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Original Research Article

A Comparative Assessment of Peripheral Blood Smears (PBS) and Automated Cell Counter Generated Parameters in Different Types of Anemias

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Abstract

Aim: The aim of the present study was to evaluate and compare the results obtained through two different methods: examination of peripheral blood smears (PBS) and automated cell counter generated parameters.

Methods: The present study was a prospective study conducted in Department of Pathology, over a period of one year. The cases included were newly diagnosed cases undergoing treatment and follow up. 200 cases were included in the study.

Results: Our study included that out of total 2000 cases; males were 960 cases (48%), while females were 1040 (52%). Among the study population, the largest proportion of patients (34%) fell within the age group of 31-45 years, followed closely by those aged 16- 30 years (31%). In this study 1400 cases (70%) were diagnosed as microcytic anemia by automated analyzer which constituted major portion of study population. In our study normocytic, dimorphic and macrocytic cases were found 14%, 7.8% and 4.5% respectively by automated analyzer. In our study on peripheral smear examination maximum number of cases (51%) belonged to microcytic anemia and normocytic anemia 30% and 8.1% cases belonged to dimorphic anemia, 3.1% cases belonged to macrocytic anemia and 3.1% hemolytic anemia and 0.3% Red Cell Agglutinins (cold) and 0.2% cases belonged to Thalassemia. Specifically, a left shift was observed in 75% of cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2.3% of cases, and multiple peaks were seen in only 1% of cases.

Conclusion: Histograms are an essential tool for the initial morphological analysis of blood samples, especially when combined with the concept of the normal curve and knowledge of CBC parameters like RDW and red cell indices. By examining the shape of the histograms, potential pathology can be identified, providing hints for cases that require detailed peripheral smear examination. Moreover, the histograms offer insight into RBC count, MCV, and RDW through their shape and shift in different directions.

Keywords: Anemia, Peripheral blood smear, Automated cell counter, Red blood cell

Introduction

Peripheral blood smear examination has been an important part of investigation for various hematological disorders since decades and also major diagnostic tool especially for etiopathological work up of different hematological disorder. The automated hematology analyzer has replaced the traditional manual methods for measuring various hematological parameters as the initial screening method in most of the hospital nowadays. [1] Along the years there have been different studies from time to time for assessing the utility and accuracy of automated cell counter generated parameters in general as well as with respect to diagnose specific types of anemia. This study is an attempt to standardize few automated red cell parameters and to compare these with microscopic examination of peripheral blood smear.

Automated cell counter provide histogram of RBCs which give us important clue regarding particle size, volume. This RBCs histogram if interpreted along with other important RBCs indices like Red cell distribution width (RDW) and mean corpuscular volume (MCV), have been found very useful in

work up of many hematological disorders and may provide major diagnostic clue in condition like anemia, thalassemia. [2-5] In addition, RBCs histogram most widely used with peripheral blood smear to monitor and interpret abnormal morphological variation of red blood cells like, dimorphic red cells.

Since decades, peripheral blood smear has been used as window to observe hematological ongoings. Analyzing peripheral blood smears routinely has facilitated interpretation of various hematological disorders and has been a major diagnostic tool. [6] Cell counters have penetrated medical laboratory services in a ubiquitous manner with increasing efficacy and decreasing cost all over the world. Over the past few years, complete blood count (CBC) by the automated and microscopic examination of haematology analysers peripheral smear have complemented each other to provide a comprehensive report on patient's blood sample. [7-9] The advent of automated hematology cell counter has improved accuracy and precision, has reduced subjective errors and safety in handling of blood specimen. There is still a need to depend on manual techniques for primary calibration despite, the sophistication of present day instruments

This highlights the importance of maintaining the manual technical skills, and to ensure this by appropriate technician training program, despite the temptation to leave it all to the machines. The aim of the present study was to evaluate and compare the results obtained through two different methods: examination of peripheral blood smears (PBS) and automated cell counter generated parameters.

