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Original Research Paper

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# CORRELATION OF AUTOMATED CELL COUNTER RBC HISTOGRAMS AND RBC INDICES WITH PERIPHERAL SMEAR IN DIAGNOSIS OF ANEMIA.

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**ABSTRACT**

**Background:** Anemia's morphological alterations can be inferred from red blood cell (RBC) indices and the histogram produced by an automated analyzer. They can be utilized to determine the cause of anemia in addition to the peripheral smear. Objective: The current study aimed to improve the method for diagnosing anemia by correlating the typing of anemia based on RBC indices and histograms acquired from an automated analyzer with peripheral smear testing. **Materials and Method:** 184 anemic patients had blood samples taken, and the blood samples were first typed for anemia using parameters from a haematology analyzer (RBC indices with red cell distribution width), and then the results of a peripheral blood smear. The significance of the mean difference in various parameters was determined by analyzing the connection between the diagnosis made by peripheral smear vs RBC indices and histogram. **Results:** Based on a peripheral smear test, the most common type of anemia was microcytic hypochromic (63%) followed by normocytic normochromic (35.9%) and macrocytic (1.1%). 62%, 37%, 1.1%, and microcytic hypochromic, normocytic normochromic and macrocytic respectively, were shown by RBC indices analysis. 114 of the 116 (63%) instances of microcytic hypochromic anemia on smears showed a left shift, and the other two cases displayed a normal curve. Sixty-six cases of normocytic normochromic anemia showed a normal curve. In two examples of macrocytic anemia, histograms exhibit a shift to the right. **Conclusion:** Although RBC and histogram indices provide useful information, peripheral smear testing is still the most crucial diagnostic method for haematological diseases.

**KEYWORDS :** Anemia, Erythrocytes, Hypochromic, Normochromic, Macrocytic, Microcytic

## INTRODUCTION

Anemia is a serious global public health concern that is particularly common in underdeveloped nations like India. Nearly two billion people suffer from anemia worldwide; with iron deficiency being the cause of half of these cases, according to the World Health Organization (WHO).In underdeveloped nations, the estimated prevalence of anemia in children under 60 is 39%. These startling numbers have significant ramifications for low- and middle-income countries' economies and health.(1)

Anemia is a reduction in the blood's ability to carry oxygen. It may occur when an individual's packed cell volume (PCV) or hemoglobin (Hb) concentration falls below the lower bound of the reference interval for their age, gender, place of residence, and physiological state. In the current clinical settings, the RBC histogram is a crucial tool for visualizing the particle size distribution that is used in the initial screening and diagnostic process for haematological illnesses.(2) As more sophisticated and accurate haematology analyzers become available, the use of manual peripheral smear examinations is gradually decreasing.(3) The automated hematology analyzers count a far higher number of cells than the manual peripheral smear testing method, and the computerized analyzers offer significantly higher accuracy and the ability to use histograms. The RBC histogram and other CBC parameters, such as mean corpuscular volume (MCV) and RBC distribution width (RDW), have been found to be abnormal in a range of haematological illnesses and may offer crucial hints for the diagnosis and management of serious red cell disorders.(4)

An essential step in the diagnosis of anemia is the automated peripheral blood count. The device can measure a number of fundamental and sophisticated characteristics, but manual microscopic scanning of peripheral smears is required to detect morphological correlation and other hints that cell analyzers are unable to detect. The quantity of cells that can be examined on a slide is usually much less than that of automated haematology analyzers, which can count cells with even greater accuracy. Histograms can be used to significantly minimize the number of slides that need to be screened. In the majority of laboratory setups, the traditional focus has been on verifying automated data—a task that has outlived its usefulness.(3)

This histogram has been abnormal in a number of haematological disorders, along with other CBC features like mean corpuscular volume (MCV) and RBC distribution width (RDW). These features may be crucial in the identification and treatment of serious red cell disorders. In theory, the red cell histogram can now show some conditions that were previously only visible with a blood film study, such as the presence of broken red cells or red cell agglutination. When treating patients with iron deficiency anemia (IDA) or megaloblastic anemia, a sequential histogram can also clearly show the rising appearance of a new erythrocyte population well ahead of other numerical indications.(5) The goal of the current study was to ascertain how automated cell counter Rbc histograms and Rbc indices correlated with peripheral smears for anemia diagnosis.

