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Correlation of Automated cell counters RBC Histogram and Peripheral smear in Anemias

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Abstract

Background and Aim: The RBC histogram visualizes particle size distribution that plays a critical role in the initial screening and detection method for haematological disorders in current clinical settings. This present study was designed to determine the relationship between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear using the blood samples.

Material and Methods: A total of 500 samples sent for CBC and PS would be used for the present study for the duration of 5 months. The CBC samples received would be analyzed in the ABOTT cell dyn ruby instrument, and a peripheral smear would be made from the same sample, using Leishman stain.

Results: Among all, 16% histograms were normal,31% had a left-shifted curve,40% showed broad-based curve, 03% showed short peak, and Bimodal peaked histogram was demonstrated by 06% of total cases. In the present study, cases of dimorphic anemia showed a normal range of MCV, MCH and MCHC. At the same time, RDW is increased due to the high degree of anisocytosis and poikilocytosis, which was observed in the PBS. Cases of Macrocytic anemia show an increase in MCV, MCH and RDW with normal MCHC.

Conclusion: Histogram plays an additional role with peripheral smear for diagnosing RBCs disorders. Haematology analyzers were very useful and reliable for evaluation of abnormal peripheral smears. Histogram was correlated with almost all peripherial smear interpretation in anemia cases.

Keywords: Histogram, Haematology Analyzer, Macrocytic anemia, Peripherial Smear

Introduction

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. Thirty to forty years ago, laboratory hematology was labor intensive and time consuming. Procedures were manual. Reagents were prepared in the laboratory from raw chemicals. Hemoglobin measurement was based on the cyanmethemoglobin method, which involved tedious procedure. The automated hematology analyzer has replaced the traditional manual methods for hematological parameters as the initial screening and detection system for hematological abnormalities in modern clinical setups.

The RBC histogram visualizes particle size distribution that plays a critical role in the initial screening and detection method for haematological disorders in current clinical settings. With the emergence of more powerful haematology analyzers with significant improvement and precision, the manual peripheral smear examination steadily declines. The number of the cells counted by the automated hematology analyzers is much more than the cells measured by manual peripheral smear examination, and computerized analyzers provide far better accuracy and with the usage of histograms.

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The RBC histogram, along with other CBC parameters like RBC distribution width (RDW) and mean corpuscular volume (MCV), has been discovered to be aberrant in a variety of haematological illnesses and may provide essential clues in the diagnosis and treatment of significant red cell disorders.[1-4]

The curve of Red cell distribution is bell shaped and peaks within 80 to 100 fl. For homogenous population of cells the curve was smaller, while for heterogenous population the curve was wider. The curve was shifted to right in megaloblastic anemia due to macrocytes; while in microcytic anemia it was shifted to left. If the patient had received treatment, Morphologically two red cell population was seen, in which multiple peaks can be observed and is referred to as Dimporhic anemia. In these conditions RDW was the better indicator than MCV to assess anisocytosis. [5,6]

A histogram can assist laboratorians in 1) monitoring the accuracy of the results provided by analyzers and 2) examining the possible cause(s) of the erroneous automated outcomes.3) establishing a tentative diagnosis [7] Certain situations, such as the presence of fragmented red cells or red cell agglutination, may now theoretically Be seen on the red cell histogram, which could not previously be seen without a blood film study. A sequential histogram can also clearly demonstrate the increasing emergence of a new erythrocyte population well ahead of other numerical indicators in patients with iron deficiency anaemia (IDA) or megaloblastic anaemia under treatment.[8,9] This present study was designed to determine the relationship between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear using the blood samples.

Objectives: 1) Interpretation of histograms in normal persons and patients with different types of anaemia. 2) Comparison of automated histogram patterns with morphological features noticed on peripheral smear examination.

Material and Methods

A total of 500 samples sent for CBC and PS would be used for the present study for the duration of 5 months.

Inclusion criteria: All patients who are diagnosed as anaemic according to WHO definition Exclusion criteria 1) Patients who are less than five years of age. 2) Inadequate quantity of blood sample for automated analyzer (< 3ml). 3) Pre Analytical errors like clotted sample.

This is a prospective cross-sectional study done on all patients diagnosed with anaemia according to WHO definition. The CBC samples received would be analyzed in the ABOTT cell dyn ruby instrument, and a peripheral smear would be made from the same sample, using Leishman stain.

Results

Out of the 500 samples, 192 samples were from Males, and 38 samples were from females. In the present study, we found that the maximum number of cases were of Microcytic hypochromic anemia and showed various histograms. Among all, 16% histograms were normal,31% had a left-shifted curve,40% showed broad-based curve, 03% showed short peak, and Bimodal peaked histogram was demonstrated by 06% of total cases. Correlation with Peripheral smear findings: In our study, we observe that cases of Microcytic hypochromic anemia with less than normal range of Mean Corpuscular Volume (MCV) &Mean Corpuscular (MCH) Hemoglobin with normal Mean Corpuscular Hemoglobin Concentration (MCHC) and increased Red cell Distribution Width (RDW) and this finding is correlated with anisopoikilocytosis which was seen on the microscopic examination of peripheral blood smear.