Materials and Methods

The present study was a prospective study conducted in Department of Pathology, Government Medical College and Hospital, Bettiah, West Champaran, Bihar, India over a period of one year. The cases included were newly diagnosed cases undergoing treatment and follow up. 200 cases were included in the study.

Inclusion Criteria

All patients, both male and female with anemia ie haemoglobin levels below WHO reference values.

Exclusion Criteria

Patients with normal Hemoglobin levels (within the normal range for that particular age)

Tools and Techniques

For this study, a blood sample of 3 ml was collected in EDTA and thoroughly mixed. The analysis will be performed using automated hematology analyzers - SYSMEX XS-800i and Sysmex XN 1000. A peripheral smear will also be prepared using Giemsa stain as per standard operating procedures. The smear will be evaluated by a pathologist who will not have access to the histogram during reporting. The typing of anemia will be considered concordant if both methods indicate the same morphological type, otherwise, the results will be considered discordant.

Results

Table 1: Distribution of study population according to gender

Gender	Frequency	Percentage
Male	960	48
Female	1040	52
Total	2000	100

Our study included that out of total 2000 cases; males were 960 cases (48%), while females were 1040 (52%).

Table 2: Distribution of study population according to age

Age groups (years)	Frequency	Percentage
Up to 1	15	0.75
1.1-15	240	11
16-30	620	31
31-45	680	34
46-60	440	22
>61	25	1.25
Total	1000	100%

Our study included patients spanning a wide age range, from 8 days to 75 years old. Among the study population, the largest proportion of patients (34%) fell within the age group of 31-45 years, followed closely by those aged 16- 30 years (31%). These findings suggest that anemia is a condition that affects individuals of various ages, and underscore the need for appropriate screening and management strategies across the lifespan.

Table 3: Comparison of cases between automation and PBF

Types of Anemia	Histogram & RBC indices	PBF
Normocytic	280 (14%)	600 (30%)
Microcytic	1400 (70%)	1020 (51)
Macrocytic	90 (4.5%)	60 (3%)
Dimorphic	156 (7.8%)	162 (8.1%)
Pancytopenia	80 (4%)	66 (3.3%)
Red Cell Agglutinins(cold)	4 (0.2%)	6 (0.3%)
Hemolytic	0 (0.0%)	62 (3.1%)
Thalassemia	4 (0.2%)	4 (0.2%)

In this study 1400 cases (70%) were diagnosed as microcytic anemia by automated analyzer which constituted major portion of study population. In our study normocytic, dimorphic and macrocytic cases were found 14%, 7.8% and 4.5% respectively by automated analyzer. In our study on peripheral smear examination maximum number of cases (51%) belonged to microcytic anemia and normocytic anemia 30% and 8.1% cases belonged to dimorphic anemia, 3.1% cases belonged to macrocytic anemia and 3.1% hemolytic anemia and 0.3% Red Cell Agglutinins (cold) and 0.2% cases belonged to Thalassemia.

Table 4: Distribution of cases asper histogram abnormality

Histogram abnormality	Frequency	Percentage
Normal curve	360	18
Left shift	1500	75
Right shift	80	4
Broad base	1600	80
Bimodal	46	2.3%
Multiple peak	20	1%

The results of our study indicate that a normal histogram (bell shape) was observed in only 18% of cases, while the majority (80%) exhibited a broad base curve, including cases with a left shift, right shift, bimodal, and multiple peaks. Specifically, a left shift was observed in 75% of cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2.3% of cases, and multiple peaks were seen in only 1% of cases.

Discussion

Anemia is one of the most common global health problem, particularly in India. It has been associated with significant morbidity and mortality. Laboratory investigations, including a complete blood count (CBC) and differential leukocyte count, are crucial in diagnosing anemia, platelet disorders, white cell disorder, leukemia, and other related conditions. Over the years, blood cell analysis has advanced significantly from manual procedures to automated instruments, providing more accurate and reliable results. [10] Automated hematological analyzers have become an integral tool in providing accurate and efficient blood cell analysis. These machines not only provide essential information on RBC indices, hematocrit, and RBC distribution width (RDW), but also give a detailed RBC histogram. Such comprehensive analysis plays a crucial role in diagnosing and managing red cell disorders. In fact, for accurate morphological diagnosis of anemia, the histogram provided by these analyzers is particularly important. Therefore, it is evident that the RBC histogram is a critical component in the laboratory evaluation of blood cells.

