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Original Research Article

RELEVANCE OF PERIPHERAL BLOOD SMEAR EXAMINATION IN DIAGNOSIS OF ANEMIA IN AN ERA OF AUTOMATION

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Abstract

Background: This study was done to compare the automated cell counter-generated parameters and histogram patterns with peripheral blood smear findings in order to provide a better approach for diagnosis of anemias. It also aims to find out if PBS examination can be replaced by automated analyzer-generated data in diagnosing anemias. **Materials and Methods:** Blood samples were collected from 914 patients of anemia, aged 18 years and above, over a period of one year, in the hematology laboratory of the Department of Pathology. RBC parameters and histograms obtained from automated hematology analyzer and PBS examination findings were evaluated and compared **Results:** Anemia was found to be more common in females (69%) as compared to males (31%). Microcytic hypochromic anemia (51.4%) was the most common followed by normocytic normochromic (32.5%), dimorphic (13.4%), and macrocytic anemia (2.7%) as diagnosed on PBS examination. On analysis of autoanalyzer derived RBC indices, microcytic hypochromic anemia comprised 53.1% of total cases, normocytic normochromic anemia comprised 38.7%, and dimorphic anemia and macrocytic anemia constituted 4.8% and 3.4% cases respectively. In the present study histogram patterns that were observed included normal curve in 24.3% of cases, left shift curve in 29% of cases, right shift curve in 4.7% of cases, broad-based, short peak, and bimodal curves were noted in 37.1%, 1.3%, and 3.6% cases respectively. **Conclusion:** RBC indices and histograms obtained from hematology analyzer help in diagnosing and categorization of anemias in most cases but peripheral blood smear examination remains an important diagnostic tool and cannot be entirely replaced especially in cases of dimorphic anemia as it provides additional morphological details which cannot be interpreted by automated analyzer. Both methods have their limitations, hence an integrated approach is advocated.

INTRODUCTION

Anemia is a global public health problem, more so in developing countries resulting in severe consequences for health, economy, and national development.[1,2] Anemia is associated with increased risks for maternal and child mortality, hence accurate and early diagnosis is essential to reduce mortality and morbidity. According to World Health Organization (WHO), anemia is a condition in which the number of red blood cells (and consequently their oxygen carrying capacity) is insufficient to meet body's physiologic need.[3] World over; 1.62 billion (24.8%) people seem to be affected by anemia.[2,4] In India, according to National Family Health Survey 5 (NFHS-5), the prevalence rate of anemia is 67.1% in children (6-59 months), 59.1% in adolescent girls (15-19 years), 31.1% in adolescent boys (15-19 years), 57% in females (15-49 years), and 25% in men (15-49 years). Nutritional deficiency is the most common cause.[5,6]

Red blood cell indices and histograms obtained by automated hematology analyzer help in the categorization of anemia to a large extent, at the same time reducing subjectivity and improving accuracy and precision.[7] In this era of automation,

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the conventional peripheral blood smear (PBS) examination under the microscope remains an important diagnostic tool as it provides additional morphological details which cannot be interpreted by numerical data and histograms obtained from automated hematology analyzer. But PBS examination is a time-consuming process and suffers from interobserver variation.[8] Therefore, if both techniques are used together, they can provide a better approach and nullify limitations.[9,10] Not many studies have taken all three parameters i.e., complete blood count, histograms, and peripheral blood smear examination into consideration and correlated their findings for the categorization of anemia.

MATERIALS AND METHODS

The present study was a prospective study carried out in the Department of Pathology, NIIMS, Greater Noida over a period of one year during January'2021 to December'2021. It is an observational study carried out on 914 patients of anemia.

Inclusion Criteria

Patients aged 18 years and above, having anemia as per World Health Organization (WHO) reference range, were included in the study.

WHO (2011) recommendations[3] for hemoglobin (Hb) levels to diagnose anemia at sea level (g/l) are

- Non pregnant females - Hb <120
- Pregnant females- Hb <110
- Men - Hb < 130

Exclusion Criteria

- Pregnant female
- Anemic patients with leucocytosis, leukemoid reaction, leukaemia and platelet disorders were excluded from the study.

The identity of patients was not revealed during the course of the study.

