International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2024; 7(3): 106-110
Received: 06-05-2024
Accepted: 08-06-2024

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Correlation of automated cell counter RBC parameters and peripheral smear findings in patients of anemia

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DOI: https://doi.org/10.33545/pathol.2024.v7.i3b.591

Abstract
Background: As one of the most prevalent disorders in our culture, an accurate diagnosis of anemia is critical to a positive clinical outcome. Morphological typing of anemia is aided by complete blood counts and automated cell counter-generated red blood cell parameters. However, it is still necessary to examine peripheral blood smears in order to determine the etiology of anemia.
Aims and Objectives: The current study compared the results of the two approaches in order to diagnose the type of anemia by analyzing peripheral blood smear and red cell parameters generated by an automated cell counter.
Materials and Methods: A total of 9500 anemic blood samples were assessed over a six-month period. Evaluation and comparison were conducted between peripheral blood smear results and RBC parameters and histograms generated by automated cell counter.
Results: Microcytic hypochromic anemia accounts for 4801 cases (50.6%) out of 9500 samples. The remaining cases are dimorphic anemia (1235, 13%), normocytic normochromic (2083, 21.87%), and hemolytic anemia (687, 7.23%). Hemolytic and dimorphic anemia exhibited a significant difference ($p<0.0001$) when compared with RBC values and peripheral smear results. In comparison to the RBC histogram, 4513 (47.51%) cases had a left shift, 779 (8.21%) cases a right shift, and 2170 (22.85%) cases a normal bell-shaped curve, indicating, microcytic, macrocytic, and normocytic normochromic anemia respectively. The bimodal and broad-base histograms in 658 (6.93%) and 1380 (14.52%) of the cases, respectively, suggested dimorphic and hemolytic anemia.
Conclusion: In most situations, the type of anemia can be diagnosed using a peripheral blood smear examination combined with an RBC histogram investigation. To improve diagnostic accuracy, each technique should be applied in a complementary manner.

Keywords: Anemia, automated cell counter, peripheral blood smear

Introduction
Anemia is a prevalent issue in our culture that is frequently disregarded in both developed and developing nations. It is a disorder that arises when the body's tissues do not receive enough oxygen from the red blood cells. Pregnant women, adolescents, and preschoolers are the most susceptible groups to anemia [1]. It's been linked to higher rates of morbidity and death. Anemia affects 1.62 billion people worldwide, or 24.8% of the total population. Preschool-age children have the highest prevalence (47.4%), whereas men have the lowest prevalence (12.7%). However, among the affected population, pregnant women make up the largest percentage (41.8%) [2].
Iron, vitamin B12, or folic acid deficiencies are the most prevalent causes of anemia. However, a number of clinical conditions can also result in anemia, such as reduced RBC production in diseases that cause bone marrow failure and increased RBC destruction in conditions that cause hemolysis. Anemia may also have multiple underlying causes. Thus, every case of anemia needs to be investigated to determine the underlying cause and the best course of action for medical care [3].
One of the basic and useful methods for identifying, diagnosing, and tracking the many types of anemias and assessing the effectiveness of treatment is the peripheral smear (PS) examination. It has been a vital diagnostic tool in the etiopathological workup of anemias for decades, serving as a window for hematological outgoings [4].

Around the globe, cell counters are becoming more and more popular in medical laboratory services, and they are also becoming less expensive. In recent years, microscopic examination of peripheral smears and complete blood count (CBC) by automated hematology analyzers have complimented each other to offer a thorough report on the patient's blood sample [5]. Despite the sophistication of modern instruments, initial calibration still requires the use of manual processes. This emphasizes how crucial it is to keep up your manual technical abilities despite your desire to trust the machines to do everything for us. Therefore, the significance of a pathologist's peripheral smear examination cannot be overstated, even with the development of cell counters.

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

- To diagnose the type of anemia by examination of peripheral blood smear and by automated cell counter generated parameters; and
- To compare the findings of peripheral blood smear examination with cell counter generated parameters.

Materials and Methods

This study was conducted prospectively in the hematology section of the Pathology Department at B.J. Medical College over a six-month period, from January 2024 to June 2024. The study comprised 9500 samples from patients who were anemic.

Inclusion criteria: All patients who are diagnosed as anaemic according to WHO definition.

