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A comparative study of anemia in peripheral blood smear and automated cell counter generated red cell parameters

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Abstract:

BACKGROUND: Complete blood count and cell counter generated red blood cell (RBC) parameters help in morphological typing of anemia. Still the importance of microscopic examination of peripheral blood smears (PBSs) cannot be excluded to interpret the cause of anemia.

AIMS AND OBJECTIVES: The present study was done to diagnose the type of anemia by examination of PBS and automated cell counter generated parameters and to compare the findings between these two methods.

MATERIALS AND METHODS: During the 6-months study, 9981 anemic blood samples were evaluated. PBS findings and cell counter generated RBC parameters and histograms were evaluated and compared.

RESULTS: Among 9941 samples, microcytic hypochromic anemia consists of the majority of cases (5048, 50.47%), followed by normocytic normochromic (2187, 21.97%), dimorphic (1297, 12.99%), and lastly, hemolytic anemia (722, 7.24%). Compared with RBC parameters and peripheral smear findings, dimorphic and hemolytic anemia showed significant difference ($P < 0.0001$). When compared with RBC histogram, 4747 (47.56%) cases showed left shift, 820 (8.21%) cases showed right shift, and 2278 (22.82%) cases showed normal bell-shaped curve suggesting microcytic, macrocytic, and normocytic normochromic anemia, respectively. 635 (6.90%) and 1447 (14.50%) number of cases showed bimodal and broad-base histogram suggesting dimorphic and hemolytic anemia, respectively.

CONCLUSION: PBS examination along with RBC histogram study can be able to categorize the type of anemia in the majority of cases. Each method should be used as complementary to each other that increases diagnostic accuracy.

Keywords:

Anemia, automated cell counter, peripheral blood smear

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Introduction

Anemia is one of the most common health problems worldwide, particularly in our country. It has been associated with significant mortality and morbidity. According to the National Family Health Survey-5, our state West Bengal has the prevalence of anemia in men aged 15–49 years is 38.7%, in all women aged 15–49 years is 70.8%, and in children aged 6–59 month is 69%.[1] Therefore, the correct diagnosis of type of anemias, especially important for treatment purpose.

Red blood cell (RBC) parameters and histograms are indispensable for the diagnosis of anemia; however, peripheral blood smear (PBS) examination also very important as it provides crucial

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information about patients' clinical conditions.[2,3] For the past few years, with the use of automated hematology analyzers, there is much improvement of accuracy and precision, reducing subjective errors in the diagnosis of anemia.[4] Automated hematology analyzers also increase safety of handling blood samples. RBC histograms along with other red cell indices such as red cell distribution width (RDW) and mean corpuscular volume (MCV) are useful in diagnosing many hematological conditions including anemia and provide clue in diagnosing thalassemia.[5-7] In spite of this, microscopic examination of PBS by the pathologist is crucial for primary calibration of cell counters and to rule out other hematological disorders such as leukemia and leukemoid reaction.[4]

RBC histogram is often used with PBS examination which increases accuracy in subtyping anemia, particularly in the presence of dimorphic and hemolytic blood pictures. Till now, to our knowledge, no study has been undertaken in this part of the country to compare results of cell counter generated parameters and PBS examination to diagnose anemia.

Hence, we have undertaken this study with the following aims and objectives:

i. To diagnose the type of anemia by examination of PBS and by automated cell counter generated parameters; and
ii. To compare the findings of PBS examination with cell counter generated parameters.

Materials and Methods

Study subjects and study area

The present study was a prospective study done for 6 months from March 2021, to August 2021, in the hematology section of the Pathology department in a tertiary care center of West Bengal, India. The study was carried out after getting ethical clearance from the institutional ethics committee. A total of 9981 samples of anemic patients were included in the study. Informed consent from all adult participants and from guardians of all minor participants was taken to enroll in the study.