Method of data collection

The venous blood sample was collected by venepuncture in EDTA vacutainers and analyzed by 5 parameter hematology analyzer, Mindray BC-5130 with standard calibration. Complete blood count which included Hemoglobin concentration, Total leucocyte count, Differential leucocyte count, Platelet count & Red blood cell (RBC) indices along with histograms were obtained. Peripheral blood smears were prepared and stained with Leishman stain and morphological abnormalities were noted. Anemia typing was done first by parameters obtained by hematology analyzer and position & shape of histograms which were then correlated with peripheral blood smear findings. The results obtained by hematology analyzer were not known to the hematologists reporting the peripheral blood smear.

Categorization of anemia based on RBC indices by autoanalyzer

- Microcytic hypochromic anemia
- Normocytic normochromic anemia
- Macrocytic anemia
- Dimorphic anemia

Patterns seen in histograms

- Normal curve
- Left shift curve
- Right shift curve
- Broad based curve
- Short peak curve
- Bimodal curve

Morphological typing of anemia based on peripheral blood smear findings

- Microcytic hypochromic anemia
- Normocytic normochromic anemia
- Macrocytic anemia
- Dimorphic anemia

Concordance was considered when categorization of anemia based on peripheral blood smear examination correlated with categorization of anemia based on histograms and RBC indices.

RESULTS

This study comprised of 914 patients having anemia, aged 18 years and above, in which cell counter generated RBC indices and histograms were evaluated and correlated with peripheral blood smear findings in different types of anemia. The study included 631 females and 283 males, with F:M ratio of 2.2:1. Out of total anemia cases reported, most affected people were in the age group of 18-30 years (37.6%). In males, maximum number of cases were seen after 60 years of age. (Table 1)

On examination of peripheral blood smears, 470 cases (51.4%) of microcytic hypochromic anemia, 297 cases (32.5%) of normocytic normochromic anemia, 24 cases (2.7%) of macrocytic anemia, and 123 cases (13.4%) of dimorphic anemia were observed. Microcytic hypochromic anemia was the predominant finding (51.4% cases) followed by normocytic normochromic anemia (32.5% cases) in both the sexes. (Table 2)

On analysis of autoanalyzer derived RBC indices, microcytic hypochromic anemia comprised 53.1% of total cases followed by normocytic normochromic anemia (38.7%) and dimorphic anemia (4.8%). Least number of cases were of macrocytic anemia (3.4%) (Table 3).

When anemia diagnosis by peripheral blood smear findings were compared with autoanalyzer derived RBC indices; discrepancy was mainly noted in the category of dimorphic anemia. A diagnosis of dimorphic anemia based on data obtained by automated analyzer was made in 4.8% cases as against 13.4% cases on PBS examination. This was because normal range of MCV i.e., 80-100 femtolitre was observed in most of these cases by autoanalyzer and hence categorized as normocytic normochromic anemia (Table 4). Rest of the

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categories correlated well on autoanalyzer and peripheral blood smear examination.

In the present study, various types of histogram patterns were observed; normal curve was seen in 24.3% cases, left shift curve in 29% cases, right shift curve in 4.7% cases, broad based, short peak and bimodal curves were noted in 37.1%, 1.3% and 3.6% cases respectively (Table 5).

Out of 470 cases of microcytic hypochromic anemia; 229 cases (25.1%) showed left shift curve, 179 cases (19.6%) showed broad based curve, 35 cases (3.8%) showed normal curve, 12 cases (1.3%) showed short peak while bimodal peaked histogram was noted in 15(1.6%) cases. Of the total 297 cases of normocytic normochromic anemia, 164 cases (17.9%) showed normal curve and 134 cases (14.7%) showed broad based curve. All cases of macrocytic anemia showed right shift curve. In cases of dimorphic anemia, we observed left shift curve in 35 cases (3.8%), broad based curve in 26 cases (2.9%), normal curve in 24 cases (2.6%), right shift curve in 19 cases (2.1%) and bimodal curve in 18 cases (2.0%). (Table 6). When we evaluated histogram patterns and correlated it with peripheral blood smear findings, we observed that the histogram patterns correlated in most cases of microcytic hypochromic, normocytic normochromic and macrocytic anemia. However, variations of histogram pattern were seen in dimorphic anemia.

