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Original Research Article

A Prospective Comparative Study of Peripheral Smear and CBCS Levels in Patients with Anemia

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Abstract

Aim: The aim of the present study was to assess the morphological findings of the red blood cells (RBCs) in peripheral blood smear in order to categorize different types of anemia and to compare it with RBC indices measured with an automated hematology analyzer.

Methods: The present study was a prospective study conducted in Department of Pathology, Nalanda Medical College and Hospital, Patna, Bihar, India over a period of one year. 200 patients were included in the study. The cases included were newly diagnosed cases undergoing treatment and follow up.

Results: Our study included that out of total 200 cases; males were 96 cases (48%), while females were 104 (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 (32%). In this study 70 cases (70%) were diagnosed as microcytic anemia by automated analyzer which constituted major portion of study population. In our study on peripheral smear examination maximum number of cases (52%) belonged to microcytic anemia and normocytic anemia (30%) and 7% cases belonged to dimorphic anemia, 2% cases belonged to macrocytic anemia and 3% hemolytic anemia and 0.5% Red Cell Agglutinins (cold) and 1.5% cases belonged to Thalassemia. The results of our study indicate that a normal histogram (bell shape) was observed in only 17% of cases, while the majority (41%) exhibited a broad base curve, including cases with a left shift, right shift, bimodal, and multiple peaks. Specifically, a left shift was observed in 35% of cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2% 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.

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

Introduction

Anemia is a condition described by insufficient red blood cells or based on hemoglobin content in the blood below a specific range estimated for specific sex and age of a person. Anemia is diagnosed using PBS where microscopic examination of blood smear provides useful information about alteration of RBC shape and size or presence of any inclusion bodies. RBC morphology is a key tool for hematologists to recommend appropriate clinical and laboratory follow-up and to select the best tests for definitive diagnosis. Anemia analysis can be done based on RBC morphology and clinical parameters. Morphological analysis using blood smear is performed by spreading a drop of blood thinly onto a glass slide and stained with coloring agents such as Giemsa, Leishman, and Wright-Giemsa. [1] The blood smear contains different types of cells, namely White Blood Cells (WBCs), RBCs and platelets.

Over the past few years, automated hematology analyzers have become increasingly popular due to their accuracy and reliability, which has significantly reduced subjective errors in diagnosing anemia. However, it is important to note that the microscopic examination of the peripheral blood smear (PBS) by a pathologist remains a critical step in the primary calibration of cell counters. This examination plays a pivotal role in ruling out other hematological disorders that may go undetected through automated analysis alone. Therefore,

despite the remarkable advancements in technology, the role of pathologists in examining the PBS remains invaluable in ensuring accurate and reliable diagnosis. [2]

It can be observed that RBCs are more in number in comparison with WBCs and platelets. During this examination of the smear, the pathologists assess the size, shape, and color of the RBCs and WBCs. Also, they estimate the number of platelets present. The quality of RBC is characterized by red cell indices and any deviation in size, volume, or shape of red cells represents an abnormal red blood cell. [3]

Anemia also could be multifactorial in origin. So, every patient of anemia should be investigated to find out the cause and the type for proper medical treatment .However there is lack of consistency in the protocols for proper screening for anemia. [4-6] The commonest laboratory test for diagnosis of anemia is hemoglobin estimation. In clinical practice, anemia is diagnosed by reduced hemoglobin concentration of the blood below the normal range and reduced hematocrit level (the ratio of packed red cells to total blood volume). [7]

Therefore, the study was undertaken to assess the morphological findings of the red blood cells (RBCs) in peripheral blood smear in order to categorize different types of anemia and to compare it with RBC indices measured with an automated hematology analyzer.

Materials and methods

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

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 were collected in EDTA and thoroughly mixed. The analysis was performed using automated hematology analyzers - ABX Pentra XL80. A peripheral smear was prepared using Giemsa stain as per standard operating procedures. The smear was evaluated by a pathologist who did not have access to the histogram during reporting. The typing of anemia was considered concordant if both methods indicate the same morphological type, otherwise, the results were considered discordant.

Results

Table 1: Distribution of study population according to gender

Gender	Frequency	Percentage
Male	96	48
Female	104	52
Total	200	100
Age groups (years)		
Up to 1	1	1
1.1-15	10	10
16-30	32	32
31-45	34	34
46-60	20	20
>61	3	3

Our study included that out of total 200 cases; males were 96 cases (48%), while females were 104 (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 (32%).

Table 2: Comparison of cases between automation and PBF

Types of Anemia	Histogram & RBC indices	Peripheral smear
	N (%)	N (%)
Normocytic	28 (14%)	60 (30%)
Microcytic	140 (70%)	104 (52)
Macrocytic	8 (4%)	4 (2%)
Dimorphic	12 (6%)	14 (7%)
Pancytopenia	8 (4%)	8 (4%)
Red Cell Agglutinins (cold)	1 (0.5%)	1 (0.5%)
Hemolytic	0 (0.0%)	6 (3%)
Thalassemia	1 (0.5%)	3 (1.5%)

In this study 70 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%, 6% and 4% respectively by automated analyzer. In our study on peripheral smear examination maximum number of cases (52%) belonged to microcytic anemia and normocytic anemia (30%) and 7% cases belonged to dimorphic anemia, 2% cases belonged to macrocytic anemia and 3% hemolytic anemia and 0.5% Red Cell Agglutinins (cold) and 1.5% cases belonged to Thalassemia.

Table 4: Distribution of cases as per histogram abnormality

Histogram abnormality	Frequency	Percentage
Normal curve	34	17
Left shift	70	35
Right shift	8	4
Broad base	82	41
Bimodal	4	2
Multiple peak	2	1

The results of our study indicate that a normal histogram (bell shape) was observed in only 17% of cases, while the majority (41%) exhibited a broad base curve, including cases with a left shift, right shift, bimodal, and multiple peaks. Specifically, a left shift was observed in 35% of cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2% 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. [8] 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. [9] 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. [10]

Our study included that out of total 200 cases; males were 96 cases (48%), while females were 104 (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 (32%). Various previous studies showed similar findings, where the maximum number of cases was in the 16–45 years age group and the majority were women. 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 due to Adolescence and adult group is an important period of nutritional vulnerability due to increased dietary requirements for 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 thus, it is extremely important for the adolescent's iron requirements to be met. Women are more affected by iron deficiency anaemia than men because they lose iron during their menstrual periods and need more when pregnant or breast feeding thus women in reproductive age group are at high risk of developing iron deficiency anemia.

In this study 70 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%, 6% and 4% respectively by automated analyzer. Microcytic hypochromic anemia was the most common type in our study and the most common cause of this was iron deficiency anemia. Iron deficiency anemia is the most common type of anemia in the world and there are various reasons for this. Causes may be due to inadequate dietary intake, increased demand mainly in pregnancy and lactation, poor absorption from gut, chronic blood loss, etc. [14] In our study on peripheral smear examination maximum number of cases (52%) belonged to microcytic anemia and normocytic anemia (30%) and 7% cases belonged to dimorphic anemia, 2% cases belonged to macrocytic anemia and 3% hemolytic anemia and 0.5% Red Cell Agglutinins (cold) and 1.5% cases belonged to Thalassemia. The results of our study indicate that a normal histogram (bell shape) was observed in only 17% of cases, while the majority (41%) exhibited a broad base curve, including cases with a left shift, right shift, bimodal, and multiple peaks. Specifically, a left shift was observed in 35% of

cases, while a right shift was present in only 4% of cases. Bimodal histograms were observed in 2% 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.

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