DOI: 10.14260/jemds/2014/4095

ORIGINAL ARTICLE

STUDY OF RBC HISTOGRAM IN VARIOUS ANEMIAS

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HOW TO CITE THIS ARTICLE:
Sandhya I, Muhasin T. P. "Study of RBC Histogram in Various Anemias". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 74, December 29; Page: 15521-15534, DOI: 10.14260/jemds/2014/4095

ABSTRACT: Over the past few years complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patients' blood sample. Numerous classifications for anemia have been established and the important parameters involved in the classifications are Hb, HCT, MCV, RDW, MCH, MCHC, reticulocytes and IRF. Many of these values are obtained only by automated heamatology analyzers. One histogram graph is worth 1000 numbers. A large collection of data, displayed as a visual image, can convey information with far more impact than the numbers alone. In hematology, these data take on several forms, one of which is the RBC histogram. Therefore a study of variation in RBC histograms in various anemias. Many times it is seen that histogram patterns show varying features when a simultaneous peripheral smear is reported. It is also seen that there are many limitations when manual peripheral smears reporting is done for example: peripheral smear reports are subjective, labor intensive and statistically unreliable. However microscopic peripheral smear examination also has their advantages. This study intends to create a guide to laboratory personnel and clinicians with sufficient accuracy to presumptively diagnose morphological classes of anemia directly from the automated hematology cell counter forms and correlate with morphological features of peripheral smear examination. **OBJECTIVE:** 1. The objective of the study is to know the utility and advantage of red cell histograms. 2. To study the automated histogram patterns along with morphological features noticed on peripheral smear examination. **SOURCE OF DATA:** All anemic patients from Central Diagnostic Laboratory of A.J.IMS. **METHOD OF COLLECTION OF DATA:** A total of about 100 patients were included in the study. Complete blood count including HB, TC, DC, Platelet count hematocrit value, RBC indices was obtained from Heama – lab life D5 Supreme automated hematology analyzers. Peripheral smear examination was also obtained wherever necessary. **RESULTS:** This is a study of histogram of various types of anemia consisting of a total of 100 cases. All cases had anemia with hemoglobin less than 12gm/dl. All cases were studied with a correlation of peripheral smear findings. The cases consisted of Normocytic normochromic anemia, Microcytic hypochromic anemia, dimorphic anemia, Pancytopenia & Thalassemia, as diagnosed by peripheral smear. This study includes predominantly females (53%) more than males (47%).The age of patients ranged from 1day to 79 years. Maximum number of patients was in 30-40 years of age range. The anemic subjects had different types of anemia. Microcytic hypochromic anemia was the most common (61%), 17% of the cases are Normocytic normochromic anemia, 15%of cases with dimorphic anemia. Macrocytic anemia and Pancytopenia seen in 3% of the cases & thalassemia is seen in 1%. Representing the histogram variation in various anemias. Out of the 17% of normocytic normochromic anemia 8% showed normal histogram and 9% showed mild broad base curve histogram. Out of the 61% of microcytic hypochromic anemia, 4% were normal histogram, 27% were left shift histogram, 26% were broad base curve histogram, 2% short peak histogram and 2% abnormal (bimodal) histogram. Out of 3% of Macrocytic anemia 2% showed right shift with broad base curve histogram and 1% showed short peak histogram where hemoglobin was 5.3g/dl and RBCs