Table 1: Age and sex distribution

Type of Anemia (PBS)	Male		Female		Total	
	Number	%	Number	%	Number	%
Microcytic Hypochromic	119	13	351	38.4	470	51.4
Normocytic Normochromic	99	10.8	198	21.7	297	32.5
Macrocytic Anemia	16	1.8	8	0.9	24	2.7
Dimorphic Anemia	49	5.4	74	8.1	123	13.4
Total	283	30.9	631	69.1	914	100

Table 2: Morphologic typing of anemia based on Peripheral blood smear examination

Type of Anemia (PBS)	Male		Female		Total	
	Number	%	Number	%	Number	%
Microcytic Hypochromic	113	12.4	372	40.7	485	53.1
Normocytic Normochromic	132	14.4	222	24.3	354	38.7
Macrocytic Anemia	20	2.2	11	1.2	31	3.4
Dimorphic Anemia	18	2	26	2.8	44	4.8
Total	283	31	631	69	914	100

Table 3: Typing of Anemia based on RBC indices obtained by autoanalyzer generated data

	Peripheral blood smear findings		Erythrocyte indices	
Type of Anemia	**Number**	**%**	**Number**	**%**
Microcytic Hypochromic	470	51.4	485	53.1
Normocytic Normochromic	297	32.5	354	38.7
Macrocytic Anemia	24	2.7	31	3.4
Dimorphic Anemia	123	13.4	44	4.8
Total	914	100	914	100

Table 4: Correlation between morphologic typing of anemia based on PBS findings with typing of anemia using RBC indices

Pattern	Number	%
Normal Curve	223	24.3
Left Shift	264	29
Right Shift	43	4.7
Broad based	339	37.1
Short peak	12	1.3
Bimodal	33	3.6

Table 5: Types of Histogram patterns

	Normal curve	Left shift curve	Right shift curve	Broad based curve	Short peak curve	Bimodal
Microcytic Hypochromic anemia	35 (3.8%)	229 (25.1%)		179 (19.6%)	12 (1.3%)	15 (1.6%)
Normocytic Normochromic anemia	164 (17.9%)			134 (14.7%)		
Macrocytic Anemia			24 (2.6%)			
Dimorphic Anemia	24 (2.62%)	35 (3.8%)	19 (2.1%)	26 (2.9%)		18 (2.0%)
Total	223	264	43	339	12	33

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DISCUSSION

Anemia is a major public health problem world over. It causes increased morbidity and mortality, especially in children and pregnant females. Timely and accurate diagnosis is therefore essential. For decades, peripheral blood smears (PBS) are being used as a diagnostic tool for the categorization of anemia.[7] The introduction of hematology analyzer has improved the accuracy and sensitivity of reporting complete blood count parameters and significantly reduced the workload in the laboratory. The present study was conducted to evaluate whether the anemia parameters and histograms obtained by the hematology analyzer correlated well with the observations made after examining peripheral blood smear manually and in case a good correlation between the two can be established, whether hematology analyzer can replace the manual reconfirmation of the result.

Our study included 914 cases of anemia, out of which 631(69%) were females and 283(31%) were males similar to observations made by Swaroop Raj et al.[4] and Singhal et al.[8] In females, the prevalence of anemia was highest in the 18-30 years age group (32%) while in males, the maximum number of cases were seen after 60 years of age (7.9%) similar to the prevalence observed by Garg et al.[7]

In the present study, in both males and females, on peripheral blood smear examination, microcytic hypochromic anemia (51.4%) was the commonest followed by normocytic normochromic anemia (32.5%) which is in accordance with other Indian studies.[1,2,5,7,8,11,12,13] Iron deficiency anemia was the most common cause of microcytic hypochromic anemia; other causes include thalassemia, sideroblastic anemia, and anemia of chronic disease.[1, 7, 14]