Inclusion criteria

All cases of anemia as per the World Health Organization criteria where both peripheral smear and cell counter reports available; and who have given informed consent.[8]

Exclusion criteria

i. Patients <6 months of age
ii. Uncooperative patients and unwilling to give consent
iii. Patients with hematological malignancies
iv. Hemolyzed samples
v. Clotted samples
vi. Samples insufficient to run in hematology analyzer
vii. Patient with history of recent blood transfusion.

Ethical consideration

The study was approved by the institutional ethics committee vide memo no IEC/2021/02/002 Dated: February 12, 2021.

Materials and methods

After getting informed consent, detailed clinical history and demographic information from each patient were recorded. 2 ml of venous blood was collected in EDTA (Ethylenediaminetetraacetic acid) vacutainers. One PBS was made, and then, the blood sample was analyzed using automated hematology analyzer SYSMEX XT-4000i. The blood film was stained using Leishman's stain.

Anemia typing was first done using automated cell counter generated RBC parameters such as RBC indices with RDW. Then, PBS was examined by the pathologist under microscope and morphological typing of anemia done. The position and the shape of RBC histograms were recorded. Pathologists were unaware of the histogram and RBC indices while reporting the PBS.

Categorization of anemia is based on RBC indices and RBC histograms by automated hematology analyzer:

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

Position (normal, left shift, and right shift) and shape (normal bell-shaped or Gaussian, broad-shaped, bimodal peak, and showing to the left or right) of RBC histograms were noted.

Final morphological typing of anemia was done based on peripheral smear examination findings and are recorded as:

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

Statistical analysis

The data were collected and complied. Comparison between the diagnosis made by cell counter generated parameters and PBS examination done using Pearson Chi-square test. The $P = 0.05$ or less was considered statistically significant.

Results and Observations

We have found that the majority of females consists of the majority of anemic patients as compared to males (5973 vs. 4008) of the total of 9981 cases [Table 1]. This distribution of anemia cases among females is statistically significant ($P = 0$). The anemia cases were microcytic hypochromic, normocytic normochromic, macrocytic, dimorphic, and hemolytic according to PBS findings and the same was tabulated in Table 2. Microcytic hypochromic anemia is the most prevalent type of anemia (50.58%) among both men and women, followed by normocytic normochromic anemia (21.92%) [Table 2].

In this study, when the diagnosis based on RBC parameters and peripheral blood film examination was compared, there was statistically significant difference seen in dimorphic anemia ($P < 0.00001$) and hemolytic anemia ($P < 0.00001$) [Table 3].

In our study, of 5048 cases of microcytic hypochromic anemia, 4668 cases showed left shift and 271 cases showed broad-based RBC histogram [Table 4]. Among 2187 cases of normocytic normochromic anemia, 2056 cases showed normal bell-shaped histogram, 727 cases of macrocytic anemia, and 701 cases showed right-shifted RBC histogram. However, in 1297 cases of dimorphic anemia, 566 showed bimodal peak while 541 cases showed broad-base histogram [Table 4].

Table 1: Age and gender-wise distribution of anemia cases

Age (years)	Sex		Total
	Male	Female	
1-10	872	630	1502
11-20	512	886	1398
21-30	640	1696	2336
31-40	354	800	1154
41-50	529	714	1243
51-60	524	490	1014
61-70	314	410	724
≥70	286	324	610
Total	4031	5950	9981

χ^2=495.94, df=7, P=0

Table 2: Types of anemia based on peripheral blood smear findings

Types of anemia	Male, *n* (%)	Female, *n* (%)	Total, *n* (%)
Microcytic hypochromic	1790 (35.46)	3258 (64.54)	5048 (50.58)
Normocytic normochromic	960 (43.90)	1227 (56.10)	2187 (21.92)
Macrocytic	337 (46.35)	390 (53.65)	727 (7.29)
Dimorphic	475 (36.62)	822 (63.38)	1297 (12.98)
Hemolytic	469 (64.96)	253 (35.04)	722 (7.23)
Total	4031 (40.12)	5950 (59.78)	9981 (100)

Table 3: Correlation of peripheral blood smear findings with cell counter generated red blood cell indices