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count was 1.30x10^6/uL. Out of 15% of dimorphic anemia 3% were of normal histogram, 3% were broad base histogram, 4% right and 3% left shift histogram and 1% each of short peak & abnormal histogram. Right shift curves correlated well with increased MCV, MCH, and RDW. Short peak correlated well with low Hb and red cell count. Out of the 3% of the pancytopenia with a 2% were broad based, and 1% were of short peak of normal MCV with low Hb (4.6g/dL), red cell count (1.37x 10^6/uL) and increased RDW. The 1% of thalassemia showed abnormal histogram with left shift, bimodal broad base curve, correlating well with low MCV, MCH, HGB (2.5g/dL) and red cell count (1.18x 10^6/uL)with normal MCHC and increased RDW. **CONCLUSION:** Histogram in conjuction with absolute counts gives valuable information about the abnormality of the sample and the need for follow up peripheral blood examination. Histogram should be used as screening tool for Hematology, but should not be considered diagnostic for any pathological condition. Shapes of histograms can identify pathology to some extent and give us hint, before the blood smear could be examined. It should be pointed out again that an evaluation of anemias is not complete without the careful examination of a well-prepared peripheral blood smear. However a study of histogram can become a new parameter along with red cell indices in the diagnosis of anemias. Histograms can be useful tool for technologists to prioritize the cases to be studied in detail and thus help in speedy disposal of samples in the laboratory. Also, Red cell indices, RDW, and red blood cell histograms will not help identify conditions such as red cell inclusions (e.g., malarial parasites) or membrane abnormalities such as spherocytosis that might be responsible for the anemia. "The manual blood film remains the definitive tool for complete heamatologic analysis''.

KEYWORDS: Anemia, RBC histograms.

INTRODUCTION: Over the past few years complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample. Numerous classifications for anemia have been established and the important parameters involved in the classifications are Hb, HCT, MCV, RDW, MCH, MCHC, reticulocytes and IRF. Many of these values are obtained only by automated hematology analyzers[1]. However microscopic peripheral smear examination also have their advantages.[2] Specific diagnosis of leukemia, and other WBC disorders are possible only with P.smear examination.

Histogram in conjuction with absolute counts give valuable information about the abnormality of the sample and the need for follow up peripheral blood examination. Shapes of histograms can identify pathology to some extent and give us hint, before the blood smear could be examined. Histograms provide a means of comparing the sizes of a patient's cells with normal populations. Shifts in one direction or the other can be of diagnostic importantce.[3]

Histogram and other associated complete blood count (CBC) parameters have been found abnormal in various hematological conditions and may provide useful information,[4] major clues for laboratories in, 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 and management of significant red cell disorders.[5]

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. 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.[6]

If the cells are larger than normal, the histogram curve will be more to right, as in megaloblastic anemia, if the cells are smaller than normal, the curve will be more to the left, as in untreated iron deficiency anemia. After appropriate treatment of the underlying cause of an anemia, the curve should move toward the normal range.

If the normal unimodal distribution is altered, the early stages of an underlying disorder may be revealed. A histogram distribution that is bimodal can be seen in various situations, such as dimorphic anemia, cold agglutinin disease, after the transfusion of normal erythrocytes into a person with abnormally sized erythrocytes, in the presence of erythrocyte fragments, or with agglutination.
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).[4]

The wide peak would represent an RDW above the reference range. The population of the cells would be variable in size. For example, some microcytic or small cells and some normal size cells would result in a higher deviation and hence a higher RDW.[6] The presence of increased numbers of smaller cells (e.g. in iron deficiency anemia) will also increase the RDW. An increased numbers of immature red cells during a degenerative response to an anemia will also increase the RDW, because immature anucleate RBC are larger than normal.[7]The general availability of RDW as a measure of anisocytosis helps further in the evaluation of anemias based on morphology Significant anisocytosis often leads to an increased RDW, whereas in its absence the RDW remains normal.[8]

Sometimes a set of spurious values may be the first clue to an otherwise unsuspected clinical condition e.g., the combination of low hematocrit, normal hemoglobin, and high MCV and MCHC is characteristic of cold agglutinins. The MCV, since it is an average value, can be normal in the presence of two different cell populations (e.g., dimorphic anemias, red cell fragmentation with reticulocyte response). It is, therefore, important to examine the peripheral smear in the evaluation of anemias. When available, RDW is a good indicator of the degree of anisocytosis. However, the red cell histogram, which offers a graphic depiction of red cell size distribution, will reveal anisocytosis even when the MCV is normal.[8] Thus histogram can be of much help in evaluating cases and allow faster disposal of within range cases.

