methods have now been vastly replaced by Automated Hematology Analyzers.

The Automated Hematolyzers are based on electronic impedance principle which explains that as each cell passes through an aperture, there is a change in conductance which leads to development of an electrical impulse. The amplitude of this impulse denotes the cell volume and the number of impulses indicates the cell count. The results are displayed as numericals and Histogram.

Histograms are the graphical representation of numerical data of cell populations in a cell counter. X-axis represents cell size and Y-axis indicates the number of cells.

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The normal RBC distribution curve is Gaussian (bell-shaped) curve, the peak of which lies within the normal MCV (mean corpuscular volume) range of 80-100fL. MCV is a perpendicular line drawn from peak of the curve to the base. The curve has two flexible discriminators: Lower Discriminator, LD (25-75fL) and Upper Discriminator, UD (200-250fL). Red cell distribution width, RDW indicates the degree of Anisocytosis and is determined by calculating the width of the RBC Histogram, expressed as CV (co-efficient of variation) or SD (standard deviation). However, RDW-CV is a better indicator of Anisocytosis than RDW-SD⁵.

RBC Histogram is a standard part and is routinely generated by Automated Hematology Analyzers^{6,7}. The well known Coulter Principle based on counting and sizing of cells forms basis of generating a Histogram⁸.

Histogram and other complete blood count parameters provide useful information in various abnormal hematological conditions by⁹:

(1) Checking the reliability of the results provided by the analyzer.

(2) Investigating the potential cause of the ambiguous automated results.

(3) Pointing towards probable diagnosis and treatment of various disorders¹⁰.

The automated analyzer generated RBC indices along with RBC Histograms based on forward scatter and side scatter in conjunction with peripheral smear examination aids in accurate morphological typing, diagnosis of Anemia and monitoring response to treatment.

However, it must be borne in mind that automation is no replacement for peripheral smear examination; it just compliments manual microscopy¹¹.

MATERIALS AND METHODS

This study was carried out in Hematology laboratory, Department of Pathology in GCS Medical College, Hospital and Research Centre, Ahmedabad over a period of one year from August, 2020 to August, 2021. Total 150 patients of Anemia were considered for the study.

The study population included all patients with Anemia as defined by WHO reference range as follows:

- Children ½ -5 years(Hb < 11 gm/dL)
- Children 5-11 years(Hb < 11.5 gm/dL)
- Children 11-15 years (Hb< 12 gm/dL)
- Pregnant females(Hb<11 gm/dL)
- Non-pregnant females(>=15 years)(Hb<12 gm/dL)
- Males (>=15 years)(Hb<13 gm/dL)

The following patients were excluded from the study:

- Patients who have received medical treatment for Anemia in past three months.
- Patients having recent history of blood transfusion.
- Patients having leukemia or Leukemoid reactions.

Venous blood samples collected in EDTA vacuette were run in SYSMEX XT-2000i 5-part Automated Hematology Analyzer and complete blood count, red blood cell indices (MCV, MCH, MCHC, RDW) and RBC Histogram was obtained. Peripheral blood smears were stained with BEACON-field stain. It comprises of Field-A (Methylene Blue) and Field-B (Eosin). The smears prepared were air dried and fixed in methanol for 2-3 seconds and were dipped in Field-B for 5-6 seconds followed by Field-A for 10-30 seconds and were examined for the morphological typing of anemia.

Anemia was categorized based on RBC indices as:

- Normocytic Normochromic(MCV between 80-100fL)
- Microcytic Hypochromic(MCV<80fL)
- Macrocytic Normochromic (MCV>100fL).

Various Histogram patterns generated were as follows:

Normal curve : Bell shaped curve from 55-125fL and peak between 80-100fL (Fig 1).

Left shift : Peak of the curve is seen before 80fL (Fig 2).

Right shift : Peak of the curve is noted beyond 100fL.

Broad Base : The curve starts before 55fL and ends after 100fL, indicates high RDW.

Bimodal : Two peaks noted.

Short Peak : The curve starts and ends within an interval of 30-40fL ie, maximum populations of cells are of same size and the height is less than half of the reference peak and a narrow constraint of Histogram is obtained indicating a single lineage or Homogenous RBC population and low hemoglobin and red cell count^{12,13}.

Morphological typing of Anemia was obtained by peripheral blood smear examination and it was categorized based on size of small lymphocyte (7-10 micron) and RBC central pallor. Anemia was classified as Microcytic Hypochromic when RBC size was less than small lymphocyte and central pallor >1/3rd, Normocytic Normochromic when RBC size was equal to small lymphocyte, Macrocytic Normochromic when RBC size was more than small lymphocyte and

Dimorphic when dual RBC population was seen.