Anemia was diagnosed in:

- Children (5-11 years of age) with Hb < 11.5gm/dl
- Children (12-14 years of age) with Hb < 12gm/dl
- Non-pregnant women (15 years of age and above) with Hb < 12gm/dl
- Pregnant women with Hb < 13gm/dl

Exclusion criteria

1) Patients who are less than five years of age.
2) Inadequate quantity of blood sample for automated analyzer (< 3 ml).
3) Pre Analytical errors like clotted sample.

The obtained CBC samples would be examined using the Horiba Yumizen H2500 Hematology Analyzer, and peripheral smear from the same sample is prepared by using Leishman stain. Anemia was first typed using automated cell counter-generated RBC parameters, such as RBC indices with RDW. Next, peripheral blood smear was examined by the pathologist under a microscope, and morphological typing of anemia was completed. Pathologists were not aware of the RBC indices or the position of the RBC histograms when they reported the peripheral blood smear.

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. On basis of parameters obtained from automated hematology analyzer and peripheral blood smear examination, anemia is catergorized.

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

Data were gathered and complied with. Comparative analysis was done using the Pearson Chi-square test between the PBS examination and the diagnosis produced by the parameters obtained by the cell counter. A P value = 0.05 or less is considered statistically significant.

Results

The peripheral blood smear and RBCs parameters of 9500 patients of anemia were analyzed and correlated. The age group of patients ranged from 6 months to 90 years. The age group of 21-30 years old accounted for the largest percentage of patients (24.2%). Out of 9500 patients, 5589 (58.83%) were female and 3911 (41.16%) were male. (Table 1).

Table 1: Age and gender distribution in the study

Age (years)	5-10	11-20	21-30	31-40	41-50	51-60	60 above	Total
Male	850	498	673	310	493	489	598	3911 (41.16%)
Female	622	789	1632	679	699	483	685	5589 (58.83%)
Total	1472 (15.49%)	1287 (13.54%)	2305 (24.2%)	989 (10.41%)	1192 (12.54%)	972 (10.2%)	1283 (13.5%)	9500

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

Type of anemia	Male	Female	Total
Microcytic hypochromic	1703 (35.47%)	3098 (64.52%)	4801 (50.6%)
Normocytic normochromic	915 (43.92%)	1168 (56.07%)	2083 (21.87%)
Macrocytic	322 (46.39%)	372 (53.60%)	694 (7.3%)
Dimorphic	453 (36.68%)	782 (63.31%)	1235 (13%)
Hemolytic	447 (65.06%)	240 (34.93%)	687 (7.23%)
Total	3840 (64.52%)	5660 (59.57%)	9500

Microcytic hypochromic anemia is the most prevalent type of anemia (50.6%) among both men and women, followed by normocytic normochromic anemia (21.87%) and the least common anemia observed was hemolytic anemia (7.23%) based on peripheral blood smear examination. [Table 2].

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

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

Type of anemia	PBS findings	RBC indices	P-Value
Microcytic hypochromic	4801 (50.6%)	5413 (56.98%)	
Normocytic normochromic	2083 (21.87%)	2246 (23.65%)	
Macrocytic	694 (7.3%)	769 (8.1%)	
Dimorphic	1235 (13%)	779 (8.21%)	<0.00001
Hemolytic	687 (7.23%)	293 (3.08%)	<0.00001
Total	9500	9500	

In our study, of 4801 cases of microcytic hypochromic anemia, 4438 cases showed left shift and 259 cases showed broad-based RBC histogram [Table 4]. Among 2083 cases of normocytic normochromic anemia, 1958 cases showed normal bell-shaped histogram. Among 694 cases of macrocytic anemia, 669 cases showed right-shifted RBC histogram. However, in 1235 cases of dimorphic anemia, 538 showed bimodal peak while 518 cases showed broad-base histogram [Table 4].