Types of anemia	PBS findings, *n* (%)	RBC indices, *n* (%)	*P*
Microcytic hypochromic	5048 (50.57)	5686 (56.97)	
Normocytic normochromic	2187 (21.92)	2356 (23.60)	0.02147
Macrocytic	727 (7.28)	805 (8.07)	0.7653
Dimorphic	1297 (12.99)	816 (8.17)	<0.00001
Hemolytic	722 (7.24)	318 (3.19)	<0.00001
Total	9981 (100.00)	9981 (100.00)	

PBS=Peripheral blood smear, RBC=Red blood cell

Discussion

Anemia has been a major health problem worldwide and West Bengal has a remarkably high prevalence of anemic people.[1] Although PBS examination along with hemoglobin report can be used as initial diagnostic tool as clue to diagnose anemia, the role of automated hematology analyzer with generated RBC parameters provides crucial information for typing of anemia.

For many decades, PBS examination has been used as a major diagnostic tool for workup of anemia.[9] With the use of automated hematology analyzers, the diagnostic accuracy and precision have improved, and it also reduces subjective errors.[10] RBC histogram is a graphical representation obtained from an automated hematology analyzer.[11] RBC histogram is an integral part of complete blood count in hematology analyzer which provides clues in various RBC disorders and gives valuable information regarding various RBC parameters such as RDW, mean corpuscular hemoglobin (MCH), and MCV.[2] RBC histogram is a vertical bar chart which reflects the size of any cell which is found within the size range. When the cell volume is in between 25fL and 250fL, the cell counter counted the cell as RBC.[12] The area of the peak is used to calculate various RBC parameters such as RDW and MCV. Normal RBC curve is symmetrical bell-shaped or Gaussian distribution. Normal curve falls within the normal range of MCV which is 80–100fL. RBC histogram along with other RBC parameters such as MCV, RDW has been found to be abnormal in various RBC disorders.[13]

In our study, we included a total of 9981 anemic cases as per inclusion criteria. Of these cases, the majority of cases fall in the age group of 21–30 years (2336) and female outnumbered male (5950 vs. 4031). Various previous studies showed similar findings, where the maximum number of cases were in the 21–30 years age group and the majority were women.[2,4,14] The increased number of cases in the 21–30 years age group is mainly due to the inclusion of anemic pregnant women as well as blood loss due to menstruation in nonpregnant women who

Table 4: Correlation of peripheral blood smear findings with red blood cell histogram curves

Type of anemia	RBC histogram pattern					Total
	Normal	Left shift	Right shift	Bimodal	Broad	
Microcytic hypochromic	99	4668	0	10	271 (5048)	
Normocytic normochromic	2056	73	32	26	0	2187
Macrocytic	20	6	701	0	0 (727)	
Dimorphic	103	0	87	566	541	1297
Hemolytic	0	0	0	87	635	722
Total, *n* (%)	2278 (22.82)	4747 (47.56)	820 (8.21)	689 (6.90)	1447 (14.50)	9981 (100)

RBC=Red blood cell

may have already in an anemic state due to poor iron store. However, this finding is in the contrary to few other studies, where they have found maximum number of cases in the 31–40 years age group.[11,12]

In the present study, the majority of cases having microcytic hypochromic anemia (50.48%) followed by normocytic normochromic (21.92%), dimorphic (12.98%), macrocytic (7.29%), and hemolytic (7.23%). Many other studies such as Shrivastava *et al*.,[12] Jain *et al*.,[15] Sandhya *et al*.,[16] and Chavda *et al*.[17] found similar results regarding the distribution of anemia cases. On the other hand, RBC histogram showed left shift in the majority of cases (47.56%) and broad base in 14.50% of cases. These findings are not like various previous studies, where the researchers found the most common histogram was broad base followed by left shift.[12,16-18]