## MATERIALS AND METHOD

### Inclusion Criteria

Every patient diagnosed with anemia in accordance with the WHO definition

### Exclusion Criteria

The study excluded participants with leucocytosis, leukemia, leukemoid reaction, parasites, and platelet abnormalities.

### Method of Data Collection

Venepuncture was used to get the venous sample in EDTA vacutainers. Blood smears were prepared and stained with Leishman stain at the same time as the samples were aspirated into the MINDRAY BC- 5000 AUTOMATED 5 part haematology analyzer. The haematology analyzer's parameters (RBC indices with RDW) were used to first type anemia, and the results of peripheral blood smears were then recorded. The RBC histogram's position and shape were noted. The RBC and histogram indices, however, were not disclosed to the pathologist at the time the peripheral smear report was made.

Auto analyzer classification of anemia according to RBC indices:

- microcytic hypochromic anemia with increased RDW
- microcytic hypochromic anemia with normal RDW
- normocytic normochromic anemia with increased RDW
- normocytic normochromic anemia with normal RDW
- macrocytic anemia

RBC histograms were analyzed for position (normal, left shift, and right shift) and form (normal bell-shaped or Gaussian, broad-based, bimodal peak with skewing to left and right). The histogram pattern was observed to be associated with RBC indices.

Anemia morphological typing according to results from peripheral smears

- microcytic hypochromic anemia
- normocytic normochromic anemia
- macrocytic anemia

### Statistical Analysis

In order to determine whether the mean difference in various RBC parameters was significant, the data were gathered and the correlation between the diagnoses given by the peripheral smear vs. RBC histogram and indices was statistically analyzed using one-way analysis of variance (ANOVA). Statistical significance was defined as a p-value of 0.05 or less.

## RESULTS

**Table 1**

| Gender | Frequency | Percentage |
|---|---|---|
| Female | 123 | 66.8 |
| Male | 61 | 33.2 |
| Total | 184 | 100 |

The above table shows the gender distribution of the sample. There are 123 (66.8%) females and 61 (33.2%) males out of 184 total sample.

**Table 2**

| Peripheral smear findings | Frequency | Percentage |
|---|---|---|
| Macrocytic anemia | 2 | 1.1 |
| Microcytic hypochromic anemia | 116 | 63.0 |
| Normocytic normochromic anemia | 66 | 35.9 |
| Total | 184 | 100 |

The peripheral smear findings shows that there were 2 (1.1%) samples of macrocytic anemia, 116 (63.0%) microcytic hypochromic anemia and 66 (35.9%) normocytic normochromic anemia samples.

**Table 3**

| Histogram interpretation | Frequency | Percentage |
|---|---|---|
| Left shift | 114 | 62.0 |
| Normal curve | 68 | 37.0 |
| Right shift | 2 | 1.1 |
| Total | 184 | 100 |

The above table shows histogram interpretation frequency which shows that out of 184 total samples, 114 (62.0%) had left shift, 68 (37%) had normal curve and right shift was seen in 2 (1.1%) of the total samples.

**Table 4**

| Peripheral smear findings | Age | |
|---|---|---|
| | Mean | SD |
| Macrocytic anemia | 66.0 | 8.45 |
| Microcytic hypochromic anemia | 46.16 | 22.77 |
| Normocytic normochromic anemia | 52.48 | 19.39 |
| p-value | 0.088 | |

The peripheral smear findings mean age was calculated in the above table. The results shows that in macrocytic anemia patients the mean age is 66.0±8.45, for microcytic hypochromic anemia mean age is 46.16±22.77 and for normocytic normochromic anemia mean age is 52.48±19.39. The mean age is statistically non-significant among the groups (p>0.05).