A study of histogram can become a new parameter along with red cell indices in the diagnosis of anemias. Histograms can be useful tool for technologists to prioritise the cases to be studied in detail and thus help in speedy disposal of samples in the laboratory. Also, Red cell indices, RDW, and red blood cell histograms will not help identify conditions such as red cell inclusions (e.g., malarial parasites) or membrane abnormalities such as spherocytosis that might be responsible for the anemia.[8]. In the past three decades sophisticated hematology analyzers are being used in all large hospitals and most commercial laboratories to perform complete blood counts (CBC). It is rather unfortunate that, because of automation in hematology, peripheral smear examination is becoming impracticable.[9,10]

However automation has also created problems relating to maintenance of expertise especially in the field of microscopic examination.[11] several attempts have been made to create an expert system with sufficient accuracy to diagnose classes of anemia directly from the automated hematology form.[12] Graphical representation of results in the form of histograms or scatter plots has

been largely ignored in favor of newer numerical parameters that have become available over the years. Some of these like the red cell distribution width, hemoglobin distribution width, and reticulocyte hemoglobin content provide very useful information in addition to the red cell indices that have been traditionally used.[13]

There have been very few studies outlining the utility of red cell histograms or scatter plots in identification of hematological conditions.[14]

A study done to evaluate anisocytosis by comparing peripheral smear morphology and red cell distribution width (RDW), showed interesting results. Quantitative RDW results correlated very well with semi quantitative microscopic reports and concluded that (RDW) can be considered a"gold standard" in evaluating anisocytosis.[15]

Patients with iron deficiency anemia, sequential histograms can show the progressive appearance of a new well hemoglobinized erythrocyte population well ahead of that indicated by the conventional numerical parameters.[16]

A histogram can provide useful information for laboratories in 1) Monitoring the reliability of the results generated by the analyzer 2) investigating the potential cause(s) of the erroneous automated results3) 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.[5]

A fair knowledge of histogram pattern can help to differentiate subclinical anemias. A study concluded that iron deficiency anemia is characterized by RBC with decreased hemoglobin concentration and can be distinguished from beta thalessemia trait which in comparison shows increase in microcytosis and preserved RBC hemoglobin concentration.[17]

Automated histograms have also been proven to identify red cell fragments more effectively than routine peripheral smear examinations. RBC fragments are commonly seen in malignancies with cytotoxic chemotherapy and severe iron deficiency.[18]

MATERIALS AND METHODS:

SOURCE OF DATA: All anemic patients from Central Diagnostic Laboratory of A.J.IMS.

METHOD OF DATA COLLECTION: A total of about 100 patients will be included in the study. They were followed of from day one to day 10 during their treatment

Complete blood count including HB, TC, DC, Platelet count hematocrit value, RBC indices was obtained from Heama – lab life D5 Supreme automated hematology analyzers. Peripheral smear examination and corrected platelet count was also obtained wherever necessary. All anemic patients with hemoglobin percentage less than 12gm% will be included in the study.

RESULTS AND ANALYSIS: This is a study of histogram of various types of anemia consisting of a total of 100 cases. All cases had anemia with hemoglobin less than 12gm/dl. All cases were studied with a correlation of peripheral smear findings. The cases consisted of Normocytic normochromic anemia, Microcytic hypochromic anemia, dimorphic anemia, Pancytopenia & Thalassemia, as diagnosed by peripheral smear.

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Types of anemia	Percentage
Normocytic	17%
Microcytic	61%
Macrocytic	3%
Dimorphic	15%
Pancytopenia	3%
Thalassemia	1%

Table 1: Distribution of case as per types of anemia

Representing different types of anemia. In our study 17% of the cases are Normocytic normochromic anemia. 61% of cases are microcytic hypochromic anemia, 3% of cases with macrocytic anemia and 15% are dimorphic anemia. Pancytopenia seen in 3% of the cases & thalassemia is seen in 1%.