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.[11] Shifting of RBC histogram depends on the size of RBC; when the RBC size is microcytic, histogram shifts toward left while the presence of macrocytes causes shift of RBC histogram toward the right. Microcytic hypochromic anemia causes decrease in MCH and MCV which causes left shift of RBC histogram. Few cases of microcytic hypochromic anemia also showed broad-based RBC histogram. Broad-base curve denotes the presence of more anisocytosis with high RDW which can be confirmed by the microscopic examination of peripheral smear. The discrepancy of results in categorizing microcytic anemia in CBC and PB may be due to various reasons such as the presence of giant platelets, formation of platelet clumps, and presence of fragmented RBCs in hemolytic anemias which are considered microcytic RBC by automated cell counter.[19]

Normocytic normochromic anemia was the second-most type of anemia seen in 2187 (21.92%) cases in PBS examination, while RBC indices showed 23.6% cases. RBC indices remain within the normal range in normocytic normochromic anemia; however, few cases show mild variation in the size of RBC. We have found normal bell-shaped curve in normocytic anemia in 2056 number of cases, while few cases showed left shift, right shift, and bimodal peak. This variation is comparable to previous studies.[4,11] Hence, the diagnosis made by cell counter based on RBC parameters and RBC histograms is quite comparable to the diagnosis made on peripheral smear examination.

Macrocytic anemia was diagnosed in 727 (7.29%) cases based on peripheral smear examination while RBC indices revealed 805 cases which are slightly higher. The variation may be because of inclusion of cases of hemolytic anemia, where the presence of polychromatic RBC and reticulocytes may cause increase in MCV value. Various other causes may also cause false elevation of MCV value such as hyperglycemia, cold agglutinins, and leukocytosis.[11] However, the diagnosis made of both RBC indices and histograms is comparable with the diagnosis of peripheral smear examination. These findings correlate well with various previous studies.[11,17,18]

Dimorphic anemia is another variety of anemia based on morphology. In this study, there is variation in the diagnosis in cell counter generated parameters and in PS examination which is statistically significant ($P < 0.00001$). As automated cell counter interpret anemia based on the presence of predominant RBC size, few dimorphic anemia cases may be misinterpreted as microcytic, normocytic, or even macrocytic depending on the presence of the predominant red cell population.[19] RBC histogram showed typical bimodal peak only in 566 cases while 541 cases showed broad-based histogram. Broad-based histogram in dimorphic anemia can be explained by that fact of the presence of a red cell population of different sizes. There are lots of causes of dimorphic blood picture which includes nutritional anemia, response to therapy to nutritional anemia, recent history of blood transfusion, sideroblastic anemia, etc.[12] In these cases, examination of peripheral smear is of great value for better interpretation of results. Our findings in

these cases were in concordance with various previous studies.[11,16,17,20,21]

In cases of hemolytic anemia, comparison of the peripheral smear finding with RBC indices, we found statistically significant difference ($P < 0.00001$). RBC hemogram in most of the hemolytic anemia cases showed broad-based curves. This finding is mainly because of the presence of fragmented RBCs which are counted as macrocytes, while the presence of polychromatic red cells was counted as macrocytes by the cell counter. The same type of problem was reported by various researchers also.[13,16] Garg *et al*. pointed out that the broad-based histograms with right skewing and elevated RDW with low hematocrit value points toward hemolytic anemia.[11] However, in these cases, examination of peripheral smear is essential for confirmation of diagnosis and other hematological pathologies.

In our study, most of the histograms in microcytic hypochromic anemia and normocytic normochromic anemia becomes useful that gives diagnostic clue. However, in dimorphic and hemolytic anemias, RBC histogram and RBC indices vary, and in these cases, the examination of peripheral smears is mandatory to confirm the diagnosis. Bain *et al*. concluded that even in the era of sophisticated modern diagnostic analyzers, the importance of peripheral smear examination could not be ruled out, and the reports of cell counters should be interpretated in the light of peripheral smear as well as clinical context.[22]

Conclusion

In this study, we have found that RBC parameters and histograms are useful in the diagnosis of various types of anemia. Each method should be used as complementary to each other that increases diagnostic accuracy. However, peripheral smear examination is still the gold standard for confirmation of diagnosis.

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Conflicts of interest
There are no conflicts of interest.

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