A diagnosis of microcytic hypochromic anemia was made in 51.4% of cases on PBS examination, similar to observations made by Garg et al (50.86% cases).[7], Singhal et al (49.8% cases).[8] and Mukaya et al (54% cases).[11] Cases of microcytic hypochromic anemia showed decreased MCV (mean corpuscular volume) and MCH (mean corpuscular hemoglobin), reduced or normal MCHC (mean corpuscular hemoglobin concentration), and increased RDW (red cell distribution width). Out of 51.4% cases of microcytic hypochromic anemia, the most common histogram pattern noted was the left shift curve (25.1%) which correlated with low MCV. A broad based curve because of high RDW was seen in 19.6% of cases and correlated with the degree of anisopoikilocytosis on peripheral blood smear examination. A normal curve was seen in 3.8% of cases which were usually mild cases of microcytic hypochromic anemia. The short peak curve represented low hemoglobin and low red cell count. These findings are in concordance with studies done by other authors.[9,13,15] On correlating the data, we observed that automated analyzer-generated data showed microcytic hypochromic anemia in 53.1% of cases as against 51.4% observed by manual peripheral blood smear examination. The explanation for this difference is that in these cases autoanalyzer considered schistocytes and giant platelets as microcytes.[7]

On peripheral blood smear examination, normocytic normochromic anemia comprised 32.4% of the total cases, similar to observations made by Kumari et al.[6], Singhal et al.[8] and Mukaya et al.[11] Automated analyzer-generated RBC indices showed normocytic normochromic anemia (MCV, MCH, MCHC within normal range) in 38.7% of cases. This discrepancy was because some cases of dimorphic anemia were included in this category by the automated analyzer. A normal bell-shaped curve was seen in 17.9% of cases while a broad based curve was seen in 14.7% of cases. In most cases of normocytic normochromic anemia showing a broad based curve, some microcytic cells were seen along with a predominant population of normal-sized cells that resulted in higher RDW and hence a broad curve.[7,13]

Macrocytic anemia was diagnosed in 2.7% of cases on peripheral blood smear examination, similar to observations made in other studies[5,7,13,15]; while RBC indices revealed 3.4% cases of macrocytic anemia (Increased MCV, MCH & RDW). Histogram findings showed a right shift curve in all the cases. This finding correlated with observations made by Chavda et al.[9] and Rao et al.[15] The right shift curve correlated well with increased MCV and MCH. RBC- based indices gave a slightly increased percentage as compared to peripheral blood smear examination because few cases of hemolytic anemia having polychromatophilic erythrocytes and agglutinins resulted in a high value of MCV and hence included in this category .[7] Also, some cases of macrocytic anemia were diagnosed as dimorphic anemia on PBS examination.

Dimorphic anemia was diagnosed in 13.4% of cases on peripheral blood smear examination while RBC parameters revealed the same in 4.8% of cases. Histogram showed a bimodal curve in only 2.0% of cases whereas the rest showed a left shift curve (3.8% cases), broad based curve (2.9% cases), normal curve (2.6% cases), and right shift curve (2.1% cases). The bimodal curve is usually associated with multiple red cell populations as seen in therapeutic transfusions and/or hematinic agent response to microcytic and macrocytic anemia, iron deficiency anemia developing macrocytic population, and folate/vitamin B12 deficiency anemia developing microcytic population.[7,9,12,16] The broad-based curve can be explained by the presence of multiple populations of cells of varying sizes (i.e., normocytes, microcytes, and macrocytes). Since MCV is an average value, hence it can be normal in presence of two different RBC populations, and such type of cases, in our study, got categorized as normocytic normochromic

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anemia when we considered automated analyzer generated data. This resulted in a discrepancy in the number of cases in this category. It is, therefore, important that morphological findings should be correlated with the graphical and numerical data for better interpretation of results. Our findings were in concordance with the studies done by other authors.[9,13,14,15]

The present study showed that the hematology analyzer provided accurate morphological categorization in most cases of microcytic hypochromic, normocytic normochromic, and macrocytic anemia. Dimorphic anemia is the category where the maximum discrepancy was noted.

Thus, we can say that blood smear examination remains an important diagnostic tool and numerical data and histograms derived from automated analyzers should be interpreted by taking the peripheral blood smear examination and clinical context into consideration.

CONCLUSION

From the present study we conclude that although, RBC indices and histograms obtained by hematology analyzer give valuable inputs, but peripheral blood smear examination still remains the gold standard in categorization of various types of anemia as they provide additional morphological details which cannot be interpreted merely by numerical data provided by automated analyzers. RBC indices and histograms can guide as to which cases peripheral examination can be restricted to, thus increasing efficiency and reducing the workload in the laboratory. Both tests are complementary to each other and an integrated approach is advocated.

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Conflicts Of Interest None

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