''Representing the relation between the anemia and the red cell indices, by taking the average of the red cell indices from different anemia.

In our study in Normocytic normochromic anemia cases show MCV, MCH and MCHC within the normal limit, with occasional cases having mildly increased RDW. In Microcytic anemia MCV, MCH, are less than normal range with normal MCHC and increased RDW due to anisopoikilocytosis. In Macrocytic anemia due to the degree of variation in size & shape of the RBCs, increase in MCV, RDW, MCH, are noted with normal MCHC. In Dimorphic anemia a MCV, MCH and MCHC were normal increased RDW due to high degree of anisopoikilocytosis. In Pancytopenia only change noticed in Red cell indices is an increased RDW with normal MCV, MCH and MCHC. In Thalassemia case MCV, MCH were decrease with a normal MCHC with increased RDW.

Based on Hemoglobin anemia is graded in to mild, moderate and severe anemia. In our study Hemoglobin with the range of above 9.0g/dl is considered as mild anemia.

Fifteen percentage of the cases were mild anemia with histogram showing normal curve. Occasionally mild broad base, left shift, Right shift Broad base curve were seen in two cases.

Sixteen percentage of the cases were moderate anemia with the hemoglobin range between 6-9g/dl. Majority showed normal curve with left shift and mild broad base curve. Two of the cases show abnormal histogram with bimodal broad base.

Sixty nine percent of the cases are severe anemic cases with hemoglobin less than 6g/dl (40%). Majority of them show broad base curve, few cases with short peak and with left or right shift. Abnormal histograms with bimodal broad peak are also noted (4%).

Type of histogram	Percentage
Normal curve	15%
Left shift	30%
Right shift	6%
Broad base	40%
Short peak	5%
Abnormal / bimodal	4%

Table 2: Types of histogram abnormality in the study

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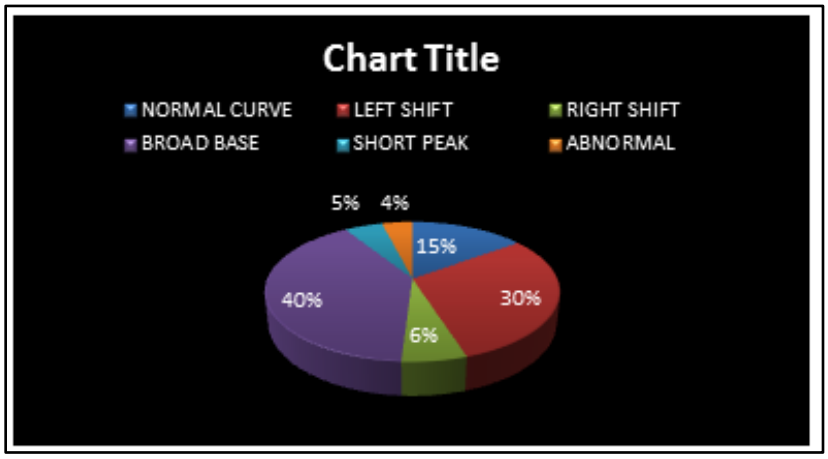

Figure 1: Types of histogram abnormality in the study

Pie chart representing the histogram abnormalities in the whole study.

In our study Abnormal histogram with 3% cases of bimodal histogram seen in microcytic anemia and thalassemia, dimorphic anemia and 1% case with right shift histogram with short broad peak and broad base curve.

Short peak histogram of 5% which is seen in microcytic anemia (2%), macrocytic anemia, dimorphic anemia and in thalassemia with mainly decreased MCV (91.8), RBC count (6.89) & Hemoglobin (4.3g/dL).

Right shift histogram with 6% cases seen in macrocytic anemia (2%) and dimorphic anemia (4%).

15% of the histogram shows normal histogram which is seen Normocytic normochromic anemia (8%), microcytic anemia (4%) and dimorphic anemia (3%).