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

Type of anemia	RBC histogram pattern					
	Normal	Left shift	Right shift	Bimodal	Broad	Total
Microcytic hypochromic	94	4438	0	10	259	4801
Normocytic normochromic	1958	69	30	26	0	2083
Macrocytic	19	6	669	0	0	694
Dimorphic	99	0	80	538	518	1235
Hemolytic	0	0	0	84	603	687
Total	2170 (22.85%)	4513 (47.51%)	779 (8.21%)	658 (6.93%)	1380 (14.52%)	9500

Discussion

The peripheral blood smear examination has been a key diagnostic technique for the workup of anemia for many years. Automated hematological analyzers have reduced subjective errors and increased both diagnostic accuracy and precision [6]. An automated hematology analyzer produces a graphical representation known as an RBC histogram [7]. The RBC histogram is a crucial component of the whole blood count in hematology analyzers. It offers important information on several RBC characteristics, including RDW, mean corpuscular hemoglobin (MCH), and MCV, as well as hints about a variety of RBC disorders. An RBC histogram is a vertical bar graph that shows the size of every cell within a given size range. The cell counter counts the cell as an RBC when the volume is between 25 and 250 FL [8] Several RBC parameters, including RDW and MCV, are computed using the peak's area. The normal RBC curve is a Gaussian distribution with a symmetric bell shape. Normal curve falls within the normal range of MCV which is 80-100fL. It has been discovered that the RBC histogram and other RBC characteristics, such as MCV and RDW, are abnormal in a number of RBC diseases [9].

In our study, we included a total of 9500 anemic cases as per inclusion criteria. Of these cases, the majority of cases fall in the age group of 21-30 years (2305) and female outnumbered male (5589 vs. 3911). 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 may have already in an anemic state due to poor iron store. In the present study, the majority of cases having microcytic hypochromic anemia (50.6%) followed by normocytic normochromic (21.87%), dimorphic (13%), macrocytic (7.3%), and hemolytic (7.23%).

Iron deficiency anemia is the most frequent cause of microcytic hypochromic anemia. There could be a number of reasons, including poor gastrointestinal absorption, prolonged blood loss, increased demand during pregnancy and lactation, and inadequate food consumption leading to iron deficiency. The RBC histogram shifts depending on the size of the RBC, a microcytic RBC causes the histogram to shift left, whereas a macrocyte-containing RBC leads it to shift right. The RBC histogram shifts to the left due to a decrease in MCH and MCV in microcytic hypochromic anemia. A broad-based RBC histogram was also present in a small number of cases of microcytic hypochromic anemia. A broad-base curve indicates greater anisocytosis with a high RDW, which can be verified by looking at peripheral smears. The presence of large platelets, platelet clumps, and the presence of fragmented RBCs in hemolytic anemias, which are classified as microcytic RBC by automated cell counter, are some of the possible causes of the discrepancy while classifying microcytic anemia in CBC and peripheral blood smear [10].

In peripheral blood smear testing, normocytic normochromic anemia was the second most common type of anemia, observed in 2083 patients (21.87%), whereas RBC indices revealed 23.65% of cases. In normocytic normochromic anemia, RBC indices stay within the normal range, nevertheless, only a small percentage of cases exhibit modest variations in RBC size. In 1958 cases, we discovered a normal bell-shaped curve indicating normocytic anemia; however, only a small percentage of cases had left, right, and bimodal peaks. Therefore, the diagnosis obtained from a cell counter using RBC parameters and RBC histograms is fairly similar to the diagnosis obtained from a peripheral smear test.

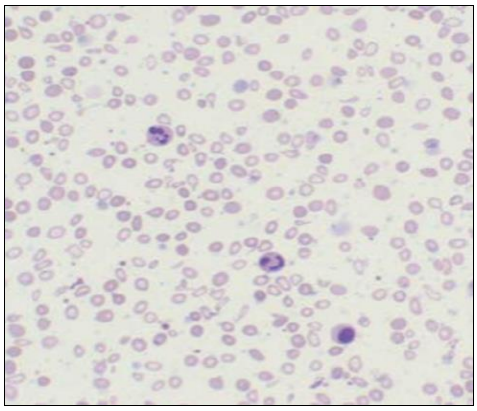

Fig 1: Peripheral blood smear of microcytic hypochromic anemia showing microcytes, teardrop cells and elliptocytes

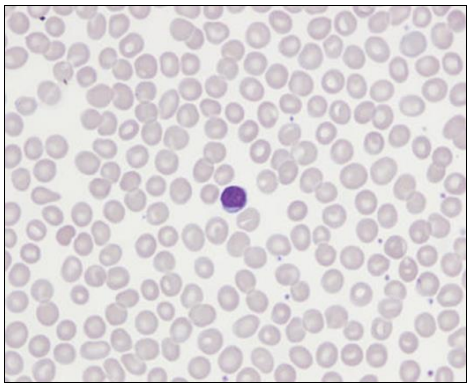

Fig 2: Peripheral blood smear of macrocytic hypochromic anemia

Macrocytic anemia was diagnosed in 694 (7.3%) cases based on peripheral smear examination while RBC indices revealed 769 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 [7]. However, the diagnosis made of both RBC indices and histograms is comparable with the diagnosis of peripheral smear examination.