The cases of Normocytic Normochromic anemia showed the standard limit of MCV, MCH and MCHC and occasional cases having mildly increased RDW. In the present study, cases of dimorphic anemia showed a normal range of MCV, MCH and MCHC. At the same time, RDW is increased due to the high degree of anisocytosis and poikilocytosis, which was observed in the PBS. Cases of Macrocytic anemia show an increase in MCV, MCH and RDW with normal MCHC.

Table 1: Age and gender distribution in the study

Age group (years)	5-10	11-20	21-30	31-40	41-50	51-60	60 above
Male	13	9	56	38	43	23	10
female	9	19	158	82	21	14	6

Table 2: Case Distribution as per types of anemia

Types of anemia	No of cases	Percentage (%)
Normocytic	77	15.4
Microcytic	364	72.8
Macrocytic	21	4.2
Dimorphic	38	7.6

Table 3: Distribution of RBC histogram in the present study

s. no.	Type of histogram	Percentage (%)
1	Normal	16
2	Left shift	31
3	Right shift	5
4	Broad base	40
5	Short peak	3
6	Bimodal peak	6

Discussion

A single histogram graph can be equivalent to 1000 numbers. The effect of a vast collection of facts represented as a visual representation is significantly greater than the impact of numbers alone. These data can take numerous forms in haematology, one of which is the RBC histogram. The range, size, shape, and other conspicuous aspects of the red cell morphology may all be seen by scanning the histogram visually.[9]

Information about RBC parameters like RDW, MCH and MCV were obtained which helped in diagnosis and typing of anemia.[3,4] Normal curve was symmetrical bell shaped or showed Gaussian distribution. Normal curve shows MCV range between 80-100 fl. [7,8]. The analyzer can recognize only those Red Blood cells 36fl to 360fl volume sizes as RBCs, and the range 24fl to 36 fl are not considered in the RBC count and not taken into consideration by the counter. The histogram begins above the baseline (36fl) indicates the presence of small particles like microspherocytes, platelet clumps, normoblast, elliptocytes, malaria parasites, bacteria, etc. The RBC count does not affect by WBCs count is increased by beyond 50000 cells / cumm.[10-13]

In the present study of 500 cases, the maximum number of instances are having Microcytic anemia followed by normocytic, Dimorphic and Macrocytic. Other studies like sandhya al[14], BynaSyamSundara Rao et al.[15], Chavda J et al [16] were also found similar findings of anemia cases regarding Distribution. Our survey of RBC histogram showed normal curve (16%), left shift (31%), right shift (5%), Broad base (40%), short peak (3%) and bimodal (6%) and these findings regarding RBC histogram were also correlated with other studies.

The most prevalent cause of microcytic RBC is iron deficiency anaemia, which affects mostly women in their reproductive years. Iron deficiency during pregnancy is a significant problem in our nation.[17,18] In macrocytic anaemia, a right shift with a broad-based curve indicates a low Hb level and a macrocytic blood image. The causes of macrocytosis range from benign to malignant, and determining the aetiology requires a comprehensive approach. Macrocytosis can strike at any age, though it is more common among the elderly.[19-21]

In our study majority of cases of macrocytic anemia showed a right shift curve. Right shift curve correlated well with increased MCV and MCH. The dimorphic blood picture shows a bimodal curve, along with some cases leading to the left and right shifting of the curve. There are wide reasons for dimorphic blood picture, including nutritional anemia, recent blood transfusion or therapy response to nutritional anemia and sideroblastic anemia.

The majority of macrocytic anaemia cases in our study had a right shift curve. Increased MCV and MCH are associated well with the right shift curve. The bimodal curve is visible in the dimorphic blood image, with some cases showing the left and proper shifting of the curve. Nutritional anaemia, recent blood transfusion or therapeutic response to nutritional anaemia, and sideroblastic anaemia are all possible causes of dimorphic blood images. To determine the specific cause, a complete examination is required.[22] These finding were correlated with study carried out by Sandhya[14] and Chavda J.[15] Our study was in concordance with the study conducted by Constantino et al.[16] in 2010. Using Fisher Exact test and comparing the two variables i.e peripheral blood smear reports with histogram patterns the p values showed very high significant difference between the two variables .This difference was largely due to dimorphic anemia cases which was in concordance with Constantino et al.

Conclusion

Histogram plays an additional role with peripheral smear for diagnosing RBCs disorders. Haematology analyzers were very useful and reliable for evaluation of abnormal peripheral smears. Histogram was correlated with almost all peripherial smear interpretation in anemia cases. When the right interpretation of the curve is paired with the findings of blood count characteristics such as red cell distribution width and red cell indices, the RBC Histogram becomes a useful diagnostic tool. Blood

indices and Hb values, as well as histograms, will help us. Histograms are a helpful tool for technologists since they may help them determine which instances require specialist peripheral smear testing.

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