Left shift histogram with 30%, which is seen in Microcytic hypochromic anemia (27%) and in dimorphic anemia (3%).

Majority of the histogram (40%) shows broad base curve with increased RDW found in all anemia from our study.

	Normal curve	Left shift	Right shift	Broad	Short peak	Bimodal
Normocytic	8%			9%		
Microcytic	4%	27%		26%	2%	2%
Macrocytic			2%		1%	
Dimorphic	3%	3%	4%	3%	1%	1%
Pancytopenia				2%	1%	
Thalassemia						1%

Table 3: Histogram variations in different anemia

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Representing the histogram variation in various anemia's. Out of the 17% of normocytic normochromic anemia 8% showed normal histogram and 9% showed mild broad base curve histogram.

Out of the 61% of microcytic hypochromic anemia, 4% were normal histogram, 27% were left shift histogram, 26% were broad base curve histogram, 2% short peak histogram and 2% abnormal (bimodal) histogram.

Out of 3% of Macrocytic anemia 2% showed right shift with broad base curve histogram and 1% showed short peak histogram where hemoglobin was 5.3g/dl and RBCs count was 1.30x10^6/uL. Out of 15% of dimorphic anemia 3% were of normal histogram, 3% were broad base histogram, 4% right and 3% left shift histogram and 1% each of short peak & abnormal histogram. Right shift curves correlated well with increased MCV, MCH, and RDW. Short peak correlated well with low Hb and red cell count.

Out of the 3% of the pancytopenia with a 2% were broad based, and 1% were of short peak of normal MCV with low Hb (4.6g/dL), red cell count (1.37x 10^6/uL) and increased RDW.

The 1% of thalassemia showed abnormal histogram with left shift, bimodal broad base curve, correlating well with low MCV, MCH, HGB (2.5g/dL) and red cell count (1.18x 10^6/uL)with normal MCHC and increased RDW.

DISCUSSION:

RBC HISTOGRAM: AN OVERVIEW: The well-known Coulter principle of counting and sizing red cells provides the basis for generating the histogram. This method relies on the change in conductance as each cell passes through an aperture. The change in conductance results in an electrical pulse, the amplitude of which is proportional to the cell volume. The 256-channel pulse-height analyzer uses a number of thresholds to sort the cells into several size (volume) channels from which the histogram is formed. Each channel on the X-axis represents a specific size (volume) in femtoliter (24–360 fL), increasing from left to right. The Y-axis represents the number of cells per channel, with each cell being stored in the channel representing its size, so that after data accumulation is completed the relative number of cells (frequency) is provided. This data is further processed by the computer, and the RBC curve is smoothed by a moving average technique and displayed on a data management system.[19]

GENERAL HISTOGRAM CHARACTERISTICS: Histograms are graphical representations of cell frequencies versus sizes. In a homogenous cell population, the curve assumes a symmetrical bell shaped or Gaussian distribution. The peak of the curve should be within the normal MCV of 80.0-100.0 fL. A wide or more flattened curve is seen when the standard deviation from the mean is increased. Histograms not only provide information about erythrocyte, leukocyte, and platelet frequency and their distribution about the mean, but also depict the presence of subpopulations. Histograms provide a means of comparing the sizes of a patient's cells with normal populations. Shifts in one direction or the other can be of diagnostic importance. In the cell counters, the size (volume in femtoliters) is represented on the X-axis. Right shift indicates increase in the size of cells and left shift indicates decrease in the size of the cells.[3,6]

THE ERYTHROCYTE HISTOGRAM: The erythrocyte histogram reflects the native size of RBC and numbers any other particles in the erythrocyte size range. The RBC histogram in the cell counter

displays the cell ranges for RBC histograms are between 24 fL and 360 fL, the instrument counts only those cells with volume sizes between 36 fL and 360 fL as red cells. Those cells counted in the 24 fL to 36 fL range are rejected and not included in the RBC count. They are enumerated and displayed in the histogram area between the 24 fL and 36 fL range, however, allowing the lower end of the histogram to be monitored. Normally, the space below 36 fL remains clear, but in certain conditions the histogram may begin above the baseline or show a high takeoff on the far left of the curve which generally indicates the presence of small particles. These particles include red cell fragments, microspherocytes, nucleated RBCs, nonlyzed RBCs, elliptocytosis, macrothrombocytes, platelet clumps, bacteria, parasitic organisms, and other interfering substances such as cryoglobulin, cold agglutinin, and macroglobinemia.[3,5,20-22]