Another morphological type of anemia is called dimorphic anemia. In this study statistically significant ($p<0.00001$) discrepancy was found in diagnosis of dimorphic anemia between the peripheral blood smear examination and the parameters generated by the cell counter. Few cases of dimorphic anemia may be mistakenly identified as microcytic, normocytic, or even macrocytic depending on the existence of the major red cell population, as automated cell counters interpret anemia based on the presence of predominant RBC size [10]. Only 538 cases had a typical bimodal peak on the RBC histogram, whereas 518 cases had a broad-based histogram. Broad based histogram in dimorphic anemia can be explained by the presence of a population of red blood cells with varying sizes. Dimorphic blood image can be caused by a variety of factors, such as sideroblastic anemia, nutritional anemia, recent blood transfusion history, and the body's reaction to treatment [8]. Examining the peripheral smear in these situations is quite beneficial for improving the interpretation of the findings.

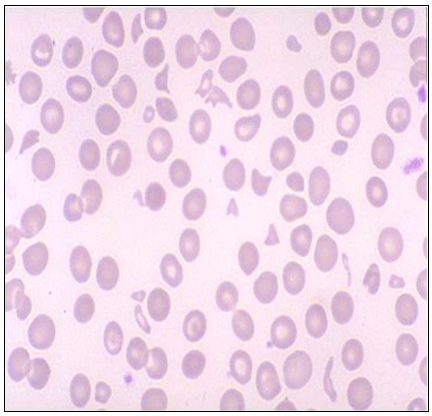

Fig 3: Peripheral smear of hemolytic anemia showing microcytes, fragmented RBCs, polychromasia

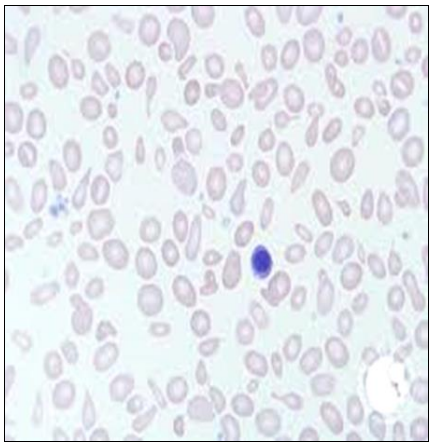

Fig 4: Peripheral smear of Dimophic anemia showing microcytes, macrocytes, teardrop cells, elliptocytes

When we compared the peripheral smear results with the RBC indices in hemolytic anemia cases, we discovered a statistically significant difference ($p<0.00001$). The majority of hemolytic anemia cases had broad-based curves on their RBC hemograms. This result is primarily due to the existence of fragmented red blood cells, which the cell counter counts as microcytes, in addition to polychromatic red blood cells, which are considered as macrocytes. Examining the peripheral smear is necessary in these situations, nevertheless, to confirm the diagnosis and rule out any further hematological disorders.

In our investigation, the majority of the histograms in normocytic normochromic anemia and microcytic hypochromic anemia become helpful in providing a diagnostic hint. However, RBC indices and histograms differ in dimorphic and hemolytic anemias, and peripheral smear analysis is required in these situations to establish the diagnosis.

Conclusion

By this study we conclude that even in the era of molecular diagnoses and automated cell counters, peripheral blood smear examination remains an important diagnostic tool. The CBC done with the help of cell counters should always be interpreted in the light of peripheral smear examination as this could help us in getting the correct diagnosis.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article
Patel AV, Shah AM, Goswami H. Correlation of automated cell counter RBC parameters and peripheral smear findings in patients of anemia. International Journal of Clinical and Diagnostic Pathology. 2024;7(3):106-110.