**Table 5**

| Peripheral smear findings | Hemo globin (gm %) | | PCV (vol%) | | MCV (fl) | | MCH (pg) | | RDW CV(%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Macrocy ticanemia | 10.50 | 0. 70 | 32.50 | 0. 70 | 102.0 | 2. 82 | 28.50 | 7. 78 | 14.0 | 0. 00 |
| Microcy tic hypo chromic anemia | 9.31 | 1. 24 | 29.86 | 3. 42 | 70.82 | 7. 40 | 21.82 | 2. 74 | 29.30 | 129. 139 |
| Normocy tic normo chromic anemia | 9.62 | 0. 87 | 30.0 | 2. 84 | 87.29 | 4. 29 | 27.85 | 2. 12 | 14.98 | 1. 45 |
| p-value | 0.082 | | 0.508 | | 0.000 | | 0.000 | | 0.660 | |

The above table shows the mean hemoglobin, mean PCV, MCV, MCH and RDW-CV of peripheral smear findings. The results shows that the mean Hemoglobin, mean PCV and mean RDW-CV had statistically non-significant (p>0.05) difference among the peripheral smear findings while mean MCV and mean RDW-CV showed highly statistically significant difference among the peripheral smear findings (p<0.05).

**Table 6**

| Histogram interpretation | Age | |
|---|---|---|
| | Mean | SD |
| Left shift | 45.96 | 22.84 |
| Normal curve | 52.65 | 19.27 |
| Right shift | 66.00 | 8.48 |
| p-value | 0.069 | |

The histogram interpretation mean age was calculated in the above table. The results shows that in left shift patients the mean age is 45.96±22.84, for normal curve mean age is 52.65±19.27 and for right shift mean age is 66.00±8.48. The mean age is statistically non-significant among the groups (p>0.05).

**Table 7**

| Histo-gram inter pretation | Hemo-globin (gm %) | | PCV (vol%) | | MCV (fl) | | MCH (pg) | | RDW-CV(%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Left shift | 9.30 | 1. 24 | 29.83 | 3. 44 | 70.59 | 7. 21 | 21.74 | 2. 68 | 29.54 | 130. 64 |
| Normal curve | 9.63 | 0. 86 | 30.04 | 2. 81 | 87.19 | 4. 39 | 27.81 | 2. 14 | 15.01 | 1.45 |
| Right shift | 10.50 | 0. 70 | 32.51 | 0. 70 | 102.0 0 | 2. 82 | 28.50 | 7. 77 | 14.00 | 0.00 |
| p-value | 0.062 | | 0.482 | | 0.000 | | 0.000 | | 0.649 | |

The above table shows the mean hemoglobin, mean PCV, MCV, MCH and RDW-CV of histogram interpretations. The results shows that the mean Hemoglobin, mean PCV and mean RDW-CV had statistically non-significant ($p>0.05$) difference among the histogram interpretations while mean MCV and mean RDW-CV showed highly statistically significant difference among the histogram interpretations ($p<0.05$).

**Table 8**

| | | Histogram interpretation | | | p-value |
|---|---|---|---|---|---|
| | | Left shift | Normal curve | Right shift | |
| Peripheral smear findings | Macrocytic anemia | 0 | 0 | 2 | 0.000 |
| | Microcytic-hypochromic-anemia | 114 | 2 | 0 | |
| | Normocytic-normochromic-anemia | 0 | 66 | 0 | |

The above table shows the distribution of peripheral smear findings based on the histogram interpretations. The results shows statistically significant distribution ($p<0.05$)