Diagnosis made by peripheral smear versus RBC Histogram and indices was compared, analyzed and correlation was done using Chi square test for their statistical significance.

OBSERVATIONS

Total 150 Anemia patients included in the study were categorized according to their age. Majority of cases ie, 59 cases (39.33%) were found in the age group of 19-36 years followed by 35 cases (23.33%) in the age group of 55-72 years (Table 1).

Out of these 96 cases (64%) were females and 54 cases (36%) were males with male:female ratio of 0.5:1. Majority of these females (62.5%) were from reproductive age group ie, 15-45 years. The male population was more commonly affected in the elderly age group of 55-72 years.

In our study, most commonly observed histogram abnormality was left shift in 40.67% cases followed by normal curve in 28.67% cases, right shift in 12% cases, broad base in 12% cases, bimodal peak in 4.67% cases and short peak in 2% cases (Table 2).

Categorization of anemia based on peripheral smear examination was done and most commonly obtained morphological type of anemia was Microcytic Hypochromic 74 cases (49.33%) followed by Normocytic Normochromic 48 cases (32%), macrocytic normochromic 22 cases (14.67%) and dimorphic Anemia in 6 cases (4%) (Table 3).

The diagnosis made by RBC indices and Histogram were compared with peripheral smear findings.

Out of 72 cases of Microcytic Hypochromic Anemia, 60 cases had a left shift, 7 cases showed a broad base, 5 cases had a normal curve, 1 case showed short peak and 1 case showed Bimodal curve.

Out of 48 cases of Normocytic Normochromic Anemia, 38 cases showed normal curve, whereas 6 cases had a broad base curve.

Out of 22 cases of Macrocytic Normochromic Anemia, 17 cases showed a right shift, 4 cases showed broad base curve and 1 case showed Bimodal curve.

Out of 6 cases of Dimorphic Anemia, 5 cases showed Bimodal peak and 1 case showed a broad base.

The impression made by peripheral smear V/S RBC histogram and indices were statistically analyzed and correlated using Chi-square test which was

Age Group	Number of Patients	Percentage (%)
≤18 years	20	13.33
19-36	59	39.33
37-54	27	18.00
55-72	35	23.33
73-90	9	6.00
Total	150	100.00

Types of Histogram Abnormality	Cases	Percentage%
Normal curve	43	28.67
Left shift	61	40.67
Right shift	18	12.00
Broad base	18	12.00
Bimodal	7	4.67
Short peak	3	2.00
Total	150	100.00

Peripheral Smear Examination	Number of Patients	Percentage(%)
Microcytic Hypochromic	74	49.33
Normocytic Normochromic	48	32.00
Macrocytic Normochromic	22	14.67
Dimorphic	6	4.00
Total	150	100.00

statistically highly significant, $p < 0.0001$ (Table 4).

DISCUSSION

In present study, amongst the Anemia cases in various age groups, maximum cases obtained were in the age group of 18-36 years with majority of them being females. It clearly indicates that Anemia is more common in adolescent girls and females of reproductive age group, either due to increased demand as in adolescence and pregnancy or loss due to menstruation. Similar findings were obtained by Sandhya *et al* in their study¹⁴.

In our study, majority of cases observed were Microcytic Hypochromic followed by Normocytic Normochromic, Macrocytic Normochromic and Dimorphic anemia. Similar findings were seen in Sandhya *et al*¹⁴ and Rao *et al*¹⁵ in their studies.

Automated analyzer generated RBC indices and Histogram Pattern							
Anemia type of Peripheral Blood smear	Bimodal	Broad base	Left shift	Normal curve	Right shift	Short peak	χ^2 test statistics (p-value)*
Dimorphic	5	1	0	0	0	0	
Microcytic Hypochromic	1	7	60	5	0	1	
Macrocytic Normochromic	1	4	0	0	17	0	300.05 (0.00001)
Normocytic Normochromic	0	6	1	38	1	2	

*Here p-value is highly significant which suggest that there is association between type of anemia, Peripheral Smear and Pattern of Histogram.

However, in our study, the cases of Macrocytic Anemia were more compared to Dimorphic Anemia as the study was carried out in Gujarat where majority of the population followed a vegetarian diet and B12 deficiency is quite common.