Our study included that out of total 2000 cases; males were 960 cases (48%), while females were 1040 (52%). Our study included patients spanning a wide age range, from 8 days to 75 years old. Among the study population, the largest proportion of patients (34%) fell within the age group of 31-45 years, followed closely by those aged 16- 30 years (31%). These findings suggest that anemia is a condition that affects individuals of various ages, and underscore the need for appropriate screening and management strategies across the lifespan. These results were in concordance with the studies conducted by Kumar et al [11], Cook et al [12] and Japheth et al. [13] This can be explained as the period of adolescence and adult group is a period of intense growth and development and iron is in high demand as it is present in all body cells and is fundamental for basic physiological processes such as Hemoglobin formation. The body needs more iron when it grows rapidly and when frequent blood loss occurs (e. g. menstruation) thus women in reproductive age group are at high risk of developing iron deficiency anemia. Visual representations, such as the RBC histogram, have a much more significant impact on clinicians than numbers alone. The newer generation of hematology analyzers generates a range of histograms that offer significant and

essential information about a patient's blood profile, even before a peripheral blood smear is examined. [14] The RBC histogram is generated by the automated hematology analyzer, which uses sophisticated technology to measure the size and number of red blood cells in the blood sample. [15] The normal histogram curve generated by the automated hematology analyzer is typically bell-shaped and symmetrical, indicating a Gaussian distribution. This normal curve represents the range of mean corpuscular volume (MCV) between 80-100fl. [16-18]

In this study 1400 cases (70%) were diagnosed as microcytic anemia by automated analyzer which constituted major portion of study population. In our study normocytic, dimorphic and macrocytic cases were found 14%, 7.8% and 4.5% respectively by automated analyzer. In our study on peripheral smear examination maximum number of cases (51%) belonged to microcytic anemia and normocytic anemia 30% and 8.1% cases belonged to dimorphic anemia, 3.1% cases belonged to macrocytic anemia and 3.1% hemolytic anemia and 0.3% Red Cell Agglutinins (cold) and 0.2% cases belonged to Thalassemia. In a cell counter's RBC histogram, cells with volumes ranging from 36 fl to 250 fl are counted as RBCs. However, if the RBC histogram begins below 36 fl, it may be indicative of the presence of small particles such as microspherocytes, parasites, platelet clumps, normoblasts, elliptocytes, bacteria, leukocyte fragments, and large platelets etc. [19] The area of the peak is used to calculate the MCV and RDW, i.e 60 fl to 125 fl. [20] The RBC distribution curves can provide valuable insights into various types of anemia. In cases of Iron deficiency anemia and beta thalassemia trait, the curves are shifted towards the left. On the other hand, a histogram with a broad base and a right- shifted curve may indicate macrocytic anemia.

The results of our study indicate that a normal histogram (bell shape) was observed in only 18% of cases, while the majority (80%) exhibited a broad base curve, including cases with a left shift, right shift, bimodal, and multiple peaks. Specifically, a left shift was observed in 75% of cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2.3% of cases, and multiple peaks were seen in only 1% of cases which were in accordance with other studies like Rao et al., Chavda et al [21] The higher incidence of normal curve in present study is due to inclusion of outpatient anemic patients only which usually have mild anemia. [22] The results of the present study were in contrast with an earlier report by Pierre 23and Novis et al. 24who reported that automated haematology analyzer are more accurate in the detection of specimens with morphological abnormality than the traditional eye count method. [23]

Conclusion

Histograms are an essential tool for the initial morphological analysis of blood samples, especially when combined with the concept of the normal curve and knowledge of CBC parameters like RDW and red cell indices. By examining the shape of the histograms, potential pathology can be identified, providing hints for cases that require detailed peripheral smear examination. Moreover, the histograms offer insight into RBC count, MCV, and RDW through their shape and shift in different directions.

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