## DISCUSSION

There is disagreement about which hemoglobin level limitations should be used to characterize anemia in the general population. Anaemia is a worldwide issue that affects people in both industrialized and developing nations.(6) Anaemia affects about one-third of the world's population.(7) Peripheral smears have been a key diagnostic technique for the workup of anemia for many years. With the introduction of automatic haematology cell counters, subjective errors have decreased and accuracy and precision have increased. For the initial calibration and the presumed diagnosis of anemia, leukemia, and other related illnesses, microscopic inspection is still necessary.(8) The goal of the current study was to connect the results of peripheral smears with red blood cell indices and histogram patterns from the MINDRAY BC- 5000 AUTOMATED 5 part hematology analyzer. The usefulness of red cell indices and histograms in identifying and contrasting anemias has not been extensively studied. Every automated cell counter has the RBC histogram, which is a standard feature of automated haematology analysis. The well-known Coulter concept of counting and sizing red cells serves as the foundation for the creation of the RBC histogram. The histogram has been found to be aberrant in a variety of haematological disorders when paired with other CBC measures like RDW and MCV.(9) It was observed that the bulk of anemic cases included female patients. Studies have shown that a number of factors, including low iron intake, prolonged blood loss, poor absorption, infections, and alterations in lifestyle, food habits, and social and behavioral patterns, contribute to the high prevalence of anemia in females. We found that females had a higher frequency of anemia (66.8%) in our study. This was consistent with the Singhal et al. study (64.9%)(10). After the sixth decade, males were seen to be more impacted than females, indicating the possibility that anemia in this age group developed due to other factors. Microcytic hypochromic anemia was the most prevalent morphological type, followed by normocytic normochromic anemia. The most frequent cause of microcytic hypochromic anemia is a reduction in the body's iron store, which can have several causes. This could be brought on by low dietary iron intake, poor intestinal absorption of iron, both acute and chronic blood loss, and increased iron requirements during pregnancy and the healing process following major surgery or trauma. The most frequent cause of microcytic hypochromic anemia was iron insufficiency.(1)

In their investigations, Sandhya et al. (2014),(11) Chavda et al. (2015),(12) and BynaSyam Sundara Rao et al. (2017)(13) also discovered a comparable distribution of anemic patients.

The most common type of anemia in both sexes, according to Basavaraj RG. et al.,(14) was microcytic hypochromic anemia, which was followed by normocytic normochromic anemia.

The study by Garg et al.(1) found that the majority of cases in both sexes were of microcytic hypochromic anemia with raised RDW (41.71%), followed by normocytic normochromic anemia with normal RDW (19.43%) and macrocytic hypochromic anemia with normal RDW (17.71%). These findings are consistent with the auto analyzer derived RBC indices, which show that the majority of cases in both sexes were of microcytic hypochromic anemia with raised RDW (29.30%), normocytic normochromic anemia with normal RDW (14.98%), and macrocytic hypochromic anemia with normal RDW (14%).

While MCV and MCH are reduced in our research of microcytic hypochromic anemia, MCHC may be normal. Low MCV RBC populations will be moved to the left. A broad-based curve indicates anisocytosis due to its high RDW. Sixty-two cases (of the sixty-three cases of microcytic hypochromic anemia) on smear showed a left shift, and two cases had a broad-based curve on the histogram. All of these results are compared to analyzer data, which shows that 62% of cases had microcytic hypochromic anemia. When the auto analyzer classifies fragmented RBCs as microcytes in hemolytic disorders, the existence of large platelets and platelet clumping can account for this slight variation. Peripheral smears therefore eliminate these mistakes. These results are consistent with research by Sandhya et al. (46%),(11)and Singhal et al. (49.8%).(10)

Peripheral smear test revealed 35.9% of cases with normocytic normochromic anemia, whereas red blood cell indices revealed 37% of cases. The red cell indices in normocytic normochromic anemia stay within the normal range, with a small percentage of cases exhibiting mildly elevated indices. The size of the cell population may vary. There could be some microcytic cells where the majority of the cells are of normal size, which would cause a bigger deviation and hence a higher RDW. Thus, a diagnosis obtained by peripheral smear testing was equivalent to a diagnostic based on RBC indices and histograms. It is similar to research conducted by Rao et al. (19.4%)(13) and Sandhya et al. (21.8%)(11). Peripheral smear test revealed 1.1% of cases with macrocytic anemia, whereas RBC indices also showed 1.1% of cases with the condition. Two examples had a right shift in the histogram findings. The results of the peripheral smear and RBC indices varied noticeably. The explanation could be that the presence of polychromatophilic erythrocytes in hemolytic anemia, which results in a high MCV value, causes it to be misinterpreted as macrocytic anemia. On the other hand, MCV using an automated blood cell counter is rarely off. False MCV elevations can be caused by leukocytosis, hyperglycemia, and cold agglutinins. Rao et al. (2.2%)(13) and Chavda et al. (3.6%) made similar observations.(12)

It is evident from this that those histograms are a helpful diagnostic tool for normocytic normochromic anemia and microcytic hypochromic anemia. On the other hand, simple to complicated curves were seen in the histogram patterns of macrocytic anemia. Consequently, peripheral smear screening is essential for detecting red cell alterations in all individuals with reduced hemoglobin levels. Even in the era of molecular analysis, blood smears remain an important diagnostic tool, and sophisticated modern investigations of haematological disorders should be interpreted in the light of

peripheral blood smears as well as clinical context, according to Bain's 2005 review on the place of peripheral smear examination in the era of automation.(15)

## CONCLUSION

An important association between RBC parameters, histogram, and peripheral smear diagnosis was found in our study. While peripheral smear is still the gold standard diagnostic test for evaluation, RBC indices and histograms are useful in the diagnosis of anemia.

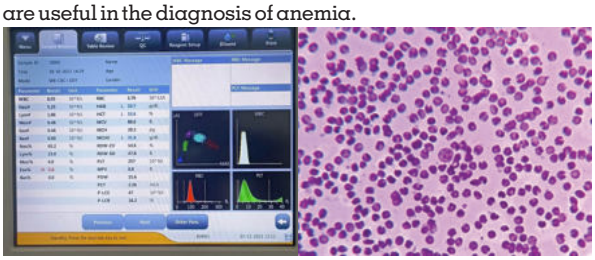

**Fig 1:** RBC indices and histogram compared with normocytic normochromic anemia(peripheral smear)

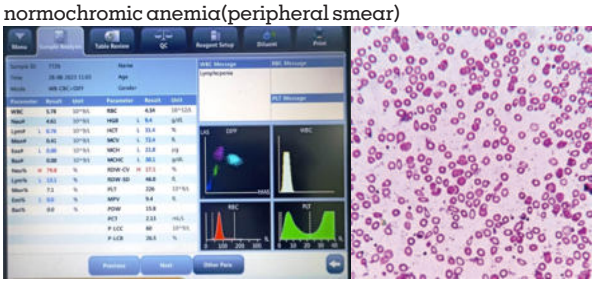

**Fig 2:** RBC Indices And Histogram Compared With Microcytic Hypochromic Anemia(peripheral Smear)

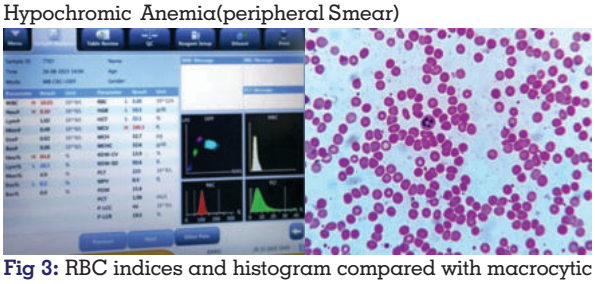

**Fig 3:** RBC indices and histogram compared with macrocytic anemia(peripheral smear)

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