The extension of the lower end of the scale from 36-24fL allows for the detection of erythrocyte fragments, leukocyte fragments, and platelets. Although normal quantities of leucocytes are presented in the RBC bath and are included in the RBC count, they are not significant in the histogram. The system can be calibrated to compensate for 7.5 X 109/L leucocytes. If the leukocyte count is significantly elevated, the RBC histogram will be affected.[3]

In our study, few cases of elevated leukocyte count show, broad base curve, short peak and bimodal RBC histogram and normal histogram, in few cases.

The red cell distribution curve will get wider as the red cell vary more in size, a narrow distribution curve indicates a homogenous the population of red cells. If the cells are larger than normal, the histogram curve will be more to right, as in megaloblastic anemia, if the cells are smaller than normal, the curve will be more to the left, as in untreated iron deficiency anemia. After appropriate treatment of the underlying cause of an anemia, the curve should move toward the normal range.

ANALYSIS AND INTERPRETATIONS OF RBC HISTOGRAM: Normal, microcytic, and macrocytic red cell histograms are symmetric, single-peaked, and "bell shaped" normal curves. The curve is considered symmetric if both sides of the curve coincide when folded in half or are approximately mirror images. When the distribution is not symmetric, it is referred to as skewed. In a normal RBC histogram, the majority of each cell falls between 55 fL and 125 fL. The tail of the distribution consists of coincident doublets and anomalous pulses.[23]

In IDA and beta thalassemia (thal) trait, the red cell distribution curves are shifted to the left, and the percentage of microcytosis is increased. Although their histograms are similar, the degree of anisocytosis, as measured by the RDW, differentiates them. Iron deficiency anemia is characterized by elevated RDW, reflecting the heterogeneity in the acquired erythrocyte populations. In that trait however, the RDW is usually within range due to the homogeneity of the inherited population of erythrocytes. Although this difference is useful to some extent in distinguishing them, cases involving severe anemia with homogenous microcytosis may give misleading results. 3 Nonetheless, patients showing severe anemia with hemoglobin <90 g/L and with hypochromic-microcytic red cells are most likely (80%) iron deficient.[24,25,26]

The distributional shape of the histogram can be classified as reflecting either single or dimorphic (multiple) red cell populations. In a single population, the histogram may have a normal Gaussian or bell-shape curve, and the peak of the curve should fall within the normal MCV range of 80-100fL. And may be widened with or without left or right shift, may be skewed to the left or right,

or may show some combination of all of these characteristics. In dimorphic populations, the histogram may be symmetric and/or bimodal, or bimodal but skewed to the left or right. The centeredness and width of the histogram also define the extent of RBC variability.[4] Shifts in the histogram will be observed in microcytic anemia (left shift) or macrocytic anemias (right shift). Depending on the causes, the width of the peak (RDW) will likely also increase. This means there are lots of cells in a whole host of different sizes and this is called anisocytosis.[27,28,29]

FOLLOWING ARE THE IMPORTANT POINTS TO CONSIDER WHEN REVIEWING/ANALYZING HISTOGRAMS.[19]

- Position of individual populations compared to normal/typical positions.
- Amount of separation between populations compared to normal/typical separation.
- Relative concentration of each population compared to normal/typical concentrations.
- Presence of unexpected or non-typical populations.