In cases of Microcytic Hypochromic anemia, MCV and MCH were decreased but MCHC was normal and hence RBC Histogram showed left shift as in majority of cases. A broad base curve was due to high RDW and indicated Anisocytosis. In such cases, presence of elliptocytes or tear drop cells points towards iron deficiency. However, a Microcytic Hypochromic picture with normal RDW and presence of significant number of target cells points towards beta-thalassemia trait. One of the cases showed presence of many platelet clumps where the lower discriminator of RBC curve didn't touch the baseline giving a left skewing and a broad base. Presence of Schistocytes in cases of Hemolytic Anemia may also lead to a histogram where the Lower discriminator fails to touch the baseline and RDW may be raised due to presence of Polychromatic RBCs. Such conditions can be ruled out by peripheral smear examination. Few cases showed a normal curve and this might be due to the fact that MCV is a mean of distribution curve and so it is insensitive to small number of Macrocytes and Microcytes¹⁶. One case showed Bimodal curve which on peripheral smear showed presence of Microcytic and Macrocytic red cells indicating mixed nutritional deficiency. The short peak correlated well with low RBC mass and low Hemoglobin¹². These findings correlated with study carried out by Sandhya *et al*¹⁴ and Rao *et al*¹⁵.

In Normocytic Normochromic Anemia, MCV, MCH, and MCHC were normal producing a normal Histogram curve. Few cases showed mild increase in RDW giving a mild broad base curve. One case showed right shift and this may be due to presence of few Macrocytes along with the normal red cell population. The decrease in RBC mass and hence the count caused short peak in some cases.

In Macrocytic Anemia, there is increase in MCV and MCH with normal MCHC and curve shifts to right due to presence of Macrocytes. Few cases showed broad base curve due to elevated RDW. However, one case showed Bimodal pattern on Histogram. This difference in the categorization was due to mis-interpretation of cases of Hemolytic Anemia as macrocytic anemia due to the presence of polychromatic RBCs which are larger in size compared to mature RBCs, and since MCV is an average of RBC size, it fell towards the higher side. However peripheral smear picture confirmed the presence of nucleated

RBCs and Schistocytes indicating hemolytic cause. Few cases observed as Macrocytic Anemia on Histogram and RBC indices analysis were actually Dimorphic on peripheral smear examination.

In Dimorphic Anemia, there were dual cell populations and hence the Histogram had two distinct peaks. There may be admixture of Normocytic and Microcytic or Normocytic and Macrocytic red cells. In the present study, in cases of Dimorphic Anemia, MCV, MCH and MCHC were nearly normal and RDW was markedly high due to severe anisopoikilocytosis. The most common reason for Dimorphic Anemia may be mixed nutritional deficiency. Out of total 6 cases of Dimorphic Anemia, majority of them showed bimodal curve and one case showed a broad based curve. The dual RBC population is usually associated with therapeutic blood transfusions, hematinic response to Anemia, etc. They may also denote other hematological conditions like early iron deficiency or folate/vitamin B12 deficiency, iron deficiency in a patient with Megaloblastic Anemia or B12/folate deficiency in a patient with iron deficiency anemia. Dimorphic Anemia is abroad entity and hence a smear is mandatory to assess the abnormal cells.

Hence, Histogram can be considered as an early predictor in identifying subclinical cases of anemia, as well as an important clue towards erroneous results. In our study, Histogram changes correlated well with peripheral smear findings in majority of the cases^{14,15}.

CONCLUSION

The present study is the comparison between automated and manual methods for accurate diagnosis of Anemia. While the automated analyzer generated indices give nearly accurate results, it must be borne in mind that various abnormal findings like Hyperglycemia, severe Leukocytosis, cold Agglutinins may lead to false rise in MCV. Likewise, presence of nucleated RBCs, Polychromatophils, Schistocytes might give erroneously normal indices with significantly elevated RDW and an abnormal Histogram pattern, in which case a peripheral smear examination can point to diagnosis of Hemolytic Anemia. Presence of platelet clumps, bacteria, malarial parasites, etc might produces flagging which should be ruled out. The indices are basically an average of the different populations of RBCs and hence presence of extreme populations might affect the indices in a way that it may fall in normal range or may show extreme deviation. The importance of Histograms comes to play here as it gives a rough idea about the deviations from normal and to some extent can help predict the likely

cause of the type of anemias. In any cases, examination of peripheral blood smear is mandatory to rule out such findings, to get a clear picture of the type of anemia as well as to monitor the response to treatment.

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