CAUSE OF ABNORMAL HISTOGRAM:

- When the curve does not start at the baseline, suspect small cells and cell fragments, giant platelets, white cell fragments etc.
- When it does not come back to the base line, suspect agglutination.[29]

In some cases when the patient has leukemia and a very high white cell count, some of these will be counted as RBCs and will also see an extended right peak. The presence of a right-sided shoulder usually corresponds to reticulocytosis, and a trailer of erythrocyte populations on the far right of the histogram correlates to red cell agglutination.[29,4]

A histogram distribution that is bimodal can be seen in various situations, are usually associated with therapeutic transfusion and/or hematinic agent response to microcytic and macrocytic anemia, but they may also indicate other hematological disorders like cold agglutinin disease, in the presence of erythrocyte fragments, in IDA (microcytic) with recent blood transfusion, in sideroblastic anemia especially in acquired forms, and megaloblastic anemia (macrocytic anemia) with recent blood transfusion.[4,29]

NORMOCYTIC ANEMIA: In our study Normocytic normochromic anemia, the red cell indices like MCV, MCH, and MCHC were within the normal limits with occasional cases showing mild increase in RDW. Out of the 17% of normocytic normochromic anemia 8% showed normal histogram and 9% showed mild broad base curve histogram correlating well with the increased RDW.

The wide peak would represent an RDW above the reference range. The population of the cells would be variable in size. For example, some microcytic or small cells and some normal size cells would result in a higher deviation and hence a higher RDW.[6]

MICROCYTIC ANEMIA: In Microcytic hypochromic anemia, a decrease in MCV and MCH are noted. The value of MCH closely parallels the value of MCV. MCHC may be normal in microcytic anaemia. RBC population with a low MCV will be shifted towards left. Increased RDW shows a mild broad base histogram. The presence of increased numbers of smaller cells (e.g. in iron deficiency anemia) will also increase the RDW.[7] The RDW is not a determination of ''normal'' red blood cell size. For example,

DOI: 10.14260/jemds/2014/4095

the population may be within the reference range. When the cells are observed on a smear, they will not be cells of typical size.[6]

In our study in Microcytic anemia MCV, MCH, were less than normal range with normal MCHC and increased RDW due to anisopoikilocytosis as observed in peripheral smear study. Out of the 61% of microcytic hypochromic anemia, 4% were normal histogram, 27% were left shift histogram, 26% were broad base curve histogram, 2% short peak histogram and 2% with bimodal histogram.

MACROCYTIC ANEMIA: A right shift histogram with broad base curve and a short broad peak broad base curve histogram would mean low Hb and macrocytic anemia. Causes of macrocytosis are many, range from benign to malignant; thus, a complete workup to determine etiology is essential.[30,31,32,33]

Macrocytosis may occur at any age, but it is more prevalent in older age groups because the causes of macrocytosis are more prevalent in older person.[34,35, 36] in our study also macrocytic anemia seen in older person.

In our study Macrocytic anemia due to the degree of variation in size & shape of the RBCs, increases in MCV, RDW MCH, were noted with normal MCHC. Macrocytic anemia with 3% shows the right shift broad base curve histogram (2%) with (1%) short peak histogram, with low hemoglobin (5.3g/dL) and low RBC count (1.30x10^6u/L).

DIMORPHIC 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).[4] These 2 distinct populations may be comprised of either a patient's own red cells (post-iron treatment) or a mixture of patient and donor red cells (post-iron transfusion). The dimorphic blood picture will look like a dual population of microcytic and normocytic or normocytic and macrocytic red cells, or an admixture of small, normal, and large cells of different sizes and forms.

In our study In Dimorphic anemia a MCV, MCH and MCHC were normal increased RDW due to high degree of anisopoikilocytosis. Out of 15% of dimorphic anemia 3% were of normal histogram, 3% were broad base histogram, 4% right and 3% left shift histogram and 1% each of short peak & abnormal histogram. Right shift curves correlated well with increased MCV, MCH. Short peak correlated well with low Hb and red cell count.

PANCYTOPENIA: Out of the 3% of the pancytopenia with a 2% were broad based, and 1% were of short peak of normal MCV with low HGB (4.6g/dL), red cell count (1.37x10^6u/L) and increased RDW.

THALASSEMIA: In IDA and beta thalassemia (thal) trait, the red cell distribution curves are shifted to the left, and the percentage of microcytosis is increased. Although their histograms are similar, the degree of anisocytosis, as measured by the RDW, differentiates them. Iron deficiency anemia is characterized by elevated RDW, reflecting the heterogeneity in the acquired erythrocyte populations.] In thal trait however, the RDW is usually within range due to the homogeneity of the inherited population of erythrocytes. Although this difference is useful to some extent in distinguishing them, cases involving severe anemia with homogenous microcytosis may give misleading results.[16] [Nonetheless, patients showing severe anemia with hemoglobin <90 g/L and with hypochromic-microcytic red cells are most likely (80%) iron deficient.[28]]

The 1% of thalassemia showed abnormal histogram with left shift bimodal, broad base curve, correlating well with low MCV, MCH, HGB and red cell count with normal MCHC and increased RDW. In our study, RDW and MCV complement in the differential diagnosis of anemia. The red cell indices were comparable with histogram changes also. High RDW showed broad base, low MCV, low HGB showed short peak & left shift high MCV showed right shift.

Hence, histogram can also be utilized in differentiating various types of anemia along with red cell indices. Histogram changes correlated well with peripheral smear findings in majority of the cases.

Out of the 17% of normocytic normochromic anemia 8% showed normal histogram and 9% showed mild broad base curve histogram. Out of the 61% of microcytic hypochromic anemia, 4% were normal histogram, 27% were left shift histogram, 26% were broad base curve histogram, 2% short peak histogram and 2% bimodal histogram. Out of 3% of Macrocytic anemia 2% showed right shift with broad base curve histogram and 1% showed short peak histogram where hemoglobin was 5.3g/dl and RBCs count was 1.30x10^6/uL. Out of 15% of dimorphic anemia 3% were of normal histogram, 3% were broad base histogram, 4% right and 3% left shift histogram and 1% each of short peak & abnormal histogram. Right shift curves correlated well with increased MCV, MCH, and RDW. Short peak correlated well with low Hb and red cell count. Out of the 3% of the pancytopenia with a 2% were broad based, and 1% were of short peak of normal MCV with low Hb (4.6g/dL), red cell count (1.37x 10^6/uL) and increased RDW. The 1% of thalassemia showed abnormal histogram with left shift, bimodal broad base curve, correlating well with low MCV, MCH, HGB (2.5g/dL) and red cell count (1.18x 10^6/uL) with normal MCHC and increased RDW.

Histogram in conjuction with absolute counts give valuable information about the abnormality of the sample and the need for follow up peripheral blood examination. Histogram should be used for screening, but should not be considered diagnostic for any pathological condition. Shapes of histograms identified pathology before the blood smear could be examined.[4]

When combined with the concept of normal curve and the knowledge of particular CBC parameters, such as RDW and red cell indices, histograms become a practical working tool in the initial stage of morphological analysis. Histogram provides a concise idea not only of the different sizes of cells but also of the distribution of cells from the center (MCV) and the spread (RDW), RBC count (indicated by the peak) & Hb% determined from the red cell histogram.[13,29,30] Whatever instrument methods are used to obtain the red cell histogram, electrical impedance or optical flow cytometry, the relationship of the different sizes of cells can be readily delineated and contrasted, when a dotted line of reference normal is superimposed on all automated abnormal red cell count histogram results.

The speed and the reliability of the modern analyzers 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 which produce high returns in terms of patient health care. Histograms can be useful tool for technologists to prioritize the cases to be studied in detail and thus help in speedy disposal of samples in the laboratory.

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DOI: 10.14260/jemds/2014/4095

ORIGINAL ARTICLE

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Date of Submission: 25/11/2014.
Date of Peer Review: 26/11/2014.
Date of Acceptance: 18/12/2014.
Date of Publishing: 